Disc haemorrhages in glaucoma

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Section 1 Overview

1.1.1 Signed declaration

I, Jibran Mohamed Noriega confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Jibran Mohamed Noriega

09 March 2021

1.1.2 Abstract

Background: Disc haemorrhages (DH) are associated with glaucomatous progression. However, the effect of lowering intraocular pressure (IOP) in patients with DHs seems controversial; it may reduce visual field (VF) progression, but it does not reduce the frequency of DHs.

Experiments and investigations: A survey of glaucoma specialists investigated the effect of new DHs on their clinical management. A method based on scanning laser ophthalmoscopy (SLO) to detect DHs was developed and compared to fundus photography and clinical examination. The detection of DHs and their role in visual field progression was investigated in the United Kingdom Glaucoma Treatment Study (UKGTS). The effect of medical and surgical reduction of intraocular pressure was investigated in the UKGTS and a cohort of normal-tension glaucoma patients, respectively. Risk factors for DHs and a probability analysis exploring how often DHs appear simultaneously in both eyes were analysed to explore possible pathogenic mechanisms.

Results: When a new DH is detected, 80% of clinicians modify their management plan, but the modifications vary. An SLO-based method for DH detection has very good within- and between-observers agreement. A comparison with fundus photography showed that photography is marginally better than SLO when the results of all observers are averaged but similar when the best observers are analysed independently. We confirmed the association between DHs and visual field deterioration. Treatment with latanoprost reduces the risk of VF deterioration in patients with and without DHs, but the frequency of DHs is not affected. Trabeculectomy reduced VF deterioration and the frequency of DHs.

Conclusion: The detection of DHs guides most ophthalmologists to modify clinical decisions. An image-based method is required to increase the detection rate of DHs and the SLO-based method is an alternative for detection. IOP lowering with medication or surgery reduces VF progression in patients with DHs.

1.1.3 Impact statement

Glaucoma has been called 'the silent thief of sight' because most individuals with glaucoma have no early symptoms; it is the most common cause of irreversible blindness worldwide. Nevertheless, not all patients with glaucoma will progress to blindness; therefore, clinicians assess the risk of disability induced by glaucoma for each patient and adjust the treatment accordingly. However, this process of risk-stratification of patients with glaucoma is based on a limited number of factors such as baseline level of IOP, exfoliation syndrome and severity of the disease. Our research on disc haemorrhages is important to patients because these bleedings in the optic nerve have been consistently identified as one of the greatest risk factors for visual field deterioration. The problem that many patients face during routine clinical examinations is that clinicians tend to miss the detection of DHs and miss opportunities to adjust the management plan accordingly.

We have identified a simple method to detect DHs with instruments that are routinely used in most glaucoma clinics in developed countries. Using data from the UKGTS, we confirmed the strong effect that DHs have on VF deterioration and identified the positive effect that treatment with the most frequently prescribed drug for glaucoma (latanoprost) has on reducing the risk and speed of VF deterioration. We also identified for the first time that the most frequently performed surgery for glaucoma (trabeculectomy) effectively reduces the IOP and the risk of VF deterioration in patients with and without DHs irrespective of DH status.

The industry could use the experience gained in our research to optimize the currently available imaging instruments to aid in detecting DHs; these updated instruments could be quickly translated into commercial products that help busy clinicians to detect DHs.

Managers of eye clinics who consider the disposal of fundus cameras to open space for other imaging instruments could use our research to evaluate the risk of reducing the detection of DHs with this decision.

Considering the substantial risk of VF deterioration that we have identified in patients with DHs, the biopharmaceutical industry could use this data to design future glaucoma trials stratifying patients at baseline based on the DH status. A balanced distribution of participants with and without DHs at baseline in all arms of clinical trials could avoid unexpected results associated with faster VF deterioration in groups with a higher proportion of DH+ participants.

The academic community would benefit from a better understanding of the role of DHs in glaucoma. We hope this research will create more interest in investigating this small and transient phenomenon that seems to have big and permanent effects on patients.

1.1.4 Dedicatory and acknowledgements

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1.1.8 List of abbreviations

Abbreviations	
AD	Alzheimer's disease
ADAGES	African descent and glaucoma evaluation study
ALC	Anterior lamina cribosa
ARR	Absolute risk reduction
AUC	Area under the curve
BE	Both eyes
BM	Bruch's membrane
BMO	Bruch’s membrane opening
CAA	Cerebral amyloid angiopathy
CCB	Calcium channel blockers
CH	Corneal hysteresis
CI	Confidence Intervals
CNTGS	Collaborative normal tension glaucoma study
CRVP	Central retinal venous pressure
CSF	Cerebrospinal fluid
CSLO	Confocal scanning laser ophthalmoscopy
DH	Disc haemorrhage
DHP	Dihydropyridine
EGPS	European glaucoma prevention study
EMGT	Early manifest glaucoma trial
ET	Endothelin
FLCD	Focal lamina cribosa defects
FU	Follow up
GON	Glaucomatous optic neuropathy

HR	Hazard ratio
HRT	Heildelberg retina tomograph
HTG	High tension glaucoma
HTN	Systemic hypertension
Hz	Hertz
ICER	Incremental cost-effectiveness ratio
IOP	Intraocular pressure
ISRCTN	International Standard Randomised Controlled Trial Number
LE	Left eye
LNPGS	Lower normal pressure glaucoma study
LoGTS	Low tension glaucoma treatment study
MMP	Matrix metalloproteinases
MPHSD	Mean pixel height standard deviation
MRA	Moorfields regression analysis
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MRW	Minimum rim width
NNT	Number needed to treat
NTG	Normal tension glaucoma
OAG	Open angle glaucoma
OCT	Optical coherence tomography
OCT-A	Optical coherence tomography angiography
OHTS	Ocular hypertension treatment study
ONH	Optic nerve head
OR	Odds ratio
ORA	Ocular response analyser

PACG	Primary angle closure glaucoma
PLWD	Prelaminar wedge-shaped defects
POAG	Primary open angle glaucoma
PPA	Peripapillary atrophy
PPV	Positive predictive value
PVD	Posterior vitreous detachment
RE	Right eye
RNFL	Retinal nerve fibre layer
ROC	Receiver operating characteristic
RR	Relative risk
RRR	Relative risk reduction
RVO	Retinal vein occlusions
SD-OCT	Spectral domain optical coherence tomography
SSRI	Selective serotonin reuptake inhibitor
TCA	Topographic change analysis
UKGTS	United Kingdom glaucoma treatment study
VF	Visual fields
2D	Two dimensional or monoscopic
3D	Three dimensional or stereoscopic

Section 2 Introduction and literature review

2.1.1 What is glaucoma

The term glaucoma is used to define a broad group of diseases that typically produce axonal loss and deformation of the optic nerve. The clinical detection of cupping, diffuse or focal narrowing of the neuroretinal rim, and peripapillary nerve fibre layer loss are usually called glaucomatous optic neuropathy (GON) and help clinicians differentiate it from non-glaucomatous optic neuropathies such as ischaemic optic neuropathies, hereditary, toxic and other types (1).

Patients with glaucoma have been historically classified into different subtypes based on the detection of other clinical signs or symptoms, such as the anterior chamber angle and the presence of other coexisting diseases. Patients can be classified with open angle glaucoma or angle closure glaucoma and primary or secondary based on examining the anterior chamber angle and coexisting disease. Therefore patients are usually categorized into one of four groups: 1) primary open angle glaucoma (POAG), primary angle closure glaucoma (PACG), secondary open angle glaucoma, and secondary angle closure glaucoma.

2.1.2 Alternate names for disc haemorrhage (DH)

Optic disk h(a)emorrhage(s)

Optic nerve h(a)emorrhage(s)

Optic nerve head h(a)emorrhage(s)

Nerve fiber (fibre) layer h(a)emorrhage(s)

Splinter disc(k) h(a)emorrhage(s)

Splinter-like h(a)emorrhage(s)

Drance h(a)emorrhage(s)

2.1.3 Definition of DHs

A DH is an intraocular phenomenon in which extravascular blood is observed in the optic nerve or peripapillary area (one disc diametre) with a flame or dot shape.

2.1.4 Reflections on the definition of DH as 'true haemorrhages'

The majority of the definitions of haemorrhage include the escape of blood, with all its components, from the vessels. Usually, after a rupture of the wall of a blood vessel. The following are some definitions:

1. Oxford dictionary (2): 'a medical condition in which there is severe loss of blood from a damaged blood vessel inside a person's body'.
2. Cambridge dictionary (3): 'A large flow of blood from a damaged blood vessel. An escape of blood from any blood vessel'.
3. Wikipedia (4): 'Bleeding, also known as a hemorrhage, haemorrhage, or simply blood loss, is blood escaping from the circulatory system from damaged blood vessels'.

Considering the previous definitions of a haemorrhage, DHs would fit perfectly in the definition of a haemorrhage except for the "rupture/damaged blood vessel". There are no visible marks of injury in the vessel that are the likely source of DHs.

In addition to the previous difference, the red colouration in DHs does not change over time similarly to other haemorrhages of the body or eye such as subconjunctival haemorrhages, vitreous haemorrhages or subcutaneous haematomas. Over time, the change of DHs is more similar to intra-retinal haemorrhages that do not change in colour over time and tend to disappear slowly.

A better definition of DHs needs to consider what is truly known about them and what is only a logic assumption (Table 1).

Table 1 Discussion about the definition of DH. With columns of points in favour and against the definition of DHs as 'true haemorrhages'.

Conventional definition	In favour	Against
Red colour	Probably from the haemo group bind to oxygen from the blood	
Damage to blood vessel	The vessels can be microscopically damaged	No visible damage to blood vessels
Duration	The local environment might preserve some of the heme red pigments in the tissue.	Last longer than bleedings in other parts of the body. Is it a continuous haemorrhage or a slow clearance?
Change in colour over time	The difference in the concentration of tissular oxygen or penetration of light might explain why DHs do not change colour	DHs differ from other haemorrhages because they do not change colour over time.

Finally, DHs tend to last longer than other haemorrhages in the body, but there is great variability in the duration between individuals. The difference between DHs and other haemorrhages could be related to the difference in the tissular pressure in other parts of the body compared to the intraocular pressure. Another option is that individuals with a higher susceptibility to develop glaucoma could have compromised clearance systems that make the blood last longer in the tissue; therefore, DHs could be considered a clinical surrogate of a compromised clearance system.

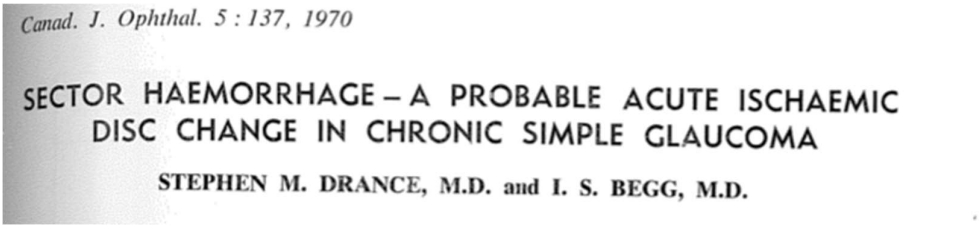
To conclude, DHs resemble many characteristics of haemorrhages in other parts of the body, but they have different clinical characteristics that need further research to improve our understanding of the role of DH in glaucoma.

2.1.5 Brief history of DHs

There is some controversy about the first description of DHs. The most frequently cited description of DHs is from 1889, when Jannik Petersen Bjerrum (Figure 1)

described DHs in a Danish Journal (5). However, in 1876 Albert Emmerich presented his medical doctorate dissertation thesis titled 'glaucoma haemorrhagicum' (6). During the early twentieth century, the term was rarely used (7) until many years later, in 1969, Feldman, Sweeney, and Drance (8), and in 1970, Drance and Begg (9) described sector haemorrhage as a probable acute ischaemic disc change (Figure 2). Afterwards, many publications have tried to identify the role that DHs play in glaucoma. How DHs affect the risk of developing glaucoma, VF progression or the response to treatment are some of the most frequent topics investigated with respect to DHs.

Figure 1 Jannik Petersen Bjerrum from a painting at the Rigshospital, Eye Department, Copenhagen (10). Image reproduced with permission of the rights holder, John Wiley and Sons.



Canad. J. Ophthal. 5 : 137, 1970

SECTOR HAEMORRHAGE – A PROBABLE ACUTE ISCHAEMIC DISC CHANGE IN CHRONIC SIMPLE GLAUCOMA

STEPHEN M. DRANCE, M.D. and I. S. BEGG, M.D.

Figure 2 Title of the paper in 1970 after the re-discovery of DHs (9).

2.1.6 Methods to diagnose DHs.

The identification of DHs has been traditionally performed with the ophthalmoscope or by indirect ophthalmoscopy during routine clinical examinations. For documentation or research purpose, stereoscopic fundus photographs have been the preferred method for diagnosis and follow up. After the introduction of high-quality cameras in mobile phones, it has become possible to adapt a high dioptre lens to the phone's camera to image the fundus and detect disc haemorrhages (11).

After the fundus photographs are acquired, the detection of DHs has been traditionally based on an expert grading a single image. Subtraction techniques have been published as an alternative to improve the ability of the grader to correctly identify the presence or absence of a DH (12). Other alternatives to aid graders to identify DHs are the digital manipulation of the image with techniques such as histogram equalisation (13).

The first non-photographic imaging technique to identify DHs was described by Dichtl A et al. using scanning laser ophthalmoscopy (SLO) (14). However, when SLO was later compared to fundus photography by Budde et al., SLO only detected 50% of the DHs that were detected by fundus photography (15). The SLO technology used by Budde et al. was first commercially available in the Heidelberg retina tomograph (HRT II and III; Heidelberg Engineering, Heidelberg, Germany); this instrument uses confocal SLO or CSLO, also known as confocal laser tomography. SLO technology has been used for the eye since the late 1980s (16). In brief, it consists of a laser beam (670nm) that scans the optic nerve with an angle of 15 x 15 degrees and is detected through a pinhole in a confocal

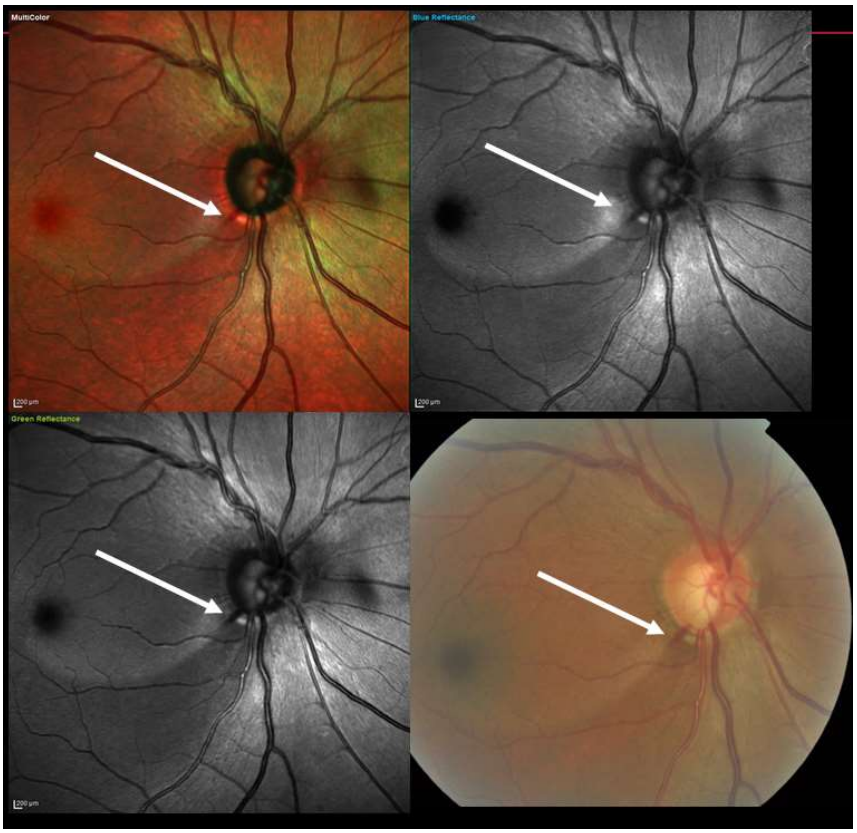
system that produces sharp images avoiding scattered light to return to the detector. The software considers the maximum reflectance point as the highest point and constructs a topography map of 384 X 384 pixels. The instrument moves the focal plane in a consecutive interval to create 64 tomographic scans (depending on the cup depth) and then constructs a three-dimensional reconstruction of the optic nerve. The HRT combines the CSLO technology with statistical, computational software and a patient reference database to aid clinicians in diagnosing glaucoma and the assessment of progression. The most recognized parameters of the HRT used to detect progression are: 1) MRA (Moorfields Regression Analysis), 2) Topography measures and 3) TCA (Topographic Change Analysis).

Ha et al. reported a method to identify small DHs ('microhemorrhage') with an area smaller than 0.01 mm^2; these DHs were not detected when graders observed normal photographs but became apparent in the enhanced fundus photographs. The enhancement of the original photograph was performed in Adobe Photoshop, and it consisted of the reduction of the media-opacity-related noise and the improvement of the contrast between the DH and the rest of the fundus. With this technique, a 'microhemorrhage' was detected in 36% of the previous photographs of patients that later developed a normal DH (17).

A different approach to identify DHs is by using a composite image of the fundus that is constructed with simultaneous confocal reflectance images obtained by different monochromatic wavelengths. The Spectralis HRA+OCT equipment (Heidelberg Engineering, Heidelberg, Germany) produces fundus images using blue (488nm wavelength), green (518nm wavelength), and infrared (815nm wavelength) light that is differentially absorbed by the ocular pigments, which could help in the identification of different characteristics of the retinal anatomy (18). The MultiColor image created with the Spectralis HRA+OCT is a blue-green enhanced fundus image that has the ability to simulate some of the characteristics of a true colour fundus photograph. Although MultiColor images are easier to acquire in patients with small pupils or media opacity, they should not be considered fully equivalent to traditional colour fundus photography (19). Figure 3 shows a patient scanned with the Spectralis HRA+OCT. The Multicolor blue-green enhanced image simulates some characteristics of how a DH would look

in a traditional colour fundus photography but with a more 'greener' background. However, there are no studies looking at the ability of this technology as a diagnostic tool to detect DHs compared to traditional colour fundus photography.

Figure 3 Multimodal imaging of a right eye DH. Top left MultiColor Spectralis, top right Spectralis blue reflectance, lower left Spectralis green reflectance, and lower right true fundus colour photograph. Courtesy of Heidelberg Engineering, Heidelberg, Germany.



More recently, OCT angiography (OCT-A) has been used to help clinicians differentiate between DHs and aneurysms at the optic nerve (20). DHs which have no moving blood would not be detected by the OCT-A as a vessel. While the aneurysm, which has red blood cells moving inside its walls, would be detected by the OCT-A as a vessel.

The use of deep learning in medical imaging has revolutionised tasks that require the detection of specific features from complex images. In glaucoma, deep learning technology has been applied in diagnosing POAG and detecting

glaucomatous progression using OCT images, fundus photography, or VFs (21). However, it has not been specifically implemented to detect DHs, although publications aiming to identify referral features of the optic nerve from fundus photography have been able to detect DHs (22).

2.1.7 Differential diagnosis of DHs

Some haemorrhages in the optic nerve have been considered non-glaucomatous. For instance, haemorrhages associated with other eye conditions such as posterior vitreous detachment (PVD), optic disc oedema, retinal vein occlusions (RVO), among others, are usually not considered as glaucomatous DHs (Table 2). In addition, other clinical characteristics make clinicians consider a haemorrhage in the optic nerve to be a typical glaucomatous DH: flame shape instead of blot shape, feathered borders instead of sharply defined borders, and localised in the inferotemporal or superotemporal sector instead of the superonasal or inferonasal sector (23).

It is important to emphasise that in a large proportion of patients, the typical shape of a glaucomatous DH is flame and not blot. Population-based studies have shown that DHs in glaucoma patients tend to be more flame shaped compared to DHs in healthy people that tend to be more blot shaped. For example, in the blue mountains eye study, 13.3% of the DHs in the glaucoma population had a blot shape compared to 36.11% in the non-glaucoma population (24).

Table 2 Differential diagnosis of DHs.

1. Anterior ischaemic optic neuropathy
2. Diabetic retinopathy
3. HIV microangiopathy
4. Intrapapillary hemorrhage with adjacent peripapillary subretinal haemorrhage (IHAPSH) (25)
5. Isolated peripapillary subretinal disc haemorrhages (26)

6. Leukaemia
7. Multilayered optic disc haemorrhages (27)
8. Optic nerve drusen
9. Other causes of optic disc oedema
10. Other retinal vascular diseases
11. Posterior vitreous detachment
12. Purtscher and Purtscher-like retinopathy
13. Retinal vein occlusions
14. Systemic hypertension
15. Systemic lupus erythematosus
16. Valsalva retinopathy

2.1.8 Prevalence of DHs in the general population

The prevalence of DHs in the general population has been widely studied in different countries, different age groups, and different levels of urbanisation (see Table 3). From these population-based studies, it can be concluded that DHs occur in healthy individuals from all regions of the world.

Table 3 Prevalence of DHs in the general population.

Author	Prevalence	Region	Year	Type	Comments
Jonas (28)	0.4% (n = 4,570)	Rural India	2013	Population-based	+30 years
Wang (29)	1.2% (n = 4,378)	Beijing	2006	Population-based	+40 years
Yamamoto (30)	0.6% (n = 13,965)	Tajimi Japan	2004	Population-based	Tajimi Project

Jonasson (31)	1.14% (n = 1,045)	Iceland	2003	Population-based	Reykjavik study
Grodum (32)	0.87% (n = 32,918)	Malmo	2001	Population-based	EMGT
Healey (24)	1.4% (n = 3,654)	West Sydney	1998	Population-based	+49 years Blue mountains eye study
Jonas (33)	0% (n = 1,505)	Germany	1994	Hospital-based	
Klein (34)	0.93% (n = 4,926)	Wisconsin	1992	Population-based	Beaver dam eye study
Diehl (35)	0 % (n = 661)	Baltimore	1990	Case series Hospital-based	Citizen and church groups
Kitazawa (36)	0.4% (n = 473)	Japan	1986	Hospital-based	
Bengtsson (37)	0.8% (n = 1,511)	Dalby Sweden	1981	Population-based	55-71 years

2.1.9 Prevalence of DHs in glaucoma patients

The prevalence of DHs in patients with glaucoma has been extensively studied (see Table 4). However, many of these publications have categorised the DH status of patients based on a single clinical or image-based examination. Prospective studies have shown that repeated examinations increase the proportion of DH+ patients compared to a single examination (36, 38, 39). For instance, in Kitazawa's study, the cumulative incidence of DHs was 43.1% compared to prevalence in each observation that varied from 5% to 13% (36).

Among patients with glaucoma, DHs have been more frequently associated with normal tension glaucoma (NTG) in hospital-based and population-based studies. The latter type of studies needs to be interpreted with caution because the definition of glaucoma as NTG in population-based studies is sometimes based on only one or two IOP measurements. Nevertheless, there is a consistent trend

toward observing more patients with DHs in NTG compared to high tension glaucoma (HTG) (see Table 4).

Table 4 Prevalence of DH in glaucoma patients.

Author	Prevalence	Region	Year	Type	Comments
Sakata (40)	31% cumulative probability at 5 years	Japan	2020	Longitudinal cohort	Healthy untreated NTG
Jonas (28)	5.7% (n = 193)	Rural India	2013	Population-based	+30 years
Wang (29)	8.8% (n = 226)	Beijing	2006	Population-based	+40 years
Yamamoto (30)	8.2% (n = 793)	Tajimi Japan	2004	Population-based	Tajimi Project +40 years
Gazzard (41)	2.99% All glaucomas (n = 167)	Singapore	2003	Hospital-based	The Singapore 5FU Study
Sonnsjo (42)	93% POAG (n = 37/40)	Sweden	2002	Hospital-based	Patients From Dalby population-based survey
Healey (24)	13.8% (15/108) 8% HTG (6/72) 25% NTG (9/36)	West Sydney	1998	Population-based. 51% un-Dx glaucoma	+49 years Blue mountains eye study
Tezel (43)	OHT 7.1%, n = 14 NTG 29.4%, n = 20 POAG 15.7%, n = 31	USA	1996	Hospital-based	
Jonas (33)	4.1 (n = 1,505)	Germany	1994	Hospital-based	
Klein (34)	1.9% (n = 104)	Wisconsin	1992	Population-based	Beaver dam eye study

Diehl (35)	2.44% (n = 123)	Baltimore	1990	Hospital-based	Citizen and church groups
Kitazawa (36)	20.5% NTG (n = 78) 4.2% POAG (n = 192)	Japan	1986	Hospital-based	
Airaksinen (44)	5.8%	Finland	1981	Hospital-based	
Gloster (45)	4.9% (n = 325)	London	1981	Hospital-based	UCL Moorfields

In PACG, there is even less information about the prevalence or cumulative incidence of DHs. Lan et al. reported a group of 770 PACG patients (from a glaucoma clinic) who were followed for nine years. The authors identified a cumulative incidence of DH+ status in 5.7% of the patients, 68% of patients had unilateral DHs, 32% bilateral (at the same or different visits), and 40% of patients had recurrent haemorrhages (46). Another study investigated retrospectively 160 Korean patients with PACG (47) with 'high' and 'normal' IOP at baseline. The patients were followed for a mean (range) of 36.2 (12.1 - 45.7) months in the 'normal' IOP subgroup and 29.7 (13.3 - 44.4) months in the 'high' IOP subgroup. During this follow-up period, 29.9% of patients within the 'normal' IOP subgroup and 14.3% within the 'high' IOP subgroup had a DH identified. The difference in the cumulative incidence of PACG DH+ patients was statistically significant when the subgroups of 'high' and 'normal' baseline IOP were compared (p=0.029). There is no evidence of the prevalence of DHs in most of the secondary types of glaucomas. However, in exfoliation glaucoma (XFG), a lower prevalence has been most frequently reported in different populations. An observational study of 1,548 patients with glaucoma in Finland identified patients with XFG to have the lowest risk of developing DHs (48). A Korean retrospective case-controlled study identified DHs in 17% of POAG patients compared to only 4% of XFG (49). A retrospective European study that investigated for five years a group of 71 patients with XFG only identified 5 (7%) with DHs (50).

2.1.10 Pathogenesis of DHs

There has been an intense debate about what causes DHs. On the one hand, some researchers favour the idea that DHs are a secondary epiphenomenon caused by the mechanical stress induced by changes in the optic nerve architecture. On the other hand, other researchers favour the idea that DHs are a vascular event that can trigger the start or the aggravation of glaucomatous damage to the optic nerve. Although multiple mechanisms are possibly involved in the pathogenesis of DHs, the two most commonly cited theories to explain their origin are the vascular (51) and the mechanical theories (52).

Despite decades of research, newer publications still favour one or the other theory without confirming a unique theory to explain the pathogenesis of DHs. In favour of a mechanical origin of DHs, the use of new imaging modalities such as the enhanced depth imaging (EDI) SD-OCT has shown an association between recent structural changes in the lamina cribrosa and patients with DHs. It has been suggested that the microvascular damage at the optic nerve head is a result of the deformation in the lamina cribrosa near its insertion (53). In favour of a vascular origin of DHs, new research on metalloproteinases-9 (MMP-9) and endothelin-1 (ET-1) have found that levels of these molecules are higher in patients with DHs. Some authors have proposed a dysfunction in the blood-retina barrier induced by MMP-9 and ET-1 as the cause of DHs (54).

Hypoxia affects the delivery of oxygen to tissues and could be involved in the pathogenesis of DHs; it is present in patients with diseases such as anaemia, chronic obstructive respiratory disease (COPD), asthma, or obstructive sleep apnoea syndrome (OSAS). Some of these diseases have been considered as risk factors for DHs. For instance, the OSAS has been associated with a higher risk of POAG, particularly with NTG (55); however, the exact mechanism of this association is still not fully understood. New imaging modalities, such as OCT-A, have identified vascular structural anomalies in patients with OSAS (56). A possible explanation for the association of OSAS and NTG is the association between OSAS and increased levels of endothelin-1, which has been associated with NTG (57), vascular dysregulation syndrome (58) and DHs. In vitro and in vivo experiments of acute and chronic hypoxia have shown a rapid activation of

endothelial cells to release inflammatory mediators (59), induce changes in cell-to-cell interaction, and enhance the release of endothelin-1; these changes are very similar to what has been identified in patients with OSAS or primary vascular dysregulation. It could be possible that in some types of DHs, hypoxic damage to endothelial cells triggers the release of high levels of ET-1 and MMP-9, which are the culminating factors that are required for vessels to bleed in and around the optic nerve. In diabetic retinopathy, which is a very different neurovascular retinal disease DHs are sometimes present; these are almost impossible to distinguish from glaucomatous DHs. However, in diabetic retinopathy, the role of the hypoxic damage to the endothelial cells has been extensively described in clinical and epidemiologic research and shown to be an independent risk factor for progression to more severe stages of the disease (60).

Irrespective of the mechanism that disrupts the blood vessels' wall, there is some evidence to support the idea that the blood released out of the vessels is from an arterial origin. A report of the colourimetric differences of the blood in DHs, microaneurysms, retinal vein occlusion, and the neighbouring arteries and veins identified more similarities between DHs and microaneurysms and arteries compared to veins and retinal vein occlusions (61). The colourimetric analysis was performed using ImageJ (NIH, Bethesda, Maryland, USA) with the histogram function that measures the intensity of each pixel inside a region of interest. The same authors further confirmed that arterial blood is the origin of DHs by applying the same colourimetric method to fundus photographs from some participants of the ocular hypertension treatment study (OHTS) (62).

2.1.11 Genetic factors and DHs

Numerous publications have identified an association between different genetic mutations or single-nucleotide polymorphism with glaucoma or intraocular pressure (63). However, there have been no publications searching for an association between those genetic differences and the glaucomatous phenotype with DHs. However, there are risk factors for DHs that have been identified in different clinical trials, such as female sex, which are likely to have a genetic influence (38). A possible association between female hormones variation and glaucoma has been reported in previous studies (64, 65). Oestrogens have an

important role in ocular physiology; they have been identified as important mediators of collagen and nitric oxide production, which are involved in vascular regulation and trabecular meshwork outflow facility (65). Oestrogen deficiency, pathologically or due to menopause, could affect the structural and vascular homeostasis of the eye and be involved in glaucomatous damage by either a mechanical or vascular mechanism (66). More recently, single nucleotide polymorphisms (SNP) in the oestrogen receptor ESR1 were identified in NTG patients with DHs (67).

A well-described mutation in the TBK1 gene has been repeatedly associated with NTG. However, despite the strong association between NTG and DHs, the largest pedigree of this mutation that has been extensively investigated never had DHs detected during the follow up of all the members of this family (68). It might be possible that this mutation has no association with DHs or that the African American ancestry of this pedigree reduced their risk of developing DHs. Patients of African American ancestry have been reported to have a lower risk of DHs (69). Another publication of the same mutations but with a single case report of progressive cupping identified DHs (70).

It is likely that an important part of the risk to develop DHs is explained by a complex interaction of different genetic characteristics. The occurrence of DHs varies significantly between glaucoma patients. A large group of patients will not develop DHs at all, some patients have few and mostly unilateral DHs, while a small group have very frequent and more bilateral DHs. A complex interaction of different genetic markers would explain the difference in how DHs occur and vary among glaucoma patients.

2.1.12 The natural history of DHs

Different research groups have analysed, in addition to the prevalence of DHs in one cross-sectional examination, the cumulative incidence of DHs in longitudinal studies. Kitazawa et al. (36), in his seminal publication about the natural history of DHs, followed a group of 58 patients with NTG every four weeks during a mean follow-up of 15.7 months and identified a cumulative incidence of 43.1% DH+ patients (25 DH+ patients with 28 DH+ eyes). Among the 28 DH+ eyes, 18 (64%)

presented a recurrence and 72% of the recurrences took place in the same quadrant (n = 13/18). Over the complete study period, 36 recurrences (some had a recurrence multiple times) occurred, and among these, 92% of recurrences (n = 33/36) occurred during the first 28 weeks after a previous DH. In Kitazawa's study, only three patients had bilateral DHs (12% of DH+ eyes). In relation to the duration of DHs, Kitazawa et al. identified 42 new DH episodes (excluding baseline DHs during the first visit of the longitudinal study) and followed these patients weekly until the DH disappeared. With this methodology, the authors identified a mean duration of 10.6 weeks, with 92% of DH episodes lasting more than four weeks. Kitazawa et al. concluded that there are two types of NTG patient: those who develop DHs and have frequent recurrences and another group with a very low risk of having a DH.

Hendrickx et al. (71) also published the results of a longitudinal study that followed patients for a mean of 7.3 years and identified a cumulative incidence of DHs in NTG (35.3% n 34) as well as in HTG (10.3% n 68), and glaucoma suspects (10.4% n 125). More recently, Bengtsson et al. (38) identified a 55% cumulative incidence of DH+ participants from longitudinal data with a median of eight years of follow-up from the early manifest glaucoma trial (EMGT).

Recurrent DHs are reported to appear in between 12% and 73% of the cases (36, 71, 72). The exact number seems to be very variable between patients and it is also affected significantly depending on the number of observations, the technology used to detect DHs and the length of the follow-up. The clinical implications of this recurrent phenomenon are subject to debate, with some reports not considering a clinical impact of recurrent DHs (73) while other reports consider recurrent DHs as an increased risk of further glaucoma progression. Park et al. coined the term' migrating hemorrhages' to refer to DHs that recur toward the healthy edge of RNFL and consider this subtype of recurring DHs as a factor associated with a higher risk of progression (74).

Sonnsjö (48) and Heijl (49) published in 1986 a series of weekly photographs of the optic nerves of patients that were followed for more than a year and developed multiple recurrences of DHs; they identified frequent recurrence before the previous bleeding would be absorbed and variable duration of each DH event.

They observed a faster absorption of the blood in the papillary tissue compared to the peripapillary area. They concluded that patients with DHs are individuals who have multiple vascular events but considered that each new DH event usually leaves no 'measurable functional or structural trace'.

From these publications, it seems clear that patients who are categorised as DH+ are part of a heterogeneous group; some might bleed regularly, while others will only occasionally develop DHs. It is also clear that DHs are a transient and dynamic phenomenon that is clinically evident only for a few weeks, disappears without leaving an easily identifiable mark and are easily missed by clinicians, as shown in the OHTS (75) and EMGT (38). An area of uncertainty is the possibility that patients with glaucoma represent two different subgroups—one with DHs and another that will never develop DHs. The concept of a 'bleeder' and 'non-bleeder' patient has been previously described (36, 71). From the evidence that has been published, it seems more likely that DHs are not a universal characteristic of glaucomatous neuropathy but instead a feature of the 'bleeder' subgroup of patients with glaucoma.

2.1.13 Location of DHs in the optic nerve

Besides the role that DHs have in patients with glaucoma, the DHs that appear in otherwise 'healthy individuals' are even more complex to understand. As reported in Table 3, DHs might appear in individuals from all ethnic and age groups. Interestingly, most of the DHs appear in the inferotemporal sector, followed by the superotemporal sector; these are the same sectors in which glaucomatous neuropathy typically produces the earliest and greatest deformation of the optic nerve. Therefore, it is possible to consider that DHs in 'healthy individuals' are precursors of future glaucoma, as suggested by Krakau (42, 76). In contrast to the concept that DHs are precursors of glaucoma, the thirteen-year follow-up report of the OHTS did not identify progression to glaucoma in 78% of the eyes that previously had a DH (69).

2.1.14 Animal models of glaucoma and DHs

Animal models of glaucoma are created either by genetic manipulation or by modification of the IOP. Despite the common detection of DHs in humans with glaucoma, the animal models of glaucoma very rarely develop DHs. However, when non-IOP factors are modified during animal experimentation, DHs can occur. The surgical reduction of cerebrospinal fluid (CSF) pressure in four monkeys produced in one of the monkeys a typical DH (77, 78). In another study, electrical stimulation to the optic chiasm produced a DH in two rabbits that later developed optic nerve cupping (79). However, human patients with spontaneous intracranial hypotension have been reported to have a deeper lamina cribosa measured with OCT but without DHs (80).

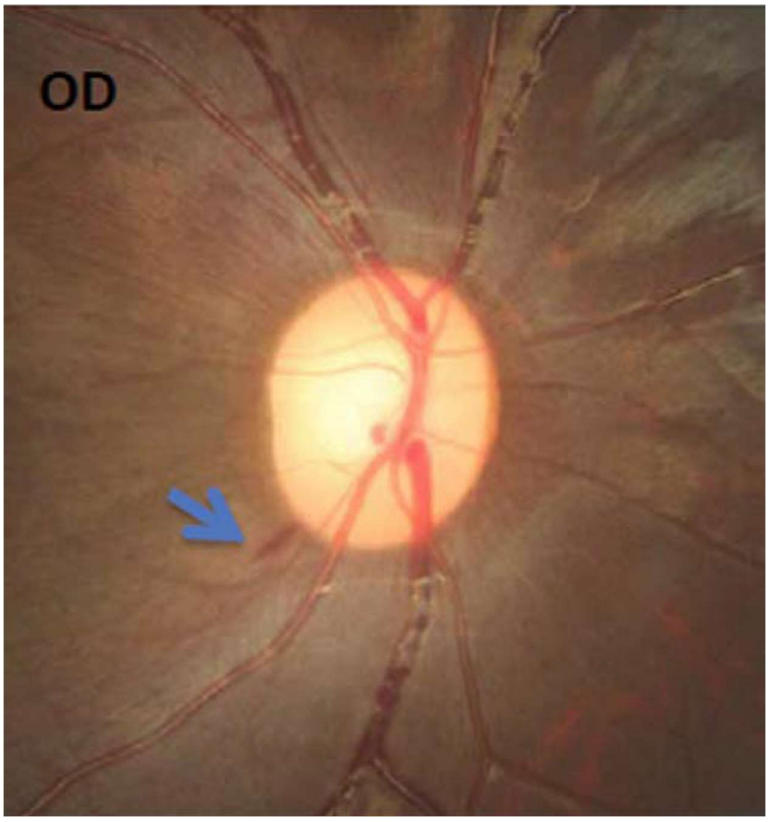


Figure 4 Photo of one of the monkeys that presented a DH after CSF pressure was surgically reduced (78). Image reproduced with permission of the rights holder, Association for Research in Vision & Ophthalmology (ARVO).

2.1.15 Animal models of cortical microhaemorrhages

Animal models in other diseases that involve neural tissue and blood have been developed. Of special interest are the animal models of stroke that have evolved dramatically since incorporating two-photon microscopy and femtosecond laser ablation technology; this technology can produce and detect the effect of microinfarcts and microhaemorrhages in vivo in the cerebral cortex (81). The cortical microhaemorrhages model (82) is of particular interest to glaucoma specialists because the microbleedings produced with this technique resemble many of the characteristics of DH:

1. The size of the haemorrhage is around 100 microns (SD = 31 microns) thirty minutes after the ablation, with a final size of an average of 300 microns
2. The haemorrhage occurs in a densely packed tissue like the retinal nerve fibre layer (RNFL)
3. The vessel that produces the haemorrhage does not develop a clot with subsequent obstruction of its lumen. Similarly, after a glaucomatous DH, there are no signs of downstream ischaemia
4. The vessels surrounding the microhaemorrhages (125 microns around the microhaemorrhage) have a minor reduction on the average flow of 20%, but this does not progress to ischaemia. Similarly, after a glaucomatous DH, there are no signs of ischaemia in the neighbouring RNFL
5. No dendrite damage is present neither immediately during the active microhaemorrhage nor two weeks later. It is very similar to DHs in glaucoma, in which no acute or early damage is evident on structure or function
6. Astrocytes become activated as far as 200 microns from the microhaemorrhage, and they remain activated until the end of the experiment (one week after the laser ablation). In glaucoma, astrocyte activation has been described as an important step in glaucoma pathogenesis (83)(84).

There are some interesting points in this model that could help our understanding of glaucomatous DHs:

1. Blood plasma penetrated the neural tissue five times more than red blood cells. At the moment, there is no technology that can assess plasma leaked around a DH in glaucoma patients
2. Red blood cells do not penetrate into the brain parenchyma; instead, they compress the surrounding dendrites. The pressure induced by the haematoma on the neighbouring dendrites is up to 1.5kPa (11 mmHg). In glaucoma, DHs take a flame shape due to compression of the RNFL axons, which are densely packed and radially organised; this pressure over a densely packed tissue could be equivalent to the compartmental syndromes that occur in other areas of the body.
3. The cortical microhaemorrhages leave a dendrite-free region of 100 microns (SD = 36) that returns to normal after the haematoma clears on average seven days after the haemorrhage. Contrary to this fast clearance, glaucomatous DHs tend to last on average 10.6 weeks (36)
4. The microglia/macrophages become activated and direct their processes toward the site of the microhaemorrhage. The microglia as far as 200 microns from the microhaemorrhage become activated, and this response lasts until the end of the experiment (two weeks). In glaucoma, there is evidence to suggest that the microglia play an important role in its pathogenesis (85).

The group that designed this model concluded that cortical microhaemorrhages do not produce active damage to neurons but could be the trigger that starts a chronic increase in which the microglia/macrophages and astrocyte activation could lead to neural dysfunction and death (86).

Another animal model of subacute cerebral microhaemorrhages was described in 2018 by Zeng et al. (87). Inflammation was induced in rats with intraperitoneal lipopolysaccharide that produced astrocyte activation that led to microhaemorrhages in the brain. The nitric oxide synthase was also activated, and the production of nitric oxide increased, inducing cerebral microhaemorrhages. In these rats, it was also documented that there was a

decrease in the levels of tight junction ZO-1 proteins, which could potentially affect integrity of the brain-blood barrier.

To conclude, brain microhaemorrhage animal models are good examples of what happens with neurons after exposure to blood. Some of these findings could be cautiously considered to play a role in glaucomatous DHs. The direct activation of glial cells by brain microhaemorrhage could support the idea that DHs are not only an epiphenomenon secondary to the mechanical deformation of the optic nerve but a critical step in the glial activation involved in glaucoma pathogenesis.

2.1.16 How could blood damage the axons? Lessons from research in intracerebral haemorrhages

Cellular death can occur after an event of intracerebral haemorrhage due to several mechanisms (88). Some of these mechanisms may also occur in glaucoma when blood from a DH is in contact with the axons of the ganglion cells:

1. Direct damage by compression of the surrounding tissue
2. Erythrocyte induced oedema (89)
3. Plasminogen and thrombin cytotoxicity (90)
4. Oxidative neuronal damage due to erythrocyte lysis (91)
5. Reduction in blood flow
6. Responsive activation of the microglia/macrophages
7. Astrocyte activation
8. Glutamate excitotoxicity due to the increasing levels of glutamate after bleeding

2.1.17 Similarities between the vascular component of glaucoma and the vascular component of dementia

The complex interaction between mechanical and vascular factors is not unique to glaucoma. In neuroscience, there has been a constant search to find how multiple factors end up causing disease. Perfect examples of the interaction of vascular and mechanical factors are cognitive impairment (92), Alzheimer's

disease (AD) and cerebral amyloid angiopathy disease, which have been associated with the presence of cortical microhaemorrhages (93). The association between vascular factors and dementia reached a tipping point in December 2013 when the Alzheimer's Association, with scientific input from the National Institute of Neurological Disorders and Stroke and the National Heart, Lung and Blood Institute from the National Institutes of Health, convened scientific experts to discuss the research gaps in our understanding of how vascular factors contribute to AD and related dementia (94). They described the state of the art research on macro and microinfarcts and concluded that cerebrovascular disease, particularly the small vessel disease, is often associated with cognitive decline. They also agreed that to move the field forward, there was a need of focus in two areas: '1) the need to identify and understand the molecular and cellular mechanisms and targets that underlie the contribution of vascular disease to AD and dementia, and 2) the need to facilitate development and validation of noninvasive biomarkers of key vascular processes related to cognitive and neurologic impairment' (94).

It is very interesting to notice the similarities between the discussions in glaucoma and neurosciences about the multiple factors involved in the pathogenesis of glaucoma and cognitive impairment. For example, the following points were highlighted as important for future research by the Alzheimer's Association and could be easily confused as areas of research in glaucoma (94):

1. Tools are needed to answer the gaps identified during this meeting:
    a. Lipid metabolism and its role in amyloid deposition and cognitive/behavioural change.
    b. Various roles of different cell types of the innate and adaptive immune systems.
    c. Vascular injury and the response to injury.
    d. Mechanisms underlying brain blood flow decrease in AD and other dementias.
    e. The role of small vessel disease and blood-brain barrier breakdown.
    f. Effects of reduced blood flow and changes in blood pressure.

g. Role of and interactions with other risk factors such as diabetes, including a study of the prediabetic brain without the confounding effects of treatment.
h. Genetic cross-talk between the vasculature and the brain.
i. Studies of mixed aetiology AD dementia.
j. Effects of interventions to control vascular risk factors on cognition.

2. Novel biomarkers are also needed both for the investigation of basic scientific research questions and to be developed as potential clinical disease markers. These markers need to be validated at an early stage in humans to ensure applicability for human studies:
    a. Better markers of blood flow, particularly for cerebral small vessels and collateral circulation.
    b. A CAA-specific or other imaging compound that recognises beta-amyloid or other markers, specifically and selectively in the cerebrovasculature.
    c. Markers that enable a more precise assessment of where pathology occurs in the brain parenchyma and blood vessels and the quantitative distribution of pathology.
    d. Biomarkers that detect breakdown or dysfunction of blood-brain barrier permeability.
    e. Biomarkers that reflect damage to brain structure and connectivity caused by microinfarcts, which are largely undetectable to current neuroimaging.
    f. Vascular biomarkers of AD/dementia risk in prediabetic and insulin-resistant adults.
    g. Improved imaging markers of cerebral vascular dysfunction.
    h. Markers of peripheral circulatory system components that contribute to neuroinflammation.
    i. Improved outcome measures and clinical diagnostic criteria that accurately reflect the range of vascular events that impact cognition and determine the effects of vascular risk factor control on cognition.

To summarise, research in both areas requires major efforts to improve the understanding of these multifactorial diseases. The clinically identifiable

phenotype (dementia or glaucoma) is induced by different mechanisms that could progress differently. There is shared enthusiasm in finding more about the role of vascular pathology as a risk factor for developing the disease or it progressing faster. Both areas require advances in imaging modalities and non-invasive biomarkers to improve the phenotypification of patients. The significant overlap between neuroscience and ophthalmology is not new, and in the near future, we will likely see more research that is applicable in both fields of medicine.

2.1.18 Associations between ocular variables and DHs

1. Disc morphology

A larger vertical cup disc ratio was associated with DHs in 'healthy individuals' but not in glaucoma patients in the blue mountains eye study (24). However, the Beijing eye study did not find an association between DHs and disc area (29).

Jonas et al. evaluated 61 photographs of DH+ patients with glaucoma at different stages of severity and found that DHs do not occur in areas without detectable neuroretinal rim. The frequency of detectable DHs increased from the early to moderate stages of the disease and then decreased from moderate to severe stages (33).

Optic disc pits were strongly associated with DHs with an odds ratio (OR) = 42 (95% CI, 9-183) in the blue mountains eye study (95).

The appearance of the optic nerve in healthy individuals or patients with glaucoma is extremely variable; however, some features of glaucomatous optic neuropathy have been used to categorize the neuropathy in different subgroups. Four subgroups of glaucomatous optic disc appearances have been suggested to investigate possible associations with different pathogenic mechanisms: focal or type 1, myopic or type 2, senile sclerotic (atrophic) or type 3, and generalized enlargement or type 4 (96). Based on the four subgroups of appearance, Broadway et al. analyzed a large cohort of patients with pure characteristics of each subgroup and reported ocular and systemic factors associated with each appearance. DHs were identified more frequently in the focal glaucomatous discs

(32%), followed by the senile sclerotic (27%) and myopic (21%); in patients with the generalized cup enlargement appearance, DHs were very rarely identified (9%) (96).

2. Exfoliation syndrome

In the blue mountains eye study, 3 of the 82 (3.7%) patients with exfoliation syndrome presented a DH with an increased risk of having DHs compared to participants without exfoliation syndrome (OR = 3.5; CI, 1.1-11.8) (24). However, other publications that included patients with exfoliation glaucoma instead of only exfoliation syndrome have identified a lower risk of having DH in patients with exfoliation glaucoma (see the last paragraph of 2.1.9).

3. Central corneal thickness

Jonas et al. followed a cohort of 223 white patients with POAG for an average of 61.3 months and found at least one visit with a DH in 63 eyes (16.2%). In this cohort, there was no association between CCT and DHs (97).

In the Beijing eye study, after Bonferroni correction for multiple analyses, there was no statistically significant difference in CCT between the DH+ and DH- individuals. However, the glaucoma population with DHs had slightly thicker corneas but with no statistically significant difference. The authors concluded that CCT is not a risk factor for DHs (98).

4. Central retinal venous pressure (CRVP)

With a contact lens ophthalmodynamometer, 22 NTG patients with bilateral disease but with unilateral DHs and 29 NTG patients with bilateral disease but with no DHs were analysed. They found that eyes with DHs had lower CRVP compared to eyes with no DHs, but it was similar to the fellow eye with no DH (99).

5. Peripapillary atrophy (PPA)

A group of 7 HTG and 37 NTG patients with unilateral DHs had HRT imaging of the optic nerve in both eyes and subsequent measurement of the PPA in the reflectance images. PPA presence and a greater area of PPA were associated with DH+ eyes (100).

Yamada et al. (101) analysed the PPA of 129 POAG patients using OCT and divided patients based on the presence or absence of Bruch's membrane (BM) in the PPA. The patients were divided into BM+, BM-, and mixed BM-and+ groups. The BM+ group and the presence of DHs were associated with faster VF progression in highly myopic patients. In addition, DHs were more common in the BM+ compared to BM- group. Nine (30%) patients in the BM+ group, 17 (29.3%) patients in the mixed BM- and + group, and 1 (7.1%) patient in the BM- group developed a DH.

A retrospective study of 46 patients with unilateral DHs found an association between DHs and the eye with the greater PPA. The authors also identified that the location of DHs was within two clock hours from the greatest PPA width in 73.8% of the patient (102).

6. Axial length and refraction

In the blue mountains eye study, there was no association between myopia and DHs (24).

In the Beijing eye study, there was no association between DHs and the refractive error (29).

The Japanese lower normal pressure glaucoma study (LNPGS) evaluated over five years' healthy', untreated NTG patients and identified spherical equivalent toward more hyperopia as a risk factor for DHs with a hazard ratio (HR) of 1.18 (95% CI 1.04–1.32; p=0.018) (40).

2.1.19 Systemic associations with DHs

1. Gender

In the blue mountains eye study, the prevalence of DHs was higher in women compared to men with an OR of 1.9 (95% CI 1.0-3.5) (24).

In the Tajimi study, women had significantly more DHs compared to men (30).

In the EMGT, males had a lower risk to develop DHs during the study in comparison to women (OR = 0.48, 95% CI 0.26–0.90; p= 0.022) (38).

In the collaborative normal tension glaucoma study (CNTGS), the effect of local and systemic factors that predict the benefit of lowering IOP was investigated, and there was no apparent association between baseline DHs and gender; however, only 23 participants had a DH at baseline (103).

2. Nailfold capillaroscopy

Three different research groups have investigated the nailbed of patients with glaucoma in different populations (Korea (104), EUA (105) and Canada (106)) and all confirmed the association between nailfold capillaroscopy changes and glaucoma. Avascular areas, nailfold haemorrhages and dilated vessels were the main changes that have consistently been associated with glaucoma.

The association between DHs and nailfold capillaroscopy changes is still under debate. From the three previous research groups, the group from EUA did not report this association (105), the Korean group reported a statistically significant association (104) and the Canadian group reported no association (106).

Nailbed abnormalities have also been associated with altered systemic MMP-9 levels but not with DH (107). It has been previously hypothesised that MMP-9 could be involved in DHs pathogenesis affecting the basal membrane of the blood-retina barrier (54).

3. Primary vascular dysregulation syndrome

This syndrome was described by Josef Flammer (58) and is defined as a systemic condition in which the regulation of blood flow is not adapted to the needs of the tissue. The syndrome has been associated with multiple medical conditions (migraine, Raynaud's phenomenon, insomnia, altitude sickness, hypothyroidism), and it is frequently associated with glaucoma and DHs.

4. Migraine

In the blue mountains eye study, 11 of the 608 subjects with a history of typical migraine headache had a DH (1.8% prevalence). In all the population the history of migraine increased the risk of DHs with an OR of 1.7 (95% CI 0.9-3.3) and in the population without glaucoma, migraine increased the risk of DHs even more with an OR of 2.2 (95% CI 1.1-4.6) (24).

In the collaborative normal tension glaucoma study (CNTGS), migraine was associated with faster time to VF progression, but there was no apparent association between baseline DHs and migraine; however, only 23 participants had a DH at baseline (103).

5. Raynaud's phenomenon

The association between Raynaud’s phenomenon and glaucoma is still under debate (108). There is no evidence to support that this common phenomenon (4.9% prevalence in the general population (109)) by itself is associated with an increased risk of DHs.

6. Endothelin-1 (ET 1) and matrix metalloproteinase 9 (MMP-9) levels

The systemic levels of ET 1 and MMP-9 have been suggested as possible factors associated with the formation of DHs (54, 110). However, the systemic levels of MMP-9 were not significantly higher in DH+ patients compared to DH- (107).

7. Glucose intolerance or diabetes

In the blue mountains eye study, diabetes was significantly associated with DHs. Six (2.3%) of 256 subjects with diabetes had a DH. Among the entire population studied, diabetes increased the risk of having a DH with an OR of 2.9 (95% CI1.4-6.3). Among the subjects with diabetes and glaucoma, the risk was higher, with a prevalence of DHs of 14.3% (n = 2/14) (24).

Glucose intolerance or diabetes was found in a retrospective study of patients with glaucoma in 50% of 62 DH+ patients compared to only 15% of 58 DH- patients (111).

A Canadian cohort of 137 patients with POAG identified a DH in 50 eyes of 38 patients. The risk of having a DH was higher in diabetic patients with a HR of 4.4 (95% CI 1.8-10.5; p=0.001).

8. Low systolic blood pressure

In the low tension glaucoma treatment study (LoGTS), low mean systolic blood pressure was associated with DHs with a HR of 1.06 (112).

In the EMGT, there was a trend toward more DHs in patients with higher baseline systolic blood pressure (OR = 1.2 per higher mmHg; CI, 1.0–1.4; p=0.098) (38).

9. Systemic hypertension (HTN)

In the blue mountains eye study, there was a statistically significant association between HTN and DHs with an OR of 1.1 (95% CI 1.0-1.3) (24).

In the Canadian cohort of 137 patients with POAG, 38 developed DHs, but there was no association between HTN and DHs (113).

10. History of vascular events (angina, heart attack or stroke)

In the blue mountains eye study, there was no association between vascular events and DHs (24).

11. Use of beta-blockers

In the LoGTS, the use of systemic beta-blockers was associated with DHs with a HR of 5.6 (112).

12. Nitric oxide

Polymorphisms of the endothelial nitric oxide synthase gene have been associated as a potential risk factor for NTG with DHs (114). However, a recent publication in a Polish population found no correlation between these polymorphisms and NTG or DHs (115).

13. Platelet function and anticoagulants

A prospective, cross-sectional study of 315 subjects with NTG with DHs, NTG without DHs and' healthy individuals' identified an association between platelet function and DHs (116). Platelet function was measured as the collagen/epinephrine closure time using a platelet function analyzer.

Animal models of cortical microhaemorrhages have shown that mice treated with warfarin had DHs, which were 1.7 times larger in diameter compared to non-anticoagulated controls (117, 118). The same animal model but under heparin had a 56% increase in the size of the haematoma.

In the blue mountains eye study, there was no statistically significant association between aspirin use and DHs (24).

In the Canadian cohort of 137 patients with POAG, 38 developed DHs, and there was a higher risk of DHs in patients treated with aspirin with a HR of 2.3 (95% CI 1.2-4.6; p=0.019) (113).

14. CSF pressure

A case report of a 27-year-old patient who had a pinealoblastoma that required eight CSF shunt revisions over 25 years opens the possibility that low CSF pressure increases the risk of NTG and DHs (119). The authors of this report

considered this case as a "Pure" human model of low CSF pressure and NTG because this patient developed an early presentation of NTG with multiple and recurrent DHs.

The relationship between CSF pressure and glaucoma has been extensively discussed (120); however, the relationship with intraocular haemorrhages is more limited. In the Beijing eye study, higher estimated CSF pressure was associated with the progression of diabetic retinopathy after adjustment of other related parameters (121). It is possible that an increased CSF pressure affects the venous pressure with a subsequent change in the capillary pressure that favours more haemorrhages and progression of diabetic retinopathy. Contrary to the findings of the Beijing eye study, a non-human primate model of induced lower CSF pressure induced in some animals the appearance of a DH with very similar characteristics to the glaucomatous DHs seen in humans (122).

2.1.20 Association between DHs and a higher risk of developing glaucoma

In a Swedish population-based study of 1511 individuals (the Dalby population survey), 'healthy participants' had DHs but without VFs defects (76). Over the years, almost 90% of these individuals developed POAG without any other risk factors for glaucoma.

The OHTS identified after a long follow-up (median of 13 years) that DHs increased the risk of conversion from OHT to POAG with a HR of 2.6 (95% CI 1.7-4.0; p=< .0001) (69).

A Korean study of patients with unilateral NTG identified conversion to glaucoma in the non-glaucomatous eye in 21 of 79 patients (26.6%). The mean number of visits with DHs in the non-glaucomatous eye between patients with and without conversion to OAG was similar, but the eyes with DHs were more likely to convert. Nine of 12 eyes that converted to glaucoma had a DH compared to only one of 54 eyes that did not convert to glaucoma (123).

2.1.21 Association with glaucomatous VF progression

During the care of patients with glaucoma, clinicians have to assess in each visit if a patient is stable or progressing. Many variables have been associated with an increased or reduced risk of progression; however, an evidence-based review (108) on prognostic factors for VF progression identified that from the 103 different prognostic factors, only five were clearly associated with glaucomatous VF progression:

1. Age
2. DH (in NTG patients)
3. Baseline VF loss
4. Baseline IOP
5. Exfoliation syndrome

Table 5 summarises some details of publications that analysed the association between DHs and VF progression. Some authors have attempted to construct formulas that include DHs to predict future VF progression. For instance, Nitta et al. constructed a formula with a multivariable regression using as independent variables DHs, the angle of the RNFL defect, vertical cup-disc-ratio, and the mean IOP percentage of reduction (124); this formula reported an area under the receiver operating characteristics curve of 0.75 to detect the patients that would progress.

Table 5 Association between DHs and VF progression.

Author	Impact on progression	Methodology	Comments
Seol (125)	HR = 1.9 (1.2-3.1; p=0.01)	Recurrent DHs (>3)	Not compared with not recurrent DH
Lee (126)	HR = 15.5 in NTG patients	Comparative of NTG and HTG	
Park (127)	Rate of progression faster in non-myopic and even faster if DH+. DHs did not affect progression in myopic eyes	POAG 101 myopic >24 mm and 78 non-myopic eyes	>5 VF

Shim (128)	HR = 2.6 (p=0.019) 92 myopic NTG only one hemifield affected	Retrospective	FU 55.8 months
Kim (129)	HR = 1.7 (p= 0.031)	127 treated pre-perimetric glaucoma	FU 5 years
Cho (130)	5/6 (83%) healthy fellow eyes from NTG patients converted to glaucoma	Cohort of 50 unilateral NTG	FU 8.7 years
Gracitelli (131)	Rates of estimated RGC loss faster in DH+. 22,233/year vs 10,704/year in DH-	Prospective observational 122 patients	FU 3.7 years
Leung (132)	RR = 3.3 (95% CI 1.2-8.8; p= 0.019)	Cohort of 256 NTG	FU for 36 months Tx simvastatin
Leung (133)	HR = 2.2 (95% CI 1.5-3.4; p=<0.001)	Cohort 286 NTG	FU for 36 months
Bengtsson (38)	HR = 1.02 per percent higher in the mean percentage of DH+ visits	RCT 129 treated 126 not treated	FU 8 years EMGT
Leske (134)	HR = 1.02 per percent higher in the mean percentage of DH+ visits	RCT 129 treated 126 not treated	FU 8 years EMGT
Budenz (75)	HR = 3.7	Cohort from the OHTS	FU 96 months
Kono (135)	No difference in progression between DH+ and DH- except for the central 10 degrees of the VF	58 NTG with no treatment	FU 60 months
Drance (136)	RR = 2.7	Prospective clinical trial	CNTGS
Ishida (137)	HR = 20.3 (95% CI 5.2-79.9; p= 0.001)	Retrospective 70 NTG with no treatment	FU 5.6 years
Rasker (138)	NTG: DH+ 80% vs DH- 32% HTG: DH+ 89% vs DH- 32% OHT DH+ 14% vs DH- 6% Unilateral NTG: DH+ 58% vs DH-11%	Retrospective Evaluated the % of patients with VF progression	FU 9 years

To conclude, DHs are clearly and strongly associated with progression in NTG. In OHT, POAG, and pre-perimetric glaucoma DHs are also associated with progression, but there is conflicting evidence.

When VF progression is categorised as rapid, intermediate or slow, DHs have also been associated with rapid progression. The advanced imaging for glaucoma study group followed 103 participants (150 eyes) and identified 23, 47, and 80 with rapid (> -0.5dB/year), intermediate (<0.5 but > -0.25dB/year), and slow (<-0.25dB/year) progression respectively. DHs were found in 39.1% of rapid progressors compared to only 7.5% of slow progression. The presence of a DH during follow-up increased the risk of rapid progression with an OR of 2.61 ($p = 0.004$) (139).

2.1.22 DHs and the location of visual field defects

Except for one publication, there has been a consistent association between DHs and paracentral VF defects. Kim et al. reported the initial VF defect in a group of 162 subjects. Superior paracentral defects were more frequently associated with DHs (18/40 45%) compared to inferior paracentral defects (12/35 34%) or superior defects (10/37 27%) or inferior nasal steps (7/50 14%) (140). In the blue mountains study, patients with DHs and glaucoma had more paracentral VF defects (95). Rao et al. observed a group of 40 NTG patients with unilateral paracentral defects and found that DHs were more common in the eye with paracentral defects (141). In contrast to the previous publications, Sakata et al. published a retrospective analysis of 92 NTG patients followed up for five years in whom there was no association between the type of VF defect and DHs. Sakata et al. divided the VF into six subfields and could not identify DHs as a risk factor for VF changes in any specific subfield (142). More recently, a longitudinal study of African American descendants (African descent and glaucoma evaluation study (ADAGES)) identified 21 eyes with DHs in whom central locations had more defects on 24-2 and 10-2 VFs compared to the 314 non-DHs eyes (143).

2.1.23 Association between DHs and RNFL loss

The association between DHs and RNFL defects needs to be analysed carefully. The complexity of this relationship is in part due to the difference in the duration of these signs; DHs are temporal and dynamic, while RNFL defects are permanent. For example, consider a hypothetical patient that is seen only on three occasions, before a RNFL defect, with a RNFL defect and finally with a RNFL defect and a DH. A naïve observer could think that the RNFL defect was the cause of the DH without considering that many DHs may have occurred prior to the first observed RNFL defect.

With the introduction of OCT, the assessment of the relationship between DHs and RFNL has been expanded beyond the RNFL defects detected in fundus photography to include earlier states with just minimal thinning in the RNFL thickness. Jeoung et al. (144) measured the RNFL thickness with OCT in subjects with 1) no glaucoma, 2) only DHs and no RNFL defects on fundus photography, 3) DHs, RNFL defects on fundus photography but normal VF and 4) DHs, RNFL defects on fundus photography and VF defect and identified RNFL reduction in thickness even when the red-free fundus photography showed a normal RNFL but DHs were present.

The proximity between the location of DHs and RNFL defects opens the possibility that both signs are related. On the one hand, it may be possible that RNFL defects are a consequence of DHs. On the other hand, DHs may be a consequence of the stretching of the vessels at the border of the RNFL defect, as suggested in the fibrous glial scar formation hypothesis of the pathogenesis of DHs (145). Airaksinen et al. (146) observed a group of 25 OHT individuals for six years and identified eight patients who developed a DH with a subsequent RNFL defect in the same location.

Irrespective of which is the first event that triggers the occurrence of DHs and RNFL defects, it seems clear that the coexistence of these two signs is a risk factor for faster RNFL loss. A retrospective review of 41 patients in whom the IOP was maintained medically under 15mmHg for a minimum of five years identified 20 patients with fast RNFL thinning (higher than −1.00 μm/year). The strongest

predictor of having fast RNFL progression was the presence of at least one DH with an OR of 37.5 (95% CI 2.9–483.1; p=0.005) (147).

In a retrospective cohort of 166 NTG patients (treated only medically), the risk factors for progression based on thinning of RNFL or GCL were DHs, diabetes, and lower minimum systolic blood pressure; with the strongest predictor being DHs with a HR for DH+ status of 2.1 (148).

To conclude, there is evidence to support the idea that RNFL defects and DHs are topographically related. It seems that patients with DHs tend to have faster RNFL loss. However, further research is needed to try to understand the order in which these signs appear.

2.1.24 Association between DHs and loss of neuroretinal rim

The deformation of the optic disc neuroretinal rim can be assessed clinically or photographically. However, newer imaging modalities, such as the SLO and OCT, have improved the ability to quantify the deformation of the optic disc. With the Spectralis OCT, radial OCT scans centred in the optic nerve are used to measure the thickness of the neuroretinal rim from the opening of the Bruch's membrane (BMO) to the closest internal limiting membrane. The minimum rim width measured from the BMO (BMO-MRW) has been extensively described for glaucoma (149). More recently, it has been compared to the RNFL thickness to detect progression after a new DH. Cho et al. (150) followed 82 patients after a new DH was detected and monitored the progression of the RNFL thickness and BMO-MRW. The authors identified that BMO-MRW progressed faster than the RNFL thickness.

2.1.25 Association between DHs and lamina cribosa deformation

Recent reports have identified an association between DHs and lamina cribosa deformation: outward deformation of anterior lamina cribosa (ALC) or focal lamina cribosa defects (FLCD) (see Table 6). Kim et al. (151), using SS-OCT, identified FLCD in 68.4% of patients with DHs. The topographical localization of FLCD and

DHs does not always coincide, and in the Kim study, FLCD were detected in the same sector as DHs in only 55.9% of the patients.

The prelaminar tissue of the optic nerve head is affected during the glaucomatous deformation of the optic nerve. In a small and retrospective study of 11 POAG patients with prelaminar wedge-shaped defects (PLWD) and 29 POAG patients without PLWD, the history of DHs was more frequent in patients with a PLWD (45.5% with DHs vs 3.4% without DHs) (152). In 80% of these cases, the PLWD was in the same hemidisc (superior or inferior) as the previous DH.

Table 6 Association between DHs and lamina cribrosa deformation.

Author	Type	Patients	Patients with LC deformation	Associations	Same location
Park (153)	Cross-sectional	148 glaucoma	45% FLCD	DH OR = 3.63 NTG OR = 4.23	88.2% hemifield
Takayama (154)	Cross-sectional	111 glaucoma 29 normals	6.6% glaucoma 0% normals	DH Longer axial length	81.8%
Lee (53)	Prospective	45 DH+ 36 DH-	88.9% DH+ 11.1% DH-	DH Longer axial length	100% 1 clock hour
Kim (155)	Cross-sectional	72 DH+ 63 DH-	80.6% DH+ 39.7% DH-	DH RNFL defects	62.1%
Kim (151)	Cross-sectional	98 DH+	68.4% FLCD	Larger area if same location	1 clock hour 55.9%
Sharpe (156)	Prospective	92 glaucoma 46 DH+ 46 DH -	96% FLCD in DH+ 58% FLCD in DH-	61% of DHs occurred in areas of no disinsertion	
Chiou (152)	Retrospective	40 glaucoma 23 control	17.5% LC defects 27.5% (PLWD)	DHs in 45% PLWD and 3% without PLWD	80% of DHs in the same hemidisc as PLWD

2.1.26 Association between DHs and retinal blood vessel positional shifts

Using sequences of optic nerve photographs automatically aligned and flickered, Radcliffe et al. (157) identified a shift in the position of temporal retinal blood vessels in patients with glaucoma. The shift of the vessels was associated with VF progression neuroretinal rim loss and the presence of DHs.

2.1.27 Association between DHs and peripapillary atrophy

Peripapillary atrophy (PPA) is a group of changes around the optic nerve that are common among healthy subjects and very common in myopia and glaucoma. PPA has been traditionally classified as alfa and beta, with the former characterized by an irregular RPE and the latter by the absence of RPE and more associated with glaucoma. More recently, two new zones of PPA (gamma and delta) were described in eyes with globe elongation (158), as seen in Figure 5. Gamma zone is defined as the area without Bruch's membrane and delta zone as part of the gamma zone that corresponds histologically to the elongated and thinned peripapillary scleral flange, which usually does not have vessels larger than 50 micrometres.

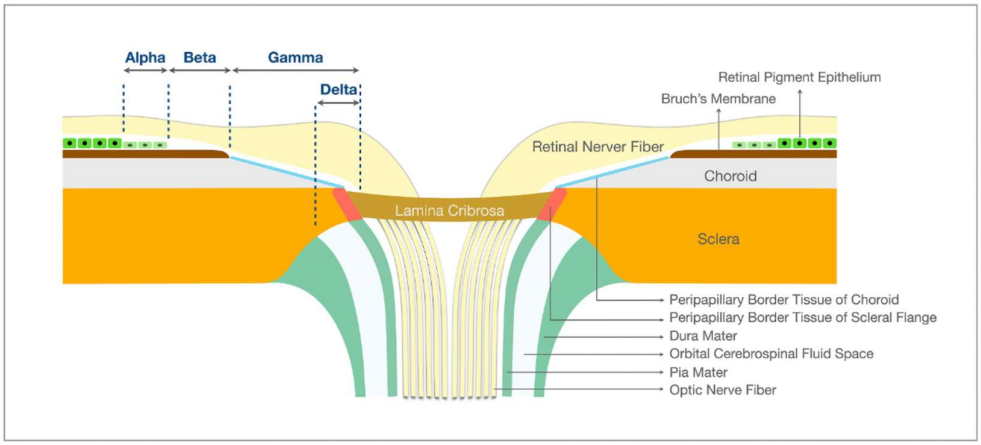


Figure 5 A schematic figure illustrating parapapillary alpha zone (Bruch's membrane present, retinal pigment epithelium irregular), beta zone (Bruch's membrane present, retinal pigment epithelium absent), gamma zone (Bruch's membrane absent), and delta zone (part of gamma zone, corresponding to an elongated and thinned peripapillary scleral flange). Gamma zone sometimes includes few large choroidal vessels. The peripapillary border tissue of the choroid connects the end of Bruch's membrane with the peripapillary border tissue of the scleral flange, and it is covered only by the retinal nerve fibres. The peripapillary border tissue of the scleral flange is the continuation of the optic nerve pia mater (159). Open access article under the CC BY-NC-ND.

Eyes with larger areas of PPA have been associated with the presence of DHs (160). Furthermore, the location of the maximum radial extent of the PPA has also been associated with the topographical location of DHs (102). However, new metrics of the PPA seem to suggest that PPA and DHs increase the risk of VF progression independently. The irregularity of the margins of the PPA has been recently associated as an independent risk factor for VF progression even when adjusted in the same model for the presence of DHs (161).

There is a basis for the interest to associate DHs and beta PPA because the possible mechanisms involved in the presence and expansion of PPA are similar to the mechanisms possibly involved in the pathogenesis of DHs. In beta PPA, there is a loss of RPE, and in the area closer to the optic disc, the choriocapillaris is closed, and photoreceptors are missing. The closed choriocapillaris in large areas of the beta zone and its association with DHs have been used as the basis to suggest possible similar vascular mechanisms involved in the pathogenesis of PPA and DHs. However, it has been suggested that RPE cells are the first lost in

PPA pathogenesis, followed by photoreceptors and the choriocapillaris, which could be considered in contrast to a possible vascular pathogenesis of PPA (159). In contrast to a vascular mechanism involved in PPA pathogenesis, Wang et al. identified folds in the RPE around the optic nerve after IOP elevation induced by dark room prone provocative test (162); these findings support the possibility that frequent mechanical deformation around the optic nerve is the mechanism involved in the damage to the RPE with subsequent loss of photoreceptors and closure of the choriocapillaris. Irrespective of the mechanism involved in PPA pathogenesis, there is a topographic correlation between DHs and PPA and between these factors and the region with the greatest loss of NRR and RNFL loss.

2.1.28 Association between DHs and OCT-A radial peripapillary capillary density

The ability to non-invasively image the vasculature of the optic nerve with OCT-A has attracted great interest from researchers interested in the role of vascular factors in glaucoma. The radial peripapillary capillary (RPC) network can be easily imaged in vivo with OCT-A for the first time. Nitta et al. (163) compared OCT-A images of 77 NTG patients who had scans in 2015 and later in 2018. A reduction in the RPC density at sectors with DHs was significantly greater than that of sectors without DHs (−4.42% in DH+ sectors vs −2.48 in DH- sectors; p=0.0469). In addition, the authors identified that the RNFL thickness of sectors with DHs declined faster than that of sectors without DHs.

2.1.29 Association between DHs and parapapillary deep-layer microvasculature dropouts

With OCT-A, it is possible to identify regions of the parapapillary capillary networks that disappear (aka dropout). An OCT-A angiography retrospective study evaluated 138 eyes of 138 patients with POAG who underwent regular OCT-A scans and VF tests for a mean follow-up of 5.5 years (164). The patients were divided into VF progressors (n = 55) and not (n = 83) based on Guided Progression Analysis (GPA) “likely progression” events. DHs were identified more

frequently in patients with VF progression, in 17 (30.9%) progressors and in 10 (12.0%) non-progressors (p=0.006). A univariable analysis evaluating risk factors for VF progression identified the ever-presence of a DH with an OR of 2.8 (95% CI 1.3–6.4;p=0.011) and the presence of parapapillary deep-layer microvasculature dropout with an OR of 15.2 (95% CI,5.5–42.0; p=<0.001). Interestingly, these two variables changed importantly their OR when both were included in a single multivariable model that included mean IOP, baseline VF PSD, presence of focal lamina cribosa defects, and beta PPA wider than 50microns. The OR of DHs increased from 2.8 to 6.7, while the OR of microvasculature dropout diminished from 15.2 to 5.7. It could be possible that some of the effect that microvasculature dropout seems to have with VF progression is caused by the effect of DHs on VF progression.

Another study in a Korean population of 68 POAG patients identified DHs in 11 (50%) of the patients with enlarged microvascular dropouts in parapapillary choroidal microvasculature and only in 8 (17.4%) of patients with stable microvasculature (no dropouts) (p=0.01) (165). The same group later published the spatial correlation between microvascular dropouts of the parapapillary choroidal microvasculature and RNFL thinning at the same location of subsequent DHs (166).

Contrary to the previous publications, a recent Korean study of pre-perimetric glaucoma patients did not identify an association between DHs and microvascular deep-layer dropout (167).

2.1.30 Association between DHs and vitreous changes

There are no publications reporting the association of glaucomatous DHs with vitreous changes. However, DHs can occur after the vitreous detaches from the optic nerve, and the close anatomical relationship between DHs and the vitreous make this interface an interesting area for further research.

For instance, the strong attachment of the vitreous with the RNFL could potentially be involved in how the retina responds to glaucomatous damage. Microcyst changes in the INL have been described in young African American patients in

areas of ganglion cell loss (168). The authors proposed that in young patients with moderately advanced glaucoma, the attached hyaloid face does not allow the retina with transsynaptic loss of cells in the internal nuclear layer to collapse.

A new technique for evaluating the elasticity of tissues is real-time elastography, which can objectively analyse the stiffness of tissues. Recently, this technique has been used in POAG, and no changes were found between cases and healthy controls at the optic disc, optic nerve, retrobulbar fat, anterior and posterior vitreous, and retina-choroid-sclera complex (169). However, the anterior vitreous/posterior vitreous strain ratios were significantly higher in glaucoma compared to normal patients; these changes in glaucoma patients could potentially affect the strain in the interface between the vitreous and the RNFL.

It may be possible that changes induced in the vitreous that make its separation with the optic nerve more aggressive could be partially involved in the pathogenesis of some glaucomatous DHs.

2.1.31 Association between DHs and reticular pseudodrusen

In a UK-based population study of elderly patients (Bridlington Eye Assessment Project “BEAP”), 3548 participants were assessed for glaucoma and macular changes. Reticular pseudodrusen were present in 5.3% and 6.1% of participants without and with DH (170). Reticular pseudodrusen are more frequently identified in females, and older individuals similar to glaucoma but its possible association with DHs remains unknown.

2.1.32 Association between DHs and corneal hysteresis

Corneal hysteresis (CH) is the name given by the company Reichert (Reichert, Buffalo, NY) to the difference between two readings of IOP obtained with the Ocular Response Analyzer; it has been extensively described as a variable that describes the cornea's viscous-elastic properties that absorbs the force of the air jet. A recent publication reported a lower level of CH in patients with DHs as compared with patients with no DHs (8.7 for DH+ vs 9.2 for DH-; p=0.002) (171).

2.1.33 Differences in progression depending on the location of DHs

The location of DHs could affect the risk of subsequent progression. Hsia et al. investigated the change in sectorial RNFL thickness in 43 patients with DHs in the temporal sector of the disc and 33 patients with DHs in the nasal sectors (172). The rate of RNFL change was significantly greater for DHs located in the temporal sectors (- 6.0 for temporal sectors vs 0.7 for nasal sectors). In addition, eyes with DHs in the temporal sectors had faster MD deterioration (dB/year) than those in the nasal sectors (- 0.32 for temporal sectors vs -0.05 for nasal sectors) (172).

2.1.34 Fellow eye of patients with unilateral DHs

Some interesting findings have emerged from cross-sectional studies that looked at the fellow eye of patients with unilateral DHs. Eyes with DHs seem to have a more advanced disease than the fellow eye, but the evidence supporting these findings is limited (173).

Chin et al. (174) identified that the DH+ eyes, in comparison to the DH- eyes, have (on planimetry) a larger cup-disc ratio (more significantly in the vertical aspect) and a thinner neuroretinal rim.

2.1.35 Effect of treatment on VF progression and the subsequent frequency of disc haemorrhages

From a clinical point of view, it is very important to know if patients with DHs respond to conventional treatment in the same way as patients with no DH. In other areas of medicine, predictive factors of poor response to treatment are available and help clinicians when choosing the most appropriate treatment for each patient.

In glaucoma, there are more than ten publications that discuss the effect of different treatments in patients with DHs (see Table 7). Some of these

publications are focussed on the effect of treatment in reducing the frequency of subsequent DHs, while others are focussed on the effect on VF progression.

From the data presented in Table 7 it seems that DHs (at least in NTG) are a predictive factor of a reduced response to treatment. It seems that the frequency of DHs is not affected by most of the therapies, except for trabeculectomy. However, these publications need to be interpreted in the context of the very heterogeneous type of research, outcome variables, type of medical or surgical IOP-lowering intervention, and methodology to identify DHs and define VF progression.

The LoGTS randomized patient with NTG to receive brimonidine or timolol and investigated the differential effect of these interventions on VF progression (175). There was no significant difference in the number of DHs between groups, but in a multivariable analysis, the risk of developing DHs in the brimonidine group had a hazard ratio of 0.3 (95% CI 0.08-1.12; p=0.072) compared to the timolol group. In the same analysis, patients on systemic beta-blockers had a HR of 5.6 (95% CI 1.5-22.7; p=0.036) (112). There is a possibility that some medications, such as brimonidine, can positively modify vascular regulation at the level of the optic nerve. On the contrary, the topical and systemic beta-blockers or the underlying disease for which patients were treated with systemic beta-blockers could negatively modify vascular regulation. The effect of future glaucoma medications on vascular regulation is important and newer glaucoma drugs are investigated for a possible positive effect on the vascular regulation of the optic nerve. An OCT-A study comparing patients under brimonidine or netarsudil (Rho-associated protein kinase, ROCK, inhibitor) identified an increase in vessel density in the netarsudil group (176).

Indirect evidence of the vascular regulation that some drugs might have in the eye comes from evaluating changes in the temperature of the cornea as a surrogate of an increased blood flow. Using thermography, brimonidine reduced by 0.5 C the temperature of the central cornea (177). Although it is impossible to know which vessels increased their flow to modify the temperature of the avascular cornea, the ciliary body or iris vessels were likely the vessels that increased their flow. If the ciliary body changed its flow under brimonidine, this

effect might also be present at the optic nerve. The thermography effect of brimonidine in the cornea and the tendency in the LoGTS study toward fewer DHs in the brimonidine group makes it possible to suggest that some antiglaucoma drugs have a differential effect on reducing or increasing the risk of new DHs. To investigate the hypothesis that brimonidine reduces the number of DHs, a Japanese group retrospectively assessed a group of patients in whom the frequency of DHs was measured before and after the administration of brimonidine; the authors identified a reduction from 0.67 DHs/year to 0.31 DHs/year (p=0.01) (178). It is possible that the reduction in the frequency of DHs was due to the mean reduction in IOP from 12.5 to 11.2 mmHg. However, in the EMGT study with a more pronounced reduction in IOP (25%), the frequency of DHs was not different between study groups (38).

Table 7 Effect of treatment on progression and frequency of DHs.

Author	Impact of lowering IOP on progression and frequency of DHs	Methodology	Comments
Furlanetto (112)	'Treatment randomization was not associated with occurrence of recurrence of DH'	Clinical trial 127 subjects	Brimonidine vs timolol
De Moraes (179)	Re-analysis of OHTS 'Eyes on treatment were less likely to have a DH during FU'. but there was no effect on recurrence of DHs	Group from a OHTS with >5 years FU and > 10 reliable VFs	2420 DH- 187 DH+
Medeiros (180)	Rate of VFI changed faster in DH+. Difference in rate of VFI post-DH was related to IOP post DH (r=-0.61). Per mmHg, 0.31%/year lower rate of VFI	Observational Cohort of 348 patients for 8.2 years	97/510 (19%) eyes had a DH
Prata (181)	76 DH+ patients had IOP reduced and VF progression continued at -1.1dB/year	Retrospective cohort	
Bengtsson (38)	The frequency of DHs over time did not differ between treated (8.4%) and control eyes (8.5%).	RCT FU median 8 year	129 treatment 126 controls

Budenz (75)	DH occurred slightly more often in the observation group. No significant (P=0.13)	RCT 1618 patients 96.3 months FU.	86.7% of DH+ eyes with no glaucoma
Miyake (182)	Probability of finding a DH pre and post trabeculectomy was reduced from 33.4 to 5.5 % in HTG and 42.1 to 23.1% in NTG	Retrospective	Reduction of 3 and 8 mm Hg in NTG and HTG.
Anderson (103)	'The most readily demonstrated treatment benefit occurred in patients with no DH'	Clinical trial	Only baseline DHs analysed
Hendrickx (71)	Treatment may reduce the incidence of initial and recurrent DHs in HTG but not in NTG.	68 HTG, 38 NTG, 125 suspect. FU for 7 years.	
Bengtsson (37)	No reduction of frequency of DHs after IOP medication was started	Prospective observation	55 DH+ patients

2.1.36 Clinical implications of DHs in healthy subjects

If an isolated DH is detected during a routine clinical examination, the risk of identifying glaucoma is variable between patients. The categorization of patients with isolated DHs as glaucoma suspects may expose many healthy patients to over diagnosis and unnecessary clinical examinations. For example, in the blue mountains eye study, only one in every four patients with a DH had glaucoma (24), which is a positive predictive value (PPV) of 25%. However, if we observe a DH in a person from a younger and healthier population, a DH could have a PPV of 65% like in the Central India Eye and Medical Study (28). Depending on the population, the PPV may vary from 10% - 20% in the Beijing eye study (29) to 65% in the Central India Eye and Medical Study. Similarly to the wide range of PPV in different populations, the odds ratio for the association between DHs and glaucoma also varies significantly between populations, with an OR of 9.3 in the Beijing eye study and an OR of 87 in the Central India Eye and Medical Study.

2.1.37 Economical impact of DHs

A cost-effectiveness analysis on the treatment of NTG patients, based on the risk ratio of the CNTG, was published, and the ICER (Incremental cost-effectiveness ratios) were US$ 34,225; however, when DHs were present, the ICER was US$24,350 (183). The strong association between DHs and VF deterioration in the CNTGS made treatment in NTG patients with DHs more cost-effective.

The timely identification of DHs has the potential to help in the following scenarios to improve health policies and budget management:

1. Glaucoma suspect/OHT:
    a. Stratify risk of conversion
    b. Lower the threshold to start treatment
2. New glaucoma patient:
    a. Stratify risk of progression
    b. Stratify risk of poor response to medical treatment
    c. Lower the threshold for surgery

2.1.38 Areas of uncertainty and controversy

Among the many areas of future work that are frequently suggested in many of the publications about DHs, the following seem to be the most frequent:

1. Basic research:
    a. Why DHs appear on the optic nerve and not in other areas where the RNFL is lost?
    b. The pathogenesis of DHs. Mechanical, vascular or another hypothesis
    c. Are DHs part of a systemic syndrome such as the so-called vascular dysregulation syndrome, or do DHs represent isolated vascular events in the eye?
2. Clinical research:
    a. How to assess the risk of glaucoma in healthy patients with isolated DHs

b. What is the best treatment for glaucoma patients with DHs
c. Should clinicians change the management plan based only on the detection of a new DH?

2.1.39 Summary of clinical implications of DHs

From a clinical point of view, there is evidence to conceptualize DHs as:

1. A risk factor for developing glaucoma in otherwise healthy individuals
2. A risk factor of conversion from OHT to glaucoma
3. A prognostic factor for VF progression in patients with glaucoma
4. A predictive factor of poor response to medical treatment in NTG.

2.1.40 DHs in international guidelines

Different clinical guidelines have been developed to aid clinicians in managing patients with glaucoma. The majority of glaucoma guidelines describe DHs to a certain extent, either in the definition of the signs of glaucoma or in the clinical assessment of the optic nerve head for diagnosis or progression. Table 8 summarises some of the recommendations about DHs.

Table 8 DHs in international guidelines.

Guidelines	Sign of unstable or active disease	Risk factor for progression	Recommendation to increase treatment if DH+
NICE (2017)	Not specified	Not specified	Not specified
EGS (5^{th} Edition)	Yes	Yes	Not specified
PPP (2020) POAG suspect	Yes	Yes	Yes
PPP (2020) POAG	Yes	Yes	Yes

Asia-Pacific 2Nd Edition	Yes	Yes	Yes
NHMRC 2010 Australia	Yes	Yes	Yes
ICO Guidelines for Glaucoma Eye Care 2015	Yes	No	Yes
(2011) WGA 8th consensus meeting: Progression	Not specified	Yes	Not specified
(2010) WGA 7th consensus meeting: Medical treatment	Not specified	Not specified	To be considered
(2005) WGA 2nd consensus meeting: Glaucoma surgery	Not specified	Not specified	To be considered

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Section 3 Investigations

Chapter 1 Impact of disc haemorrhages on clinical practice.

3.1.1 Abstract

Purpose: To investigate the impact of DHs on clinical practice.

Methods: The national survey on the impact of disc haemorrhages in clinical practice was designed based on a focus group of glaucoma specialists and the responses of a pilot version of the survey. The final survey presented four clinical scenarios in a web-based survey manager to all self-declared glaucoma specialists members of the United Kingdom and Éire Glaucoma Society (UKEGS).

Results: 76 (63.3%) glaucoma specialists consented to participate and confirmed to be trained as ophthalmologists. When a new DH was identified, 81.6% of clinicians responded that they would modify to a certain degree the management of patients. In the four clinical scenarios, most of the participants changed the management of patients irrespective of the stability of other clinical parameters if a new DH was detected. There was wide practice variation in how participants would change clinical management; the most frequently selected option never passed 50% of participants. There was no association between age, deanery of training, the proportion of glaucoma patients in clinical practice and the responses.

Conclusions: Most glaucoma specialist members of the UKEGS change the management of patients if a new DH is detected, but there is wide practice variation. There is limited information about DHs in guidelines to aid clinicians in changing clinical management.

3.1.2 Introduction

Since the description of DHs, a great debate has arisen concerning why DHs appear, what DHs mean in relation to the stability of glaucoma and the best management of patients after a clinician identifies a DH. The debate about DHs is exemplified in a case in controversy publication in the Journal of Glaucoma in 2000 (1); this report presented a woman with NTG and no evidence of RNFL progression but with a new DH. Two invited ophthalmologists described a diverse range of potential diagnostic and therapeutic options to manage the patient. Almost two decades later, clinicians still have a wide range of options and little evidence to support their decisions.

Clinical guidelines are a good source of guidance for clinicians, but there is limited information (see page 66, DHs in international guidelines) on how to better diagnose, manage, and follow up patients with DHs. For instance, the 2017 United Kingdom NICE guidelines (2) only mention DHs to describe the difference in their detection between optometrists and ophthalmologists and never in areas related to treatment or follow-up. Outside the guidance of clinical guidelines, clinicians are left with evidence from individual publications or systematic reviews which report conflicting evidence about the role of reducing IOP in patients with DHs. Some publications have reported no effect of medical intervention in patients with DHs (3-6), while others reported significant benefits (7). The inconsistency of these clinical findings and the limited understanding of the general role of DHs in glaucomatous damage and its natural history (8) make it likely that there is a wide variation in management in the current care of patients with DHs.

Surveys about clinical practice are a common method used to describe the differences in how clinicians manage patients under conditions with high levels of uncertainty or no evidence. It is also common that after a gap in the evidence of any type of clinical activity is identified, a consensus of experts is established to guide management more homogeneously. It is still debated if national or regional guidelines should include only evidence from the highest standards (meta-analysis or randomised clinical trials) or also include evidence from other types of research methodologies or even the consensus of a group of experts. In the

United Kingdom, multiple clinical surveys have been reported (Table 9 Previous UK national surveys in ophthalmology) about different fields of ophthalmology. Some surveys have helped increase the awareness of a change in practice that was required, such as the 2004 survey about the use of antimetabolites in glaucoma surgery (9). In this survey, the authors identified a much lower use of antimetabolites in the United Kingdom in comparison to the United States of America and Japan. Over the years after the publication of this survey results and thanks to ophthalmologists who actively promoted the use of antimetabolites, the number of surgeons using them increased significantly, and a change in management to a more standardised way occurred.

Table 9 Previous UK national surveys in ophthalmology

Subject	Author	Date Journal	To whom	Pilot Incentives	How	Length	Response Rate
Pachymeter use and disinfection (10)	Jasan	2020 JoG	Training ophthalmic units in UK	No	Telephone	4 items	70%
Trabeculectomy bleb needling (11)	Mercieca	2018 BJO	UKEGS (60/72) Glaucoma consultants	No	Online SM	10 items	83% glaucoma consultants
Glaucoma surgery (12)	Rodriguez-Una	2017 Clin & Exp Ophth	UKEGS (64/116)	No	Online SM	8 items	55% glaucoma consultants
Glaucoma drainage device usage in UK (13)	Feyi-Waboso, A	2016 J J Ophthalmol	UKEGS (200)	No	Online SM	No details but 15 clinical scenarios	42%
Outcomes measures for glaucoma effectiveness trials (14)	Ramsay, C	2016 Journal of Glaucoma	UKEGS senior (122) EGS (198)	Yes	Delphi online	Two phases: 1 (25 items) 2 (26 items)	25.4% UKEGS 18% EGS
Importance of day 1 Post Phaco in glaucoma (15)	Gupta, A	2015 Eye	UKEGS website email database	NR NR	Online	7 questions	21%

Pre-op management of ocular surface (16)	Tailor, R	2014 Semin Ophthalmol	146 RCOph self-declared glaucoma specialist	Yes No	Post	1 page yes no sometimes	43.8%
Equipment by UK optometrists (17)	Dabasia, P	2014 Ophthalmic Physiol Opt	Random 1300 UK College of Optometrists members	Yes 100	Online SM	21 questions	35%
VF intervals (18)	Malik, R	2013 BMJ Open	UKEGS meeting 2011 and post 150	NR NR	Hand Post	5 questions and 3 clinical scenarios	46.6%
Attitudes of management in advance glaucoma (19)	Stead, R	2011 Clin & Exp Ophth	All consultants practising in the UK	NR NR	Post	1 page 7 questions	68.8%
Diagnostic tests for glaucoma (20)	Myint, J	2011 Ophthalmic Physiol Opt	All electronic database of association of optometrist	Yes No	Online SM	27 items	28%
UK diode (21)	Agrawal, P	2011 Eye	All consultants members of RCOphth	NR NR	Online Post	31 questions	53.6%
Glaucoma management in hospitals (22)	Gordon-Bennett, P	2008 Eye	All RCOphth members	NR NR	Post	10 questions	49%
Glaucoma and pregnancy (23)	Vaideanu, D	2007 Eye	All UK consultants from Medical Directory	NR NR	Post	4 questions	47%
Prophylactic YAG iridotomy (24)	Sheth, H	2005 Eye	All UK ophthalmologist from RCOphth	NR NR	Post	8 questions	84%
Antimetabolite in glaucoma surgery (9)	Siriwardena, D	2004 BJO	All UK ophthalmologist from RCOphth	Yes NR	Post	12 questions	82%

NR=Not reported. SM= survey monkey.

3.1.3 Methodology

The National Survey on the impact of disc haemorrhages in clinical practice was designed to understand the impact of new DHs on everyday clinical practice. The primary hypothesis was that the clinical management of patients with glaucoma is modified after a new DH is detected despite the stability of other clinical parameters. It was also hypothesised that the modification in clinical management would be homogeneous among glaucoma specialists. It was assumed that glaucoma specialists would have the greatest clinical experience and the most standardised approach to managing patients with glaucoma and DHs among all eye care providers.

We conducted a focus group with six glaucoma specialists from different countries to discuss common and relevant clinical scenarios that could have a high level of variability in clinical management. The discussion of the focus group was transcribed and analysed using content analysis. During the discussion, the clinical scenarios were condensed to report the severity of glaucoma and how stable were parameters such as VF, IOP, and RNFL. Subsequently, codes were assigned based on the severity of glaucoma, and finally, related codes were grouped in categories that were reported in the survey as clinical scenarios. Four clinical scenarios were selected and included in the pilot version of the survey:

1. Healthy patient with new DH discovered as an incidental finding
2. Stable OHT patient with low risk and no treatment, with new DH
3. Stable POAG patient with early/moderate disease under one drop, with new DH
4. Stable POAG patient with early/moderate disease under three drops, with new DH

Following the selection of the clinical scenarios, three pilot surveys (see 5.1.1) were constructed differing mainly on the amount of clinical data presented and the type of responses required. The first pilot survey presented a full clinical case (with the results of multiple investigations that are common in glaucoma clinics such as OCT, CCT, IOP, VF) and the domains of responses then ranged from a modification in the frequency of follow-up visits to treatment escalation. The second pilot survey presented only the clinical scenario, and the domains of

responses were similar to the first pilot survey. The third pilot survey presented statements about the most relevant clinical dilemmas that clinicians could face in each clinical scenario, and the responses were five levels of agreement with proposed actions on a Likert-type scale.

The content of the pilot and final versions of the survey were uploaded to an online survey manager (Survey Monkey®, San Mateo, CA, USA) and distributed to a representative sample of UK eye health care professionals and started in June 2016. Three optometrists, three glaucoma fellows, three recently appointed consultants, and three senior consultants were invited to participate via email. During the pilot survey, in addition to the clinical questions, the participants were asked to respond to three additional general questions about the survey:

1. How well do you think these questions represent the diversity of scenarios that clinicians might face in relation to DH?
2. How relevant do you think it is to survey the impact that DHs have on clinical practice?
3. Among the three methods to question the clinical scenarios, which one would you prefer?

The focus group and the results of the pilot survey showed high variability in the responses despite having only participating clinicians with a special interest in glaucoma. Due to the interest in using the survey results as a future consensus framework, it was decided to distribute the final survey only to glaucoma specialists registered in the UKEGS. It was decided that the best method to distribute the survey was through the society's mailing list of self-declared glaucoma specialist members willing to receive surveys that have been previously used for glaucoma surveys (13). The final survey with consent form as it was seen by participants on the Survey Monkey platform (5.1.3), email invitation, and email reminders were approved by the UCL research ethics committee with the registration number 8335/001 (5.1.4). The following questions were included in the final version of questions to which participants in the survey responded.

Case 1

You receive a new referral from an optician who discovered a new DH in a healthy 50 year old patient with no past ocular history and no family history of glaucoma or blindness. IOP 13 mmHg on both eyes. Disc haemorrhage in his right eye and otherwise normal eye examination including a healthy optic disc with no RNFL defect or rim thinning. No previous history of vitreous detachment.

What would you do?

1. Discharge
2. Ask for imaging scans of the optic nerve (OCT or other)
3. Follow-up yearly for 3-5 years and then discharge
4. Start treatment to reduce the IOP

Case 2

50 year old ocular hypertensive patient that has been under observation yearly for 5 years. Maximum IOP 23 BE with CCT 560 BE. Healthy optic disc VCDR 0.3, no RNFL defects. Normal visual fields and normal OCT. On the last visit you observed a new DH not associated with PVD on the left eye, what would you do next?

1. Discharge
2. Continue monitoring every 12-24 months
3. Monitor every 4-6 months
4. Start treatment to reduce the IOP

Case 3

65 year old patient with early/moderate glaucoma on both eyes. Glaucoma was diagnosed 3 years ago after an optician found and IOP of 28 RE and 26 LE. During the last 3 years the IOP has been controlled under PGA treatment with 40% reduction and no side effects. On the last visit you observed a new DH not associated with PVD on the left eye, what would you do next?

1. Continue with same treatment and monitor in 6-12 months.
2. Continue with same treatment and monitor in 2-6 months.

3. Increase treatment to reduce the IOP (add a second drop)
4. Offer surgery to increase the reduction in IOP

Case 4

75 year old patient with early/moderate glaucoma on both eyes diagnosed 6 years ago after an optician found and IOP of 30 RE and 28 LE. During the first 3 years of treatment the patient had a labile IOP and tried many different treatments. Finally during the last 3 years the IOP has been between 12-14 on BE with no progression on visual fields. The patient is using 3 drugs to reduce the IOP with no side effects. On the last visit you observed a new DH not associated with PVD on the right eye, what would you do next?

1. Continue with same treatment and monitor in 6-12 months.
2. Continue with same treatment and monitor in 2-6 months.
3. Increase treatment to reduce the IOP (add a fourth drop or systemic treatment)
4. Offer surgery to increase the reduction in IOP

Finally, as a general rule, in your clinic when a new DH is present what do you do. (Question for three options of surveys).

1. Continue same treatment and monitoring interval
2. Reduce the monitoring interval
3. Increase treatment
4. Offer surgery

3.1.4 Results

All the invited participants to the pilot survey responded and they preferred the questions that explained the clinical scenario in more detail. The most common explanation to support this type of questioning (based on full clinical cases) was that participants felt that it was most similar to the way in which clinicians would regularly assess a patient and make a clinical decision. All participants considered the survey relevant and agreed with the selection of clinical scenarios. The results of the pilot version are in 5.1.2.

The final survey was disseminated to the full mailing list of UKEGS members who consented to receive surveys (196 members). However, we targeted only the UKEGS members who were self-declared as ophthalmologists (120 members), excluding optometrists and other scientists with an interest in glaucoma. The survey was first emailed on the 05th June 2017, a reminder email was sent on the 10th July 2017, the 07th August 2017, and finally closed on the 21st August 2017. The survey was open for 11 weeks, and the link to the survey was clicked by 84 participants. One participant did not accept the consent, and the survey was automatically terminated, and seven only partially completed the survey. All the participants who partially responded to the survey stopped at the question that asked about ophthalmology training. Feedback from participants revealed that some of the partial responders were non-ophthalmologist UKEGS members. The following results represent the opinion of the 76 participants who consented to participate and confirmed to be trained as ophthalmologists. The response rate obtained was of 63.3%.

3.1.4.1 General characteristics of the participants

The participant's general characteristics were surveyed in a categorical fashion (each deanery, groups of age, or proportion of glaucoma patients in their practice), but due to the limited number of participants in some categories, the results are also presented in a dichotomised fashion as follows:

1. Participants trained in a London deanery (n= 19) or outside of a London deanery (n= 57)
2. Participants who are younger (n= 39) or older than 50 years (n= 37)
3. Clinical practice of mostly/only glaucoma patients (n= 61) or few/half of glaucoma patients (n= 15).

Seventy-six per cent of the participants were between 40 and 60 years old, and the remaining were equally distributed below 40 and over 60 (see Figure 6).

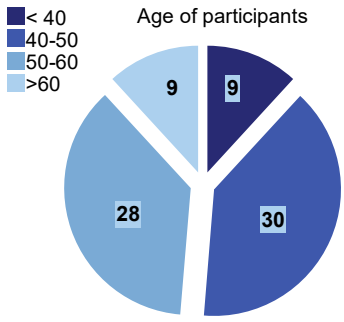

Figure 6 Age of participants, reported as the total number of participants in each category.

When asked about their type of clinical practice, 41 participants (54.0%) mentioned that they mostly saw glaucoma patients, while only 4 participants (5.3%) saw a few glaucoma patients. Seventy-two (94.7%) participants described their practice to have more than half of the patients with glaucoma.

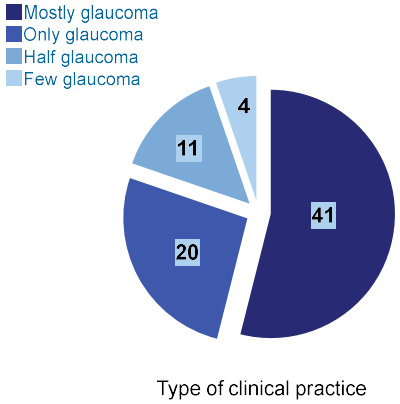

Figure 7 Proportion of glaucoma patients under participants' care, reported as the total number of participants in each category.

The place of ophthalmology training was asked of participants and the options offered were each of the UK deaneries or outside the UK. One-fifth of participants were trained in a London deanery (Figure 8) and, among the 22 deaneries, only two deaneries had no participants (Figure 9). Six participants were trained outside the UK.

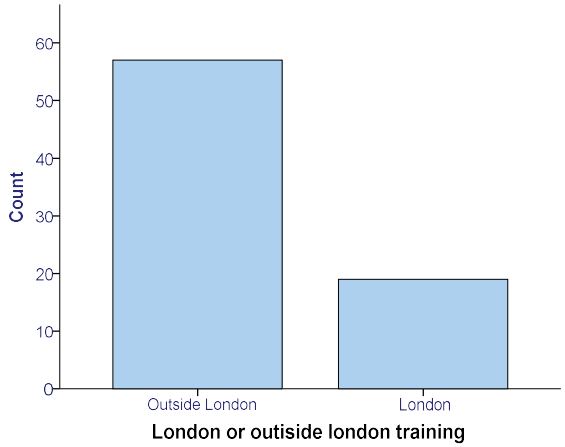

Figure 8 Participant's ophthalmology training. In a London deanery or outside London.

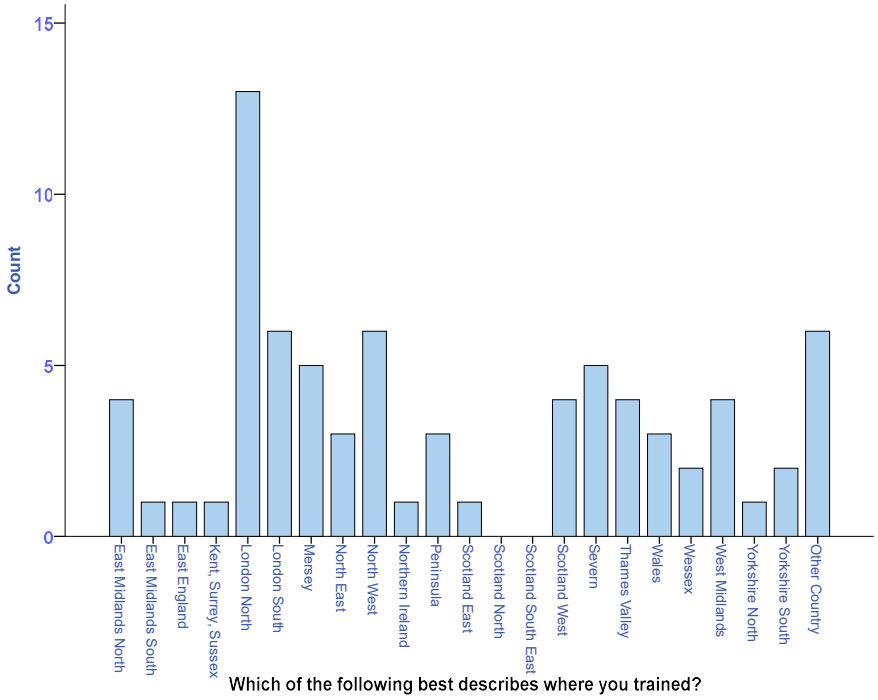

Figure 9 Deanery of participant's ophthalmology training.

A final general characteristic of the participants was the time and date of their responses (Figure 10 and Figure 11). The most common day of the week to respond was Friday and the most common time of the day was around midday followed by 11 pm.

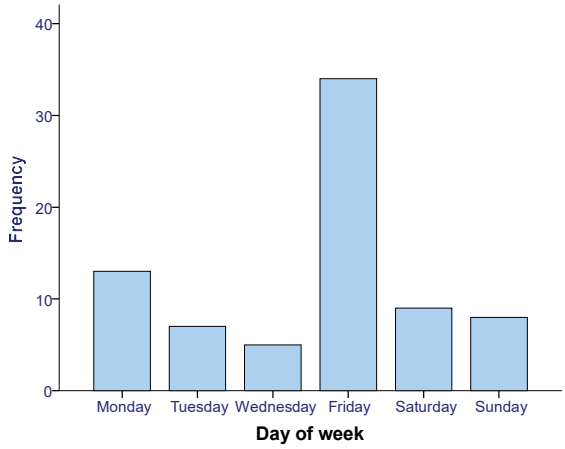

Figure 10 Day of the week when participants responded to the survey.

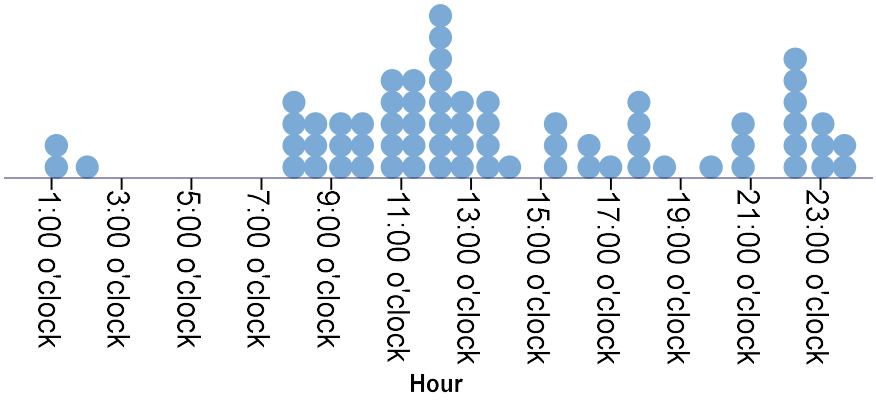

Figure 11 Time of the day when participants responded to the survey.

3.1.4.2 Results of clinical questions.

The first clinical question presented a healthy patient who visited an optometrist for new glasses and had a DH detected in an otherwise healthy eye. The results are shown in Figure 12. Around 10% of participants decided to discharge the patient while the rest would have requested further imaging or visits.

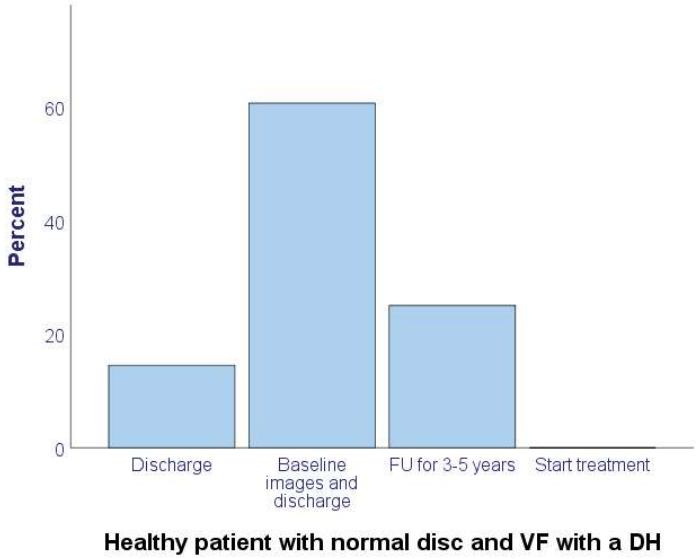

Figure 12 Responses from the first clinical question.

The second clinical question presented a patient with ocular hypertension under clinical monitoring with no medication for the last five years with IOP, VF, and RNFL stable but a new DH. The results are shown in Figure 13. A similar number of participants would have either discharged the patient or started treatment, while the majority would have continued with regular clinical appointments.

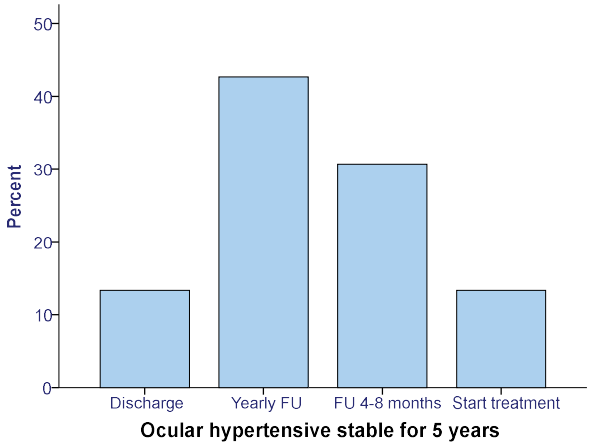

Figure 13 Responses from the second clinical question.

The third clinical question presented a patient with early/moderate glaucoma under medical treatment with Latanoprost for the last three years with IOP reduction and stable VF and RNFL but a new DH. The results are shown in Figure 14. Thirty per cent of participants would have continued the same management while the rest would have decreased the time between appointments, increased the medical treatment, or 3% would have offered surgery.

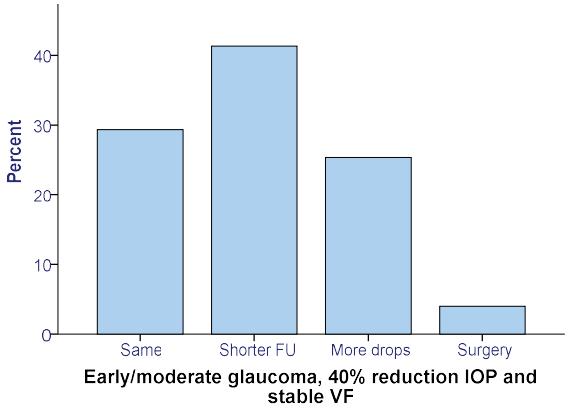

Figure 14 Responses from the third clinical question.

The fourth clinical question presented a patient with early/moderate glaucoma under maximum medical treatment with stable IOP, VF, and RNFL during the last three years but a new DH. The results are shown in Figure 15. Half of the participants would have shortened the follow-up interval while the rest would have either maintained the same management, increased the medication, or offered surgery.

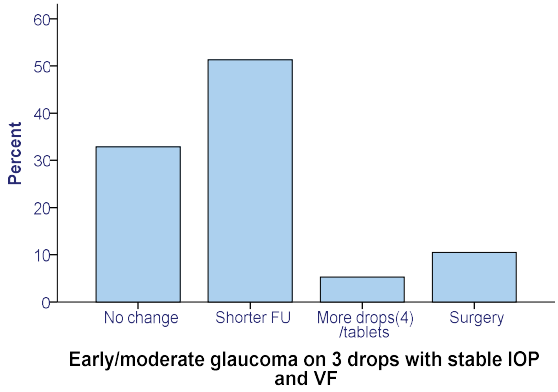

Figure 15 Responses from the fourth clinical question.

Finally, the fifth clinical question asks in a more general way what the most common way would be to respond clinically when a new DH is detected. The results are shown in Figure 16, and they clearly show that most of the clinicians were regularly changing the management of their patients when a new DH was detected.

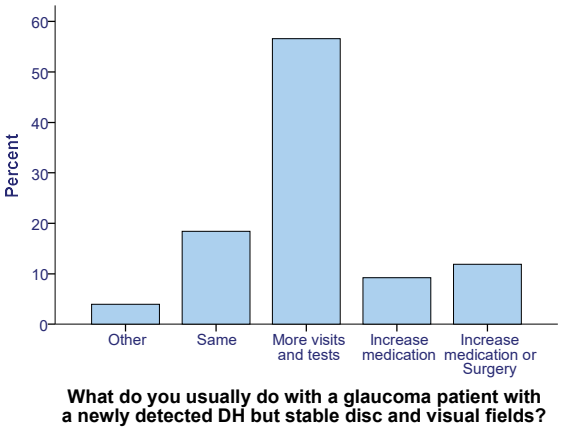

Figure 16 Responses from the fifth clinical question.

3.1.4.3 The impact of the general characteristics of participants on the response to the clinical questions.

The relationship between the results of the five clinical questions and the general characteristics of participants was analysed. An analysis of frequencies using

crosstabulation and Chi-square tests showed no difference for all comparisons. The relationship between the fifth clinical question about how clinicians usually managed patients with new DH and the deanery of training is reported in Table 10 to exemplify the results.

Table 10 Comparison between deanery of training in or out of London and the usual clinical management of patients with new DHs.

Usual management of patients with a new DH	Percentage trained in London	Percentage trained not in London
Other	5.3%	0%
Same management	15.8%	26.3%
More visits and tests	57.9%	52.6%
Increase medication	10.5%	5.3%
Increase medication or Surgery	10.5%	15.8%

A multinomial logistic regression did not show a significant effect of any of the general characteristics of participants on the responses to the clinical questions. Table 11 reports the multinomial logistic regression results for the relationship between the fifth clinical question about how clinicians usually manage patients with new DH and the deanery of training; this exemplifies the lack of relationship between all the participants' general characteristics and the response to the clinical scenarios.

Table 11 Multinomial logistic regression analysing the effect of the deanery of training in or out of London and the usual management of patients with new DHs.

Usual management of patients with a new DH	OR*	p-value
Other	NA	
Same management	0.90	0.907
More visits and tests	1.65	0.528
Increase medication	3.0	0.395
Increase medication or Surgery	NA	

OR of consultants trained in London
NA= not enough data to run the analysis

3.1.5 Discussion

The results of the national survey on the impact of disc haemorrhages in clinical practice confirmed the hypothesis that self-declared glaucoma specialist members of the UKEGS change the clinical management of patients with OHT and POAG and new DHs, irrespective of the stability of other clinical parameters such as visual fields, IOP, or other optic nerve characteristics. The change in management was found to be variable among different glaucoma specialists and it was not related to the age, deanery of training, or type of clinical practice.

The change in management observed in the survey had two important characteristics: firstly, all patients had in common the presence of a new DH, and secondly, it appeared when all other clinical parameters such as VF, IOP, or optic nerve were reported as stable or normal. The four clinical scenarios used in the survey were constructed to fit the NICE guidelines characteristics of a patient who has no glaucoma or stable glaucoma and would require continuing with the same treatment and follow-up interval, extending follow-up interval, or even consideration of discharge of the patient from a consultant-led clinic. However, NICE guidelines, as with many international glaucoma guidelines, do not make recommendations of how to modify the management of patients when a new DH is identified. If clinicians who participated in the survey had aligned and practised following only clinical guidelines, the four clinical scenarios would have been answered very differently. Only 11 (14.5%), 10 (13.3%), 22 (29.3%), and 25 (32.9%) of the participants responded as clinical guidelines suggested in the four clinical scenarios (incidental finding, OHT, POAG stable on one drop, and POAG stable on three drops). The lack of compliance with clinical guidelines in these four clinical scenarios is expected, considering the large amount of clinical evidence which has reported the importance of DHs in the diagnosis and progression of glaucoma. For this reason, in the last general question, 81.6% of clinicians responded that they would modify to a certain degree the management of patients when a new DH was identified. Among this 81.6%, 56.6% would have shortened the follow-up and added auxiliary tests, 9.2% would have increased the number of medications, 11.8% would have suggested glaucoma surgery, and 3.9% would have suggested other extra interventions (diurnal phasing of IOP and other systemic evaluations).

There was great variability in how participants would have changed their clinical management. The management option most frequently selected in the four clinical cases of the survey never exceeded 50% of the participants. It is interesting to notice the amount of variability in management among glaucoma specialist members of the UKEGS, despite having been trained in the same region and who practise under a similar health care system. The fifth clinical question, which surveyed more broadly about the management of patients with new DH, had the greatest homogeneity with 43 (56.6%) of the participants reducing the interval between visits and adding additional tests. Variability in clinical management, also known as practice variation or inconsistency in healthcare, among other terms, is a well-known characteristic of clinical practice that has been reported in all regions of the world and for almost all medical conditions (25). Inconsistencies have been described for different aspects of healthcare systems, such as availability of services in different geographic regions (26, 27), variations in diagnostic practices (28) and even use of surgical procedures (29).

Publications from other areas of medicine have identified an even wider practice variation between clinicians from different countries such as USA and UK (30). It is likely that the practice variation among specialists trained in different regions of the world would be even wider than that identified among glaucoma specialist members of the UKEGS. The variation would also be expected to increase when the level of training of the eye care provider is lesser. For instance, general ophthalmologists or non-glaucoma specialist optometrists would be expected to have a wider practice variation if the same clinical scenarios were to be presented. Harper et al. reported the agreement between six ophthalmologists and six optometrists in optic disc assessment and identified among the optometrists a systematic under-reading of cup disc ratios, as well as worse identification of DHs (31). Although this publication had a small number of participants, it supports the possibility that the practice variation might be even wider when general ophthalmologists, optometrists, and other eye-care specialists are compared to glaucoma specialists.

Variation in management might be harmless in many of the cases and it is expected to exist to a certain degree due to differences among patients'

preferences and the risk factors present in each population. However, when the variation is unwarranted, it has the risk of increasing the cost of management due to unnecessary treatments and referrals or causing harm due to late diagnosis and incorrect medical/surgical treatment and its inherent risk of complications (25). In the management of patients with an incidental finding of a DH, there was a strong tendency toward requesting additional tests and visits to monitor progression despite the limited evidence of DHs' role as possible precursors of POAG and POAG progression. The exposure of patients to extra clinical care might end in overdiagnosis of other diseases or incorrect interpretation of normal variability/fluctuation on examinations as progression. The harmful effect of extra clinical care has been extensively described in other diseases like breast cancer (32), in which patients with a higher income have more screening visits at an earlier age with the consequence of identifying a higher number of patients with cancer at younger ages and in earlier stages. When these patients are treated at earlier stages, the harmful effect of treatment remains the same while the beneficial effect of reducing the risk of death or disability from cancer that would have progressed into a stage that can produce clinical problems is reduced because the chances of progressing from a very early stage to a clinically significant disease are lower. In ophthalmology, a similar situation could happen in patients that are regularly seen in the clinic to assess the risk of glaucoma. Clinicians might suggest glaucoma treatment for lower levels of OHT or suspicious optic discs, or consider cataract surgery at an earlier age and with less or no visual dysfunction.

In the second clinical scenario, 13.3% of participants would have started treatment in a 50-year-old patient with OHT who had been stable during the previous five years. In addition to the risk of overdiagnosis of other ophthalmic conditions, overtreating chronic diseases in young patients has a high risk of causing harm despite the mild or rare complications related to medical treatment due to the long treatment period that is expected in young patients. In the third clinical scenario, 29.3% of participants would have increased treatment with an additional drug despite the higher risk of complications, polypharmacy, and reduction in compliance. Finally, in the fourth clinical scenario, 10.5% of participants would have suggested surgery that involves a small but higher risk of

abrupt complications and change in the quality of life for the patient and family/carer.

Although the benefits of standardisation of clinical management seem to outweigh potential criticisms, it is also true that clinical freedom and the “art of medicine” might be at risk (33-35). The controversial variation in clinical practice that characterises the romantic stereotype of the doctor-patient relationship could be positive and help to individualise treatments when it is based on the clinician judgment of each patient individually. Doctors practising more individualised care that is well-informed and free from biases is highly appreciated by patients who want expert advice on how to make the best decision for their preferences and beliefs. Another caveat of standardisation of clinical management, based on the available evidence, is that the “mean effect” found on many clinical trials might not be generalised to many individual patients. The heterogenicity of treatment effect has been extensively described in randomised clinical trials and cohort studies; it is often assessed by subgroup analysis, and it is defined as the difference between groups in the risk of an outcome. However, systematic reviews addressing the frequency and the quality of how the heterogenicity of treatment effect is reported identified that only 58% of cohort studies (36) and 44% of randomised clinical trials reported it (37). Despite the frequent underreporting of possible heterogenicity of treatment effects, mean effects from these studies are translated into clinical guidelines and applied to individual patients. In the opposite scenario, overused/misuse of subgroup analysis has also been associated with harm to patients (38). The complexity and high risk of biases of the heterogenicity of treatment effect is another factor that makes standardisation of care more challenging, especially when average clinical results from hundreds of ideal participants are applied to an elderly individual patient with multiple comorbidities, such as the patients seen in glaucoma clinics.

A reduction of variability in clinical management, despite its criticisms, stands as a practical option to improve healthcare across all areas of medicine. Possible options to reduce variability are the development of a consensus of experts, the implementation of national or international clinical guidelines, the utilisation of alerts on electronic patient records to aid clinicians when filling them, clinical audits and feedback, and decision support tools, among others. However, clinical

guidelines need to apply to the majority of patients; otherwise, doctors will identify in their patients multiple contraindications to apply the guidelines and provoke intentional non-adherence to clinical guidelines with levels as high as 65.3% reported by Arts et al. (39) in a systematic review. Finally, even if clinical guidelines are ideal, surveys to health professionals have reported only a small effect of clinical guidelines on change in practice or as the source of practice variation (40). Some hospitals have integrated health information technology to reduce practice variations successfully (41), but these benefits might not be the same when applied at a national or international level. Audit and feedback is a strategy designed to improve clinical practice based on the continuous audit of important clinical outcomes that are compared to predefined targets and then are transformed into feedback to the healthcare professionals with the idea that variability in practice that is driving underperformance is reduced (42) However, the use of audits have proved to have only a small effect on improving clinical outcomes. Finally, with the current increase in artificial intelligence research, it is possible that clinical decision support systems based on properly labelled real-world data could support clinicians in the decision-making process that is best for each patient (43).

To conclude, there is practice variation among glaucoma specialist members of the UKEGS on how glaucoma specialists manage patients with a new DH. There were no demographic characteristics of clinicians which explained the practice variation. There is little mention of DHs in NICE guidelines and, based on the clinical scenarios presented in the survey, there was wide intended non-adherence to the guidelines.

*Part of this work was presented as a paper presentation in the UKEGS 2017 meeting.

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Chapter 2 A method based on scanning laser ophthalmoscope for the detection of disc haemorrhages.

3.2.1 Abstract

Purpose: To evaluate the diagnostic accuracy of a new method to detect disc haemorrhages (DH) in patients with glaucoma.

Methods: Patients with newly diagnosed open angle glaucoma were included in the United Kingdom glaucoma treatment study (UKGTS), and during the 24 months follow-up, patients were imaged with monoscopic fundus photography (FP) and Heidelberg retina tomograph (HRT) on the same day over 11 visits. From the 516 enrolled patients, 122 were classified as DH+ based on the analysis of the full series of HRT images and photographs using a flickering method between baseline and each follow-up scan. Twenty-five DH+ and 25 DH- participants were randomly selected from the participants with FP and HRT on the same day. Anonymised flicker pairs of HRT and FP were converted into a GIF file and presented in a random order to 18 glaucoma specialists to classify images as DH+ or DH- and the location in clock hours. Accuracy to detect DH+ patients and within- and between- observers agreement was compared between FP (reference standard) and HRT (index test).

Results: The between-observers kappa agreement was 0.69 and 0.86 for HRT and FP, respectively. The mean within-observer kappa agreement for the 18 observers was 0.87 and 0.86 for HRT and FP, respectively. The pooled area under the curve (AUC) to detect DHs was 0.87 and 0.95 for HRT and FP; there was slightly better accuracy using FP than HRT (0.08 greater AUC favouring photography, CI 95% 0.00-0.15; p=0.04).

Conclusions: HRT images could be used to identify DHs in patients with glaucoma with only a slightly lower accuracy compared to FP. The SLO technology incorporated in some OCT instruments could be used to aid in the detection of DHs in clinical practice without the requirement for additional equipment.

3.2.2 Introduction

The diagnostic instruments supporting the identification of DHs have not changed since Stephen Drance described them in 1969 (1). Clinical examination and fundus photography are still the only methods available for DH detection; the former is the most commonly used and the latter is considered the gold standard. Careful clinical examination has the potential to be as sensitive as fundus photography to detect DHs, but, unfortunately, every time it has been compared to fundus photography, it fails to detect the same number of DHs. Even in the setting of clinical research trials, when clinicians are asked to report precisely if DHs are present or absent, DHs are frequently missed. The Ocular Hypertension Treatment Study (OHTS) (2) and the European Glaucoma Prevention Study (EGPS) (3) identified that 84% and 80% of eyes respectively would have been wrongly categorised as not having a DH (DH-) if clinical examination would have been used alone. The Early Manifest Glaucoma Trial (EMGT) found that in 29% of the visits DHs were missed by clinical examination in comparison to fundus photographs read in a reading centre (4).

The acquisition of a good quality fundus photograph is not the complete solution to the problem of under detection of DHs. After the photograph is acquired, the next challenge is to correctly identify the presence of the DH and facilitate the process of detection to increase the agreement among different observers. Table 12 summarizes publications that have analysed the agreement between observers to detect DHs. In these publications, the most common factors that have been investigated to affect the agreement among health care professionals seem to be the type of training (ophthalmologist compared to optometrists), level of training (glaucoma specialist compared to ophthalmology residents), type of photography (film compared to digital), the dimensions of the photography (3D or 2D), the use of sequential images (side-by-side or flicker compared to single), among others. From these publications, there seems to be a tendency toward better agreement among graders with 3D photography. In addition, the publication with the largest number of eyes with a DH found a better sensitivity to detect small DHs with an automated flicker technique irrespective of the level of training of the grader (5) and better agreement between graders with this technique. However, they did not compare the flicker technique with 3D photographs. The flicker

technique to compare fundus photographs was originally described by Goldmann with the name of stereo-chronoscopy (6). Stereo-chronoscopy has been repetitively confirmed to improve the ability of clinicians to detect changes in the optic nerve (7-10) and to improve the detection of DHs (11); it consists in the alignment of two photographs taken at different times and the alternate "flicker" presentation of one and then the other photograph in rapid succession (2-4 Hz).

Table 12 Publications that investigated the agreement between observers to detect DH using different techniques.

Author	Type of observers	# Eyes (# DH+)	Type of Images	Kappa Inter	Kappa Intra
Li (12)	1 optometrist	26 (ND)	3D film, digital	1.0	ND
Harper (13)	6 ophthalmologists	48	3D digital	0.54	0.77
	6 optometrists	(ND)		0.35	0.60
Murdoch (14)	8 optometrists	50	direct	0.67	ND
	1 ophthalmologist	(ND)	ophthalmoscopy		
O'Brien (15)	2 ophthalmologists	53 (ND)	Slit lamp	0.31	ND
Scheetz (16)	46 orthoptists	42	2D digital online	0.77	
		(4)		0.89*	
Kong (17)	197 ophthalmologists	42	2D digital online	0.69-0.83	ND
		(4)			
Radcliffe (5)	4 ophthalmologists	40	2D flicker	0.43	ND
		(6)	3D digital	0.78	
Chee (18)	2 glaucoma	103	2D flicker	0.7	ND
	specialists	(8)			
Sandhu (19)	4 glaucoma	192	3D digital	0.77	ND
	specialists	(9)	2D digital	0.56	
			3D film	ND	
Shah (20)	2 glaucoma	399	3D digital	0.59	ND
	specialists	(ND)		0.80	
	2 optometrists			All 0.65	

*Increase after online education, ND = not described.

Regular fundus photography graded by a glaucoma specialist aided by a flickering technique seems to be the ideal method to detect the majority of DHs. Unfortunately, this is not feasible in most clinical scenarios and the tendency in many clinics is to reduce the number of fundus cameras or even decide not to have them to allow space for other imaging equipment not based on photography. In the last decades, there has been a steady increase in the use of OCT and OCT angiography and a reduction in the number of fundus photographs and fluorescein angiographies (21). Unfortunately, OCT technology was not designed to detect DHs and modern OCT images are incapable of detecting even large DHs. The reduction in availability of the basic equipment required to increase the detection of DHs requires the development of new approaches that either combine fundus photography and OCT or use characteristics of the OCT signal that can be used to simulate fundus photography. The DRI OCT Triton (Topcon, Tokyo, Japan) and Cirrus photo 600 (Carl Zeiss Meditec, Inc, Dublin, CA) opted for the combining of a fundus camera with an OCT while Spectralis (Heidelberg Engineering, Heidelberg, Germany) invested in the development of a proprietary technology called multiColor image (22). The latter is based on scanning laser ophthalmoscopy and creates a pseudocolour image that resembles fundus photography. Although these strategies are promising, the majority of OCT instruments do not provide images from which DHs can be detected by clinical observers.

An interesting alternative to fundus photography is the use of scanning laser ophthalmoscopy (SLO); it was the first non-fundus photography imaging technique to be used widely in glaucoma clinics. Dichtl et al., using the Heidelberg retina tomograph (HRT) pseudocolour reflectivity images, reported this instrument's ability to detect almost half of the DHs that were detected by fundus photography (23). Later, the same group expanded their original findings into 73 eyes with DHs detected by fundus photography and identified that 78% of the DHs extending into the parapapillary region, and 10% of those restricted to the intrapapillary region, were identified by HRT (24). The authors concluded that “For detection and documentation of optic disc hemorrhages, clinical examinations with detailed documentation or evaluations of optic disc photographs are recommendable.” However, as previously mentioned, clinical examination detects fewer DHs than was reported by the authors using the mean reflectivity

images of a single HRT acquisition session. The version of the software used by the authors did not allow flickering between different HRT reflectivity images.

Aware of the trend toward using more imaging instruments for screening, aid in the diagnosis and to assess progression in glaucoma clinics, it would be ideal to enhance the current imaging techniques with the ability to detect DHs. One approach is to optimise the previous experience with HRT detection, adding a flickering technology, and allowing future automization of the detection. Despite the fact that the use of the HRT instruments is declining in most glaucoma clinics and the company no longer sells it for glaucoma purposes, the SLO technology employed by the HRT could be used to detect DHs. The SLO technology is incorporated in many of the current OCT instruments as an eye-tracking system (25) and some of the OCT instruments even create an image similar to the SLO reflectance image constructed with the HRT or Spectralis. The SLO technology already present in commercially available OCT instruments could potentially be used to identify DHs. If the diagnostic capacity of the SLO technology is able to detect a higher proportion of DHs compared to clinical examination, it would be an important improvement for routine clinal practice in which a large number of patients fail to have their DHs identified with subsequent risk of missing an opportunity to escalate treatment, have more frequent monitoring or assess with more detail other characteristics of the optic nerve.

Due to the abundance of good quality HRT scans acquired during the United Kingdom Glaucoma Treatment Study (UKGTS), the ability of HRT to detect DHs was explored using data acquired during the scheduled study visits. The agreement within and between observers to detect DHs using the flicker method based on SLO images was investigated and then compared to DH detection from fundus photography. We hypothesised that DHs could be detected in SLO images as well as in fundus photography if we combined sequential SLO images with a flicker methodology.

3.2.3 Methodology

3.2.3.1 Participants

All the participant's data were collected prospectively during scheduled visits of the 516 participants of the United Kingdom Glaucoma Treatment Study (UKGTS). The complete study design and baseline characteristics have been published previously (26, 27). In brief, it was the first multicentre, randomised, placebo-controlled trial of medical treatment for glaucoma and showed preservation of visual fields with latanoprost treatment in newly diagnosed glaucoma patients (28). The participants were enrolled between December 2006 and March 2010 for 11 visits over a 24-month period. One of the secondary objectives of the UKGTS was "to evaluate whether risk profiling and stratifying patients is able to identify which patients do not need immediate treatment and which patients may benefit from more vigorous treatment" (26). To address this secondary objective, DHs were planned to be identified among the characteristics assessed to risk profile patients. The UKGTS followed the good clinical practice recommendations and the Declaration of Helsinki and was approved by Moorfields and Whittington Research Ethics Committee on June 1, 2006 (reference 09/H0721/56) and registered at ISRCTN registry with the reference ISRCTN96423140. All patients signed written informed consent before the screening visit. An independent Data and Safety Monitoring Committee (DSMC) was appointed by the trial steering committee. Adverse events were monitored and reported to the operational DSMC at Moorfields Eye Hospital. Serious adverse events were reported to the Medicines and Healthcare Products Regulatory Agency (26).

During the 24-months follow-up, patients had fundus photography with various commercially available cameras on a variable number of visits, and SLO scans with Heidelberg retina tomograph 3 (HRT 3; Heidelberg Engineering, Heidelberg, Germany; image acquisition software version 3.0.60, Heyex 1.6.2.0) at each of 11 visits. All technicians were trained to perform each examination and it was permitted for different members of the staff to acquire images from different participants on different visits. All staff were masked to the treatment allocation. A standard operative procedure was written and all sites encouraged technicians to follow the standards.

The HRT II and III instruments use a diode laser (670nm wavelength) and a confocal optical system centred at the optic nerve with a scan angle of 15 x 15 degrees to produce a tomographic series of up to 64 tomographic scans at different depths with a resolution of 384 X 384 pixels. The tomographic series of up to 64 tomographic scans (depending on the cup depth) are combined and automatically aligned to correct for any movement during the approximate 1.6-seconds acquisition time. The final images that are seen in the software report (topography and reflectance image) are the mean of three tomographic series that are automatically acquired during each session. We repeated two or three sessions at baseline,18 and 24 months (clustering) to reduce the test-retest variability (29, 30) and acquired single series in the remaining visits. Images were excluded if any part of the optic nerve was not visible or if the image quality of the mean pixel height standard deviation (MPHSD) was greater than 40 microns. Fundus photography was monoscopic, digital, under pharmacological mydriasis, and centred at the optic nerve and macula with a view of 15 or 30 degrees depending on the preferences and equipment of the site. Images were excluded if any part of the optic nerve was not visible or if the quality did not allow the correct visualization of the main vessels.

3.2.3.2 Image analysis

All the SLO scans were analysed with the Topographic Change Analysis (TCA) algorithm of the Heidelberg Eye Explorer HRT3 (Heidelberg Engineering, Heidelberg, Germany; version 3.0.60, Heyex 1.6.2.0). The TCA automatically aligns all the follow-up images with the baseline and analyzes the probability that each superpixel (4X4 pixel) has a change in height (topographic change) (30). The software has a flicker function which allows good visual comparison between the baseline and each of the follow-up images. The speed at which baseline and subsequent images were flickered was set at two frames per second to best highlight the DH in the reflectance scans. The progression feature of the software that analyses the change at each superpixel was not used for the DH identification.

Fundus photographs were minimised or maximised to match the image with the best quality in the few cases where baseline and follow up images did not have

the same magnification. All photographs were aligned with built in software from the Beijing Institute of Ophthalmology previously used to identify changes in series of disc photographs (31) (the fundus photography alignment process was done by Yaxing Wang's team at the Beijing Institute of Ophthalmology). The aligned images were then flickered using ImageJ at two frames per second.

The grading of HRT images to assess the presence of DHs followed a standard operating procedure (5.1.5). A DH was defined as the appearance of a dot or flamed shaped change in reflectivity or red colouration in the optic nerve rim or peripapillary area within one disc diameter of the rim. The location of the DH was defined as the clock hour in which the centre of the DH crossed the optic nerve border. One glaucoma specialist experienced with the analysis of optic nerve HRT images and photographs assessed all photographs and HRT data, masked to treatment allocation and outcome status. The 516 participants were labelled as DH+ or DH- based on the detection of a DH on either HRT or fundus photography in at least one eye at any visit. All eyes labelled DH+ using HRT with good quality fundus photographs also had the DHs confirmed by fundus photography. However, in some visits, DHs were only detected by fundus photography, although this never changed a patient's label because, over the series of visits, the DH was also detected by HRT in another visit. All DH+ eyes identified by fundus photography or HRT were re-graded by a second glaucoma specialist, and a consensus of DH status was reached on all participants using all the sequences of HRT and fundus photographs data. The reference standard for labelling a participant as DH+ was the consensus reached between the first and second glaucoma specialist.

3.2.3.3 Agreement within and between observers study

Twenty-five trial participants with a DH and 25 without a DH were randomly selected from the original 516 UKGTS participants; the presence/absence of DH was validated from same-day fundus photography. The reference standard to define the DH+ or DH- label was based on the fundus photography graded by two glaucoma specialists. Anonymized flicker pairs of HRT reflectivity images were converted into a GIF file and uploaded into an online survey manager (Survey Monkey®, San Mateo, CA, USA). An example of how the images were displayed

in the online survey manager can be seen in 5.1.6. Five glaucoma specialists masked to treatment allocation and outcome status participated in the study. Participants were asked to classify the flickering HRT reflectivity image as DH+ or DH- and then locate the DH in clock hours. Twenty-five images were presented twice to assess within-observer agreement (Figure 17).

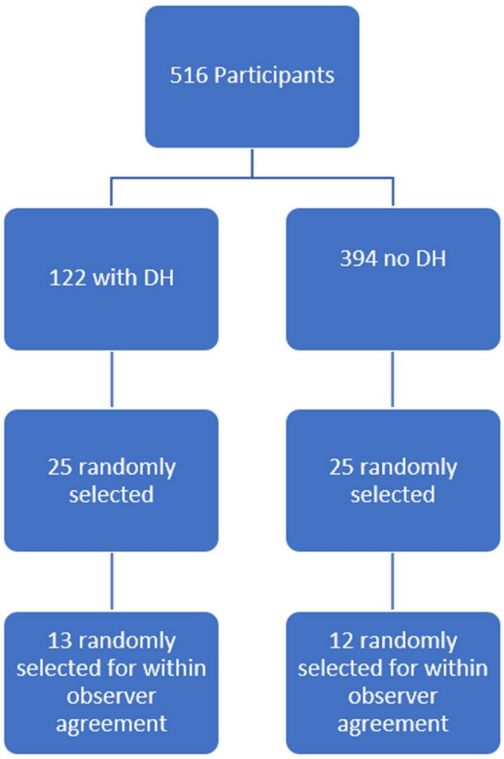

Figure 17 Flow chart of the selection of participants from the UKGTS to the agreement and accuracy studies.

Images were presented in random order. The area (mm^2), angular extent (degrees), association with retinal nerve fibre layer (RNFL) defects, location (clock hour), and shape (dot or flame) of the DH were compared between the ten eyes with the best and worst agreement. The area and angular extent were calculated using the PPA Zone Analysis software (Heidelberg Engineering GmbH) (Figure 18). Within- and between-observer agreement for identifying the presence of a DH was calculated using kappa statistics and the t-test was used to compare the mean of the DH characteristics (area and angular extent) between the groups of best and worst agreement.

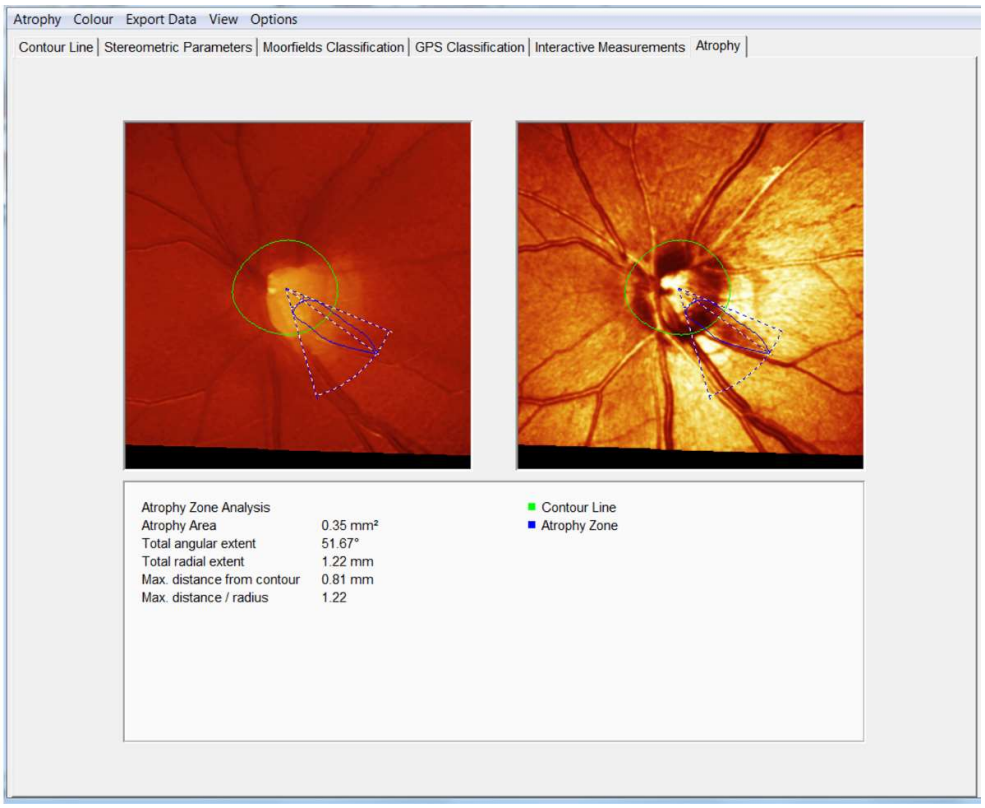

Figure 18 Example of measurements of the DH area and angular extent using the PPA Zone Analysis software (Heidelberg Engineering GmbH).

3.2.3.4 Comparison of DH detection from HRT images compared to fundus photography (Diagnostic accuracy study)

A new group of 25 participants with a DH and 25 without a DH with fundus photography and HRT scans on the same day were randomly selected from the 466 UKGTS participants that were not included in the agreement study. A flickering pair (GIF file format) of fundus photographs and HRT images were constructed and presented to the observers. The flickering images from 25 randomly selected participants were presented twice to assess the within-observer agreement (Figure 17). In total, each observer looked at 150 flickering images.

The observers were glaucoma specialists with ophthalmological training and interest in imaging from different parts of the world. All participants received the

same written instructions on how to identify DH presence and location, and there was no extra training provided on how to identify DHs. All participants were unaware of treatment allocation and outcome status. The invitation to participate, instructions for the study and for the grading of the images was done electronically by email and the use of an online-based survey manager (Survey Monkey®, San Mateo, CA, USA). HRT scans and fundus photographs were presented in blocks of five images in random order with no option of going back but with the opportunity to save the work done and later complete the analysis of the 150 images.

Within- and between- observer agreement for HRT images and fundus photographs was analysed. Agreement per each observer, all pairs of observers, and for all observers was compared between HRT images and fundus photography to compare the performance of each platform. Between-observer agreement is reported between all pairs of observers with Cohen's kappa and the overall agreement was reported with Fleiss' kappa statistics. Accuracy was analysed using Receiver Operating Characteristic (ROC) curves and the areas under ROC curves (AUC); they were constructed for each observer detecting DHs based on HRT and fundus photography. Overall accuracy was constructed for HRT and fundus photography and compared. Areas under the ROC curve were further analysed by setting a fixed threshold (partial AUC) of 0.75-1.0 sensitivity. Comparisons were made between platforms to detect DHs and observers. All statistical analyses were performed using SPSS (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.). All multi-observer agreement analyses were calculated using the mean of all pairwise Cohen's kappa, Fleiss kappa, and Krippendorff's alpha (32). Cohen's kappa was prefered for comparison between two observers, and Fleiss's kappa and Krippendorff's alpha for comparisons between multiple observers. Fleiss kappa and Krippendorff's alpha have the advantage of being more flexible in analysing agreement or reliability studies with multiple raters or observers (33). The pooled estimates of AUC were calculated using iMRMC (Multi-Reader, Multi-Case Analysis Methods) (34). Sensitivity, specificity, predictive values, likelihood ratios, and accuracy were calculated with Medcalc (Ostend, Belgium). Likelihood ratios are presented using the nomogram described by Fagan (35).

3.2.4 Results

All of the 516 participants enrolled in the UKGTS had at least three HRT scans and the flicker analysis was performed successfully on all of them. However, the observation period was less than 24 months in some patients; 55 patients did not attend any post-baseline visit, and 127 patients had fewer than 21 months of follow-up. In 77% of the patient's visits, the DHs were identified by both methods, fundus photograph and HRT. In the remaining 27 % of visits, DHs were identified based only on HRT due to the absence of fundus photography for that visit. The HRT images were clustered at baseline,18, and 24 months of the trial. The large number of scans produced 14,236 independent scans, 7,156 of the RE and 7,080 of the LE. On 3,913 (94.2%) of all study visits, an HRT image was acquired. Across the 11 scheduled visits, participants had a mean of 1.7 HRT scans per visit. One hundred and twenty-two (23.45%) participants had at least one visit with a DH in either or both eyes. Assessing each eye of the participants, 139 eyes (13.47%) had at least one visit with a DH. The mean prevalence of visits categorised as DH+ per participant was 9.1%.

For the agreement study, the five invited observers accepted the invitation and participated in the study. All were fellowship-trained glaucoma specialists from Europe and Asia. For the accuracy study, 25 glaucoma specialists from around the world were invited by email to participate, and 18 signed consent and graded the images (response rate of 72%). The nationality of training varied, as shown in Figure 19 with the UK being the most common place for training.

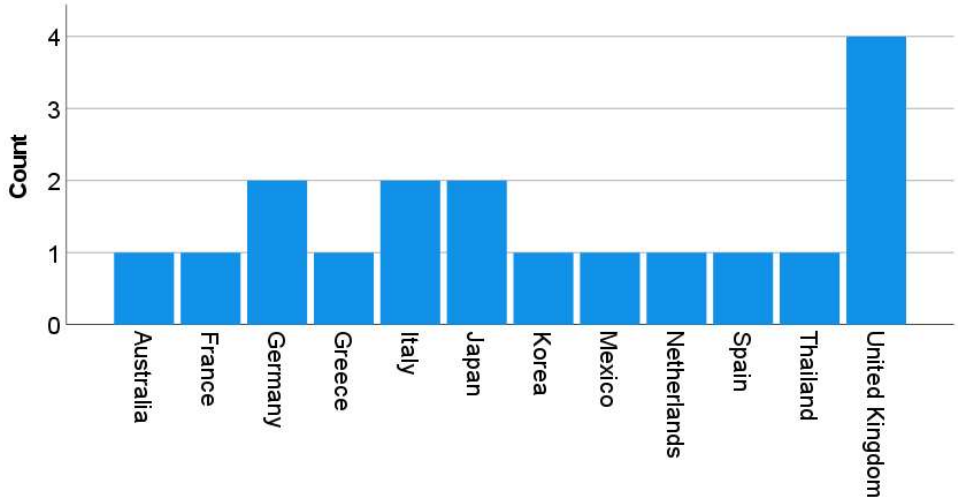

Figure 19 Country of ophthalmic training of glaucoma specialists who collaborated as observers.

Among the five glaucoma specialists who participated in the agreement study, the mean between-observer agreement kappa was 0.64 (range 0.40–92) for DH presence. In the eyes in which the observers agreed that there was a DH, the between-observer agreement kappa for the location in the same clock hour was 0.80 (range 0.62-1.0). In addition, 98.9% of responses agreed on the location if only the eyes in which the observers agreed on the DH presence were analyzed and they considered the location as clock hour ± the neighbouring clock hour. The mean within-observer agreement kappa was 0.79 (range 0.28–1.00) for DH presence and 0.81 (range 0.5-1.0) for the location in the same clock hour. In addition, 100% of responses agreed on the location if only the eyes in which the observer agreed on the DH presence were analyzed and they considered the location as clock hour ± the neighbouring clock hour. The details for all kappa agreements between observers are reported in Table 13 for the detection of the presence of DHs and in Table 14 for the location of DHs.

Table 13 All between and within observer agreement Kappa (95% Confidence Interval) for the detection of the presence of DHs.

	Observer 1	Observer 2	Observer 3	Observer 4	Observer 5
Observer 1	**1.00 (1.00–1.00)**	0.68 (0.48–0.88)	0.80 (0.64–0.96)	0.40 (0.20–0.60)	0.92 (0.81–1.00)
Observer 2	0.68 (0.48–0.88)	**0.84 (0.62–1.00)**	0.55 (0.31–0.78)	0.51 (0.29–0.75)	0.76 (0.58–0.94)
Observer 3	0.80 (0.64–0.96)	0.55 (0.31–0.78)	**0.84 (0.62–1.00)**	0.55 (0.32–0.77)	0.78 (0.63–0.97)
Observer 4	0.40 (0.20–0.60)	0.51 (0.29–0.75)	0.55 (0.32–0.77)	**0.28 (-0.12–0.69)**	0.45 (0.24–0.67)
Observer 5	0.92 (0.81–1.00)	0.76 (0.58–0.94)	0.78 (0.63–0.97)	0.45 (0.24–0.67)	**1.00 (1.00–1.00)**
Mean Kappa (range)	**0.64 (0.40–0.92)**				**0.79 (0.28–1.00)**

Table 14 All between and within observer agreement Kappa for the location of DHs.

	Observer 1	Observer 2	Observer 3	Observer 4	Observer 5
Observer 1	**0.82**	1	0.75	0.62	0.9
Observer 2	1	**0.87**	0.84	0.79	1
Observer 3	0.75	0.84	**0.88**	0.64	0.8
Observer 4	0.62	0.79	0.64	**0.5**	0.67
Observer 5	0.9	1	0.8	0.67	**1**
Mean kappa (Range)	**0.8 (0.62-1.0)**				**0.81 (0.5-1.0)**

For these five observers, there were factors associated with good and poor between-observer agreement. A significant difference was identified in the mean area and angular extent of the DH between the groups with the best and worst agreement. There was no difference in the location, association with RNFL defects, or shape of DHs (Table 15).

Table 15 Differences in DH between groups with the best and worst agreement.

	Worst agreement	Best agreement	p value
Mean, SD (IQR) area of the DH in mm^2	0.07, 0.04 (0.04-0.10)	0.15, 0.04 (0.09-0.18)	0.01
Median (IQR) angular extent in degrees	13 (8-20)	26 (19-29)	0.02
% of DHs associated with RNFL defects	50%	60%	0.73
Mode of location in clock hours (median)	7 (7)	7 (7)	
Shaped as flame:dot	10:0	9:1	

SD = standard deviation, IQR = interquartile range.

Among the 18 glaucoma specialists who participated in the accuracy study for the detection of DHs, the mean between-observer agreement kappa was 0.69 (95% CI 0.67 - 0.71) for HRT and 0.86 (95% CI 0.84 - 0.88) for fundus photography. The mean within-observer agreement kappa was 0.87 and 0.86 for HRT and fundus photography, respectively. For the DH location, the mean between-observer agreement kappa was 0.76 and 0.75 and the mean within-observer agreement kappa was 0.65 and 0.73 for HRT and fundus photography, respectively (Table 16).

Table 16 Cohen's kappa agreement among 18 observers.

	Between observers	Within observers
HRT detection	0.69	0.87
Photography detection	0.86	0.86
HRT location	0.76	0.65
Photography location	0.75	0.73

Among the 18 observers, all pairwise kappa agreements are reported in Table 17 for HRT and Table 18 for fundus photography. Red boxes represent the worst five agreements, while blue boxes are the best five agreements. The kappa agreement between HRT and fundus photography was 0.72. All the pairwise multi-observer agreement analysis presented in Table 17 and Table 18 were calculated using three different methods: Cohen's kappa, Fleiss Kappa, and Krippendorff's alpha. However, the results were almost identical, with only marginal differences at the level of $1 \times 10^{-5,}$ and it did not affect the current results that were rounded up or down to two decimal points.

Table 17 All pairwise kappa agreement among 18 observers for the detection of DH on HRT images.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	0.75	0.72	0.55	0.57	0.71	0.77	0.67	0.83	0.71	0.75	0.70	0.39	0.55	0.79	0.71	0.41	0.61	0.68
2		0.82	0.63	0.49	0.71	0.95	0.67	0.74	0.62	0.67	0.78	0.47	0.63	0.78	0.71	0.42	0.53	0.60
3			0.68	0.52	0.68	0.59	0.56	0.72	0.60	0.72	0.76	0.40	0.60	0.68	0.60	0.41	0.72	0.64
4				0.68	0.60	0.53	0.64	0.72	0.76	0.72	0.68	0.48	0.68	0.68	0.68	0.56	0.64	0.72
5					0.92	0.75	0.88	0.88	0.84	0.88	0.84	0.57	0.60	0.84	0.76	0.57	0.72	0.80
6						1.00	0.71	0.79	0.67	0.71	0.83	0.51	0.68	0.83	0.75	0.45	0.57	0.64
7							0.76	0.84	0.88	0.84	0.80	0.53	0.64	0.88	0.80	0.69	0.68	0.76
8								1.00	0.88	0.92	0.88	0.53	0.72	0.96	0.80	0.54	0.76	0.84
9									1.00	0.88	0.75	0.49	0.60	0.84	0.76	0.57	0.72	0.80
10										1.00	0.88	0.61	0.72	0.88	0.88	0.61	0.84	0.92
11											0.84	0.65	0.76	0.92	0.84	0.58	0.72	0.80
12												0.75	0.48	0.57	0.65	0.58	0.51	0.60
13													0.92	0.76	0.68	0.64	0.72	0.80
14														0.92	0.84	0.58	0.72	0.80
15															0.96	0.65	0.72	0.80
16																0.76	0.51	0.68
17																	0.92	0.76
18																		1.00

Table 18 All pairwise kappa agreement among 18 observers for the detection of DH on fundus photography.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	0.92	0.80	0.88	0.80	0.92	0.84	0.88	0.80	0.76	0.92	0.80	0.80	0.88	0.84	0.92	0.92	0.80	0.92
2		0.92	0.92	0.84	0.80	0.88	0.92	0.84	0.80	0.88	0.76	0.76	0.84	0.88	0.80	0.80	0.68	0.80
3			0.92	0.92	0.88	0.88	0.92	0.84	0.88	0.96	0.84	0.84	0.92	0.96	0.88	0.88	0.76	0.88
4				0.92	0.88	0.88	0.84	0.92	0.88	0.88	0.84	0.92	0.92	0.96	0.88	0.88	0.84	0.88
5					1.00	0.92	0.88	0.88	0.84	0.92	0.80	0.88	0.88	0.92	0.92	1.00	0.88	1.00
6						0.84	0.88	0.88	0.84	0.84	0.80	0.80	0.88	0.92	0.84	0.92	0.80	0.92
7							0.76	0.76	0.80	0.88	0.84	0.76	0.84	0.88	0.88	0.88	0.76	0.88
8								0.92	0.80	0.88	0.76	0.92	0.84	0.88	0.88	0.88	0.84	0.88
9									0.68	0.84	0.80	0.80	0.88	0.92	0.76	0.84	0.72	0.84
10										1.00	0.80	0.88	0.88	0.92	0.92	0.92	0.80	0.92
11											0.67	0.76	0.76	0.80	0.88	0.80	0.76	0.80
12												0.75	0.84	0.88	0.88	0.88	0.84	0.88
13													0.84	0.96	0.80	0.88	0.76	0.88
14														0.92	0.84	0.92	0.80	0.92
15															0.92	0.92	0.88	0.92
16																1.00	0.88	1.00
17																	0.68	0.88
18																		0.84

The area under the ROC curves (AUC) among the 18 observers (Figure 20) ranged from 0.80 to 0.96 for HRT (pooled average of 0.87; 95%CI 0.82- 0.93) and from 0.89 to 1.00 for fundus photography (pooled average 0.95; 95%CI 0.92-0.98). When the average AUC (Figure 21) of both techniques was compared, there was a marginal, but significant, difference of 0.08 (p=0.04, CI 95% 0.00-0.15). However, when the AUC of each observer was compared between HRT and fundus photography, it was significant in only one observer with a difference in the AUC between HRT and fundus photography of 0.2 (p=0.02). The significant difference was driven by a single observer's performance. However, if a more clinically relevant range of sensitivity is used (75% to 100%) the difference in the accuracy between techniques gets wider. The partial AUC for a fixed sensitivity of 0.75-1.0 (pAUC) for HRT was 0.78 compared to 0.90 for fundus photography.

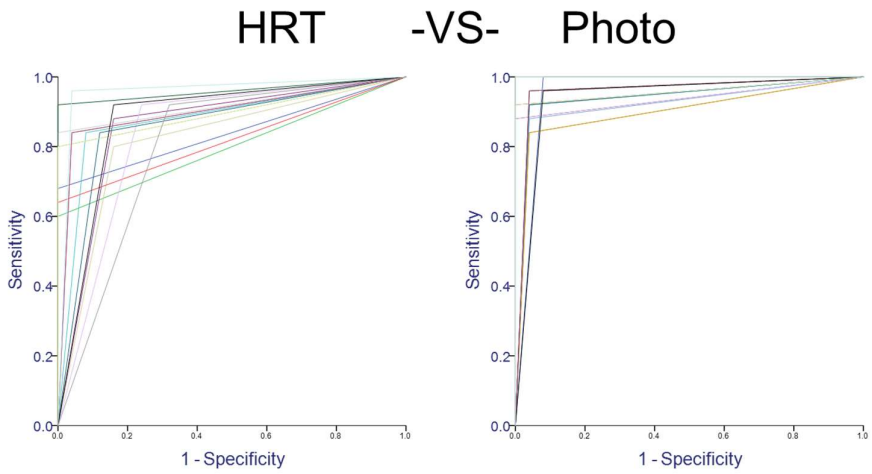

Figure 20 Area under ROC curves of the 18 observers for HRT (left) and fundus photography (right) to detect DH. Each line represents each of the 18 observers.

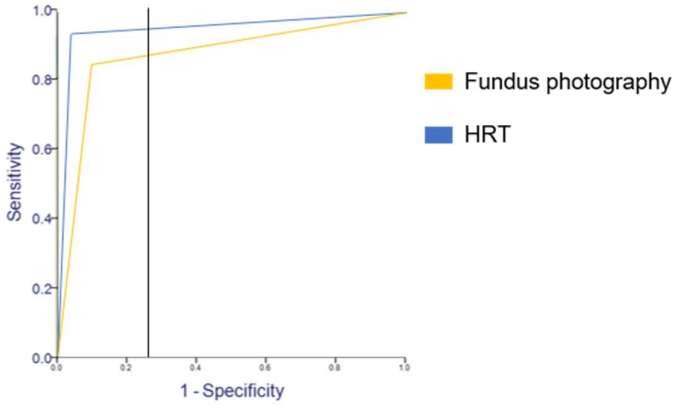

Figure 21 Pooled average of the area under the ROC curves for the 18 observers to detect DH using HRT or fundus photography.

The sensitivity and specificity were 84% and 92% for HRT, and 93% and 97% for fundus photography (Table 19).

Table 19 Diagnostic performance of HRT and fundus photography to detect the presence of DH.

	HRT	Fundus photography
Sensitivity	84%	93%
Specificity	92%	97%
Positive likelihood ratio	10.5	33.3
Negative likelihood ratio	0.17	0.07
Positive predictive value	0.92	0.97
Negative predictive value	0.86	0.94
Accuracy	88%	95%

The positive likelihood ratio was 10.5 for HRT and 33.3 for fundus photography. Figure 22 and Figure 23 show Fagan's nomograms with a pre-test probability of 50% as presented to the observers and 10% that would be more similar to real-life clinical practice and the complete study sample of the UKGTS that presented a prevalence of DH+ per visit of 9.1%.

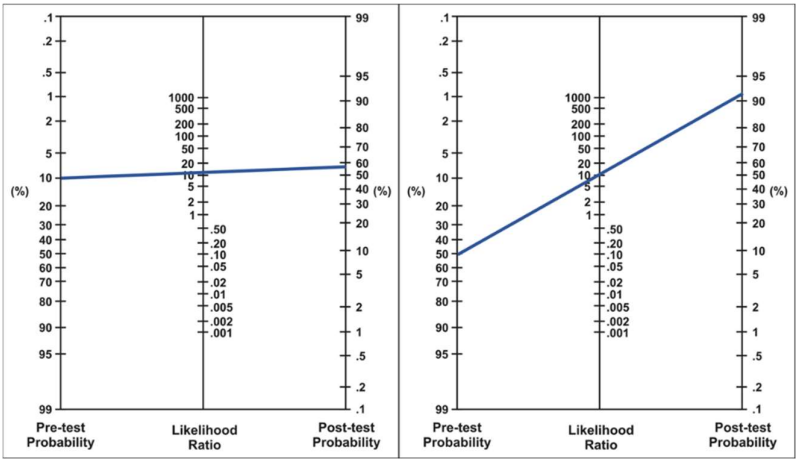

Figure 22 Positive likelihood ratio for HRT detection of the presence of DH (right with 10% pre-test probability and left with 50%).

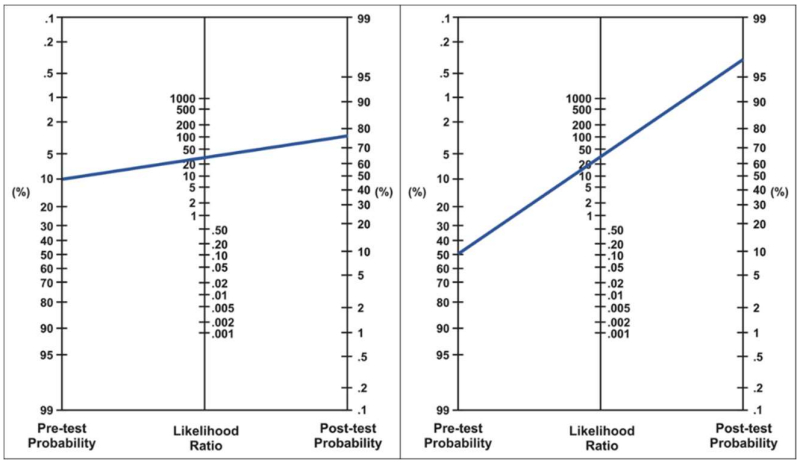

Figure 23 Positive likelihood ratio for fundus photography detection of the presence of DH (right with 10% pre-test probability and left with 50%).

3.2.5 Discussion

This multi-observer, multicentre study confirmed the potential for identifying DHs on flickering HRT images. The within-observer kappa agreement for the eighteen observers was 0.87 and 0.86 for HRT and fundus photography, respectively. The between-observer kappa agreement was 0.69 and 0.86 for HRT and fundus photography, respectively; it was slightly, but significantly, better for fundus photography. In addition, the difference between the AUC for HRT and fundus photography detection of the presence of DHs showed better accuracy using fundus photography (0.08 greater AUC favouring photography, CI 95% 0.00-0.15; p=0.04).

All observers had previous training to assess fundus photography to identify glaucomatous signs such as DHs, but they were not trained to use the reflectivity image of the HRT to detect DHs. Despite this discrepancy in training, the detection based on HRT seems to be a repeatable method that reported almost the same within-observer agreement compared to that for fundus photography. An important aspect of any new method to aid diagnosis is its repeatability. The within kappa agreement was 0.87 and 0.86 for HRT and fundus photography which is arguably considered an almost perfect agreement (36), and it was a promising result that should motivate more research on the possible clinical application of

this method. The slightly lower accuracy and between-observer agreement of the HRT-based detection of DHs compared to fundus photography-based detection could be related to the lack of experience to detect DHs using HRT images. When the results of each observer were compared for HRT and fundus photography, only one had significantly lower accuracy and agreement using HRT. It might be possible that this observer came from a country where the use of HRT was not widespread and he may have been completely unfamiliar with the HRT images. If the best performing observers of each technique are compared, both methods of DH detection had very similar results.

The kappa agreement in detecting DHs has been published previously for different types of images and observers (Table 12). The within-observer agreement ranged from between 0.60 and 0.77 (13). Considering the different methodologies of these previous publications, they are not very different from our results of 0.87 and 0.86 for HRT and fundus photography. The between-observer kappa agreement in previous publications ranged from 0.35 (13) to 1.0 (12). These very wide ranges of results could be explained by the heterogenicity of methodologies. It would be better to compare our results to the study by Chee et al. (18) which used a flickering technique, had a similar design and had glaucoma specialists as observers. Chee et al. reported a kappa agreement of 0.70 between two observers, which is consistent with the current study of 0.86 for fundus photography and 0.69 for HRT. Our agreement results based on HRT images cannot be compared with other publications that used HRT because the only previous publications did not use a flicker technique, had a previous version of the HRT and did not evaluate agreement (23, 24). The factors associated with a lower agreement between observers were as expected, size, and angular extent of the DHs. Despite that, eyes with small DHs and angular extent had agreement that ranged from ‘moderate’ to ‘almost perfect’ and was at least ‘substantial’ for all the observers.

The pooled AUC to detect DHs was 0.87 and 0.95 for HRT and fundus photography. Commonly used standards to judge how good a diagnostic instrument can perform would consider both HRT and fundus photography as good or excellent test instruments. However, these results may overestimate performance because the sample of eyes that the observers assessed had a DH

50% of the time, although the observers were unaware of the proportion of eyes that would have a DH. Previous publications have evaluated the diagnostic ability of different types of fundus images to detect glaucoma and some of the optic disc characteristics. Syed et al. reported a sensitivity of 88%, 71%, and 76% for detecting the presence of DHs using 2D flickering photos, 2D side by side photos, and 2D single photos (11). Syed et al. results were similar to the present results from the UKGTS of 84% and 93% pooled sensitivity for the 18 observers using HRT and fundus photography, respectively. It is possible that, in real-life clinical practice, the diagnostic performance of these instruments would be worse due to the much lower prevalence of DHs (1.9% to 29.4%, Table 4). Fagan's nomogram employing likelihood ratios is a better method to represent the impact that the pre-test probability has on the post-test probability. For instance, if the pre-test prevalence of DHs is 10% and a DH is detected by HRT or fundus photography, the post-test probability will go up to around 60% and 75% respectively. Very different if the pre-test prevalence of DHs would be 50% (as presented to the observers), the post-test probability would go up to over 90% and 95% for HRT and fundus photography. Considering the very low sensitivity of clinical examination (20% (3) to 71% (4) following clinical trial protocols that ask specifically for DHs) and the harmless nature of the detection of a probable DH that can be later confirmed or ruled-out with a careful clinical examination, the use of an SLO-based imaging method to detect DHs seems like a very reasonable alternative. An instrument that has the potential to detect DHs with a sensitivity similar to the current study of 84% would have a positive clinical impact especially for busy glaucoma clinics that no longer have fundus photography as part of their regular clinical visits but do have regular imaging with HRT or OCT instruments that have an SLO, or similar, image included.

The SLO technology that has been incorporated for tracking purposes (25) by some of the most commonly used OCT instruments could be a good source of data to detect probable DHs in patients who routinely visit glaucoma clinics and have their eyes only scanned with an OCT; this could improve the real-life detection rate of DHs in glaucoma clinics without the requirement of extra equipment such as a fundus camera or a combined OCT with a fundus camera. Current work is exploring the automatization of the detection technique to reduce the variability seen between observers and obtain results similar to the best

performing observers. It is interesting to notice that if automation of the detection of DHs gets closer to the best performing observers, the results would be very close to, if not the same as, fundus photography which is the current gold standard. Traditional imaging analysis techniques or artificial intelligence could be implemented for the automation of detection. Among the artificial intelligence technologies, deep learning has recently been widely researched to detect other eye diseases, including conditions that require the identification of haemorrhages in the retina, such as diabetic retinopathy (37). There are even commercially available instruments that use this technology to automate the diagnosis of DR using multiple features of the fundus photography including the haemorrhages in the retina (38) (IDx-DR, IDx Technologies Inc).

It is possible that the diagnostic performance of observers to detect DHs with the HRT will be different when applied to the SLO embedded in the OCT of different manufacturers. Differences in the optics, A-scan density, postprocessing algorithms or the laser wavelength could affect positively or negatively the subsequent diagnostic performance. For instance, the SLO for the HRT is 670nm, which is similar to, but not the same as, the same manufacturer's (Heidelberg Engineering, Heidelberg, Germany) Spectralis OCT that uses an SLO of 840nm. Studies that evaluate the diagnostic performance of each instrument will be required. However, it is possible that newer instruments with better post-processing capacity will be able to better detect the blood in and around the optic nerve. Figure 24 is an example of the ability to detect more clearly a DH in the SLO image obtained with an OCT/SLO Spectralis scan.

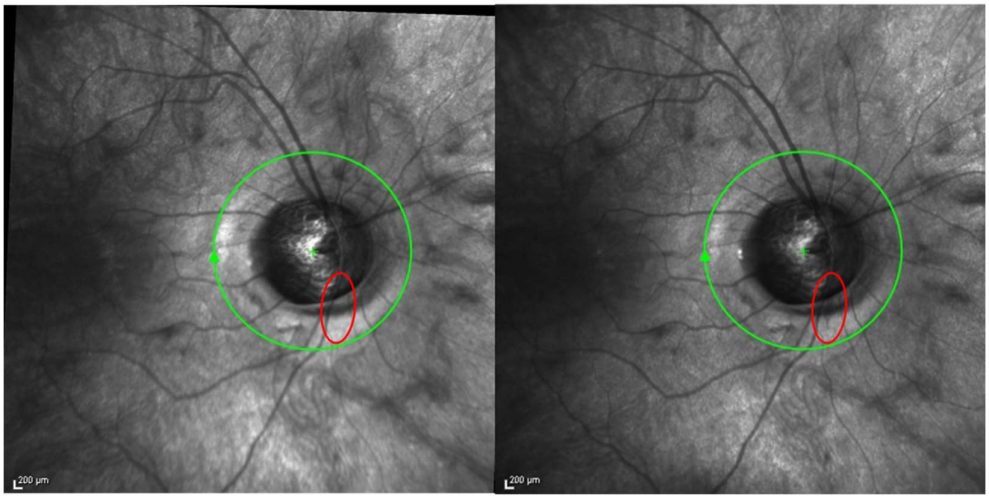

Figure 24 SLO image acquired with Spectralis OCT on the same patients in two visits. Left with no DH and right with a DH (DH inside the red oval).

Another important limitation with the HRT-based method to detect DHs is the inability to detect DHs that are only located in the cup of the disc. However, DHs that are present only inside the cup margins represent the minority of the DHs as reported by Yoo et al. in a Korean population, in which only 8% of all DHs extended to the cup base (39).In addition, newer SLOs that combine different wavelengths might be able to resolve the cup's characteristics better. A limitation with the set of fundus photographs and HRT images used for this study is that the technicians acquiring them for the UKGTS were more familiar with the use of HRT than with the acquisition of good quality fundus photos. In addition, a good quality HRT (mean pixel height standard deviation (MPHSD) 40 μm or less) was an inclusion criterion to participate in the UKGTS while there was no inclusion criterion for fundus photography quality. Therefore, all patients included had good HRT images while some might have had bad fundus photos since baseline. These factors may have resulted in fundus photographs with a lower quality compared to the HRT images. The lower quality of fundus photographs may have made it more difficult to detect some DHs, such as very small DHs or DHs only located in the cup.

The difference in the quality of the images might have caused a relative overestimation of the ability of HRT to detect DHs because the DHs that are

harder to detect by HRT were less common in the study sample of images compared to other populations. However, this might better represent real-life clinical practice in which the acquisition of good quality fundus photos is more challenging to technicians compared to other imaging modalities such as HRT or OCT. Finally, it could be argued that the glaucoma specialist does not represent the majority of eye care professionals who diagnose and manage most of the patients with glaucoma worldwide and that our results might be worse for other eye care professionals, as has been previously shown for trainees and optometrists (11, 13). However, if this technology is translated into clinical use, it will probably be automated to aid any clinician to focus his/her attention to a location of the disc with a likely DH.

To conclude, the hypothesis that DHs can be detected on SLO images as well as in fundus photography with the flickering technique was not supported when human observers were required to identify the DHs. However, the diagnostic performance of HRT-based detection of DHs was very close to the gold standard of fundus photography and could significantly improve the detection rate in clinical practice without the requirement for additional equipment.

*Part of this work was accepted as a paper presentation in the IMAGE 2016 and 2017 meetings of the EGS, as a poster presentation in ARVO 2017 (40) and as a paper presentation in the 2018 EGS Congress (41).

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Chapter 3 The effect of implementing an imaging-based method for disc haemorrhages detection in routine clinical practice.

3.3.1 Abstract

Purpose: To evaluate the effect of implementing an imaging-based method for DHs detection in routine clinical examinations.

Methods: The notes from all consecutive patients who underwent trabeculectomy between May 2007 and September 2013 in the normal tension glaucoma (NTG) clinic at Moorfields eye hospital were included in a clinical audit. The clinical notes of all visits and Heidelberg retina tomograph (HRT) scans before and after trabeculectomy were retrospectively assessed and classified as DH+ or DH-; the HRT scans were assessed using a flickering method between baseline and each follow-up scan by an experienced observer masked to the DH status of the clinical notes.

Results: The 97 included patients had 3740 clinical examinations (7489 eye examinations) with a mean (SD) of 38.6 (12.2) visits during a follow-up time of 16.5 (5.2) years. In 26.6% of visits (994 visits with 1988 eye examinations), patients underwent an HRT scan and clinical examinations on the same day, and 123 (6.2%) eye examinations had a DH detected with either method. Among the 123 DH+ eye visits, the DH was detected by both methods in 32 (27.6%) visits, only by clinical examination in 7 (4.2%) visits, and only by HRT in 84 (72.5%) visits.

Conclusions: The implementation of a flickering method of HRT scans increased the detection of DHs. A non-photographic method to improve and speed up DH detection is possible using the SLO technology built in many modern OCT instruments.

3.3.2 Introduction

A disc haemorrhage (DH) is an important clinical sign that has been associated with visual field progression and has the potential to be used for risk stratification of glaucoma patients (1). A UK partnership collaboration between patients, carers, and health professionals elaborated research priorities using a survey process for different eye diseases. One of the top ten priorities for glaucoma was 'How can glaucoma patients with a higher risk to progress rapidly be detected?' (2). Multiple possible options have been considered to stratify the risk of progression, and genetic characteristics seem to be the most promising for the future. However, the early detection of patients with DHs could be implemented immediately because the instruments required are already available in the clinics at no extra cost to patients or the healthcare system.

The most difficult challenge to correctly categorise a patient as DH+ or DH- is the short time DHs are visible. Professor Kitazawa followed 70 patients every four weeks until a DH was identified and then weekly until it disappeared and concluded that DHs were visible for a mean of 10.6 weeks (3). Another challenge for DH detection is that if only clinical examination is used to detect a DH, up to 84% of cases confirmed by fundus photography are missed by clinical examination (4) under research clinical trial conditions. It would be expected that during routine clinical practice, without a standard protocol to follow, more DHs would be missed. Therefore, it would be desirable to avoid missing the opportunity to detect a DH by regularly observing glaucoma patients and acquiring images to improve the detection rate.

The first reported non-photographic method to detect DHs was with the SLO (HRT) (5). An adaptation of a flickering technique to the SLO scans of the HRT has been described in Chapter 2 as an alternative method to detect DHs with only slightly inferior results compared to fundus photography. However, the technique based on SLO reported in Chapter 2 has never been compared to routine clinical examinations. It was hypothesised that the SLO-based method for DH detection would identify more DHs in comparison to clinical examination in routine clinical practice.

3.3.3 Methodology

This study was approved in 2015 as a clinical audit (CA15/GL/12) by the Clinical Audit Assessment Committee from the NIHR Biomedical Research Centre for Ophthalmology, UCL Institute of Ophthalmology & Moorfields Eye Hospital, London, UK. The study was a re-audit that followed the same consecutive cohort of patients used to report in 2015 the outcomes of the Moorfields Safer Surgery technique of trabeculectomy in normal-tension glaucoma (NTG) patients (6). The original audit was approved in 2014 and focused on the surgical outcomes of trabeculectomy. The DH status based on clinical examination or HRT was not assessed for the original audit. The present re-audit looked at the effect of DHs in the same cohort of the original 2014 audit of patients that were followed over a long period in the NTG clinic and required a trabeculectomy in at least one eye. The NTG clinic was designed by Professor Roger Hitchings to provide tailored care to this specific group of patients and to provide new insight into this pathology. The patients seen in the NTG clinic had a standardised set of examinations that included regular visual field testing and HRT imaging, and diurnal IOP measurement. Patients were seen regularly over a long period by the same consultants (Deborah Kamal and Roger Hitchings) and treated medically and surgically with a similar approach over their clinical course. All the clinicians that collaborated in this clinic were under the direct supervision of these consultants.

The notes from all consecutive patients that underwent trabeculectomy by Miss Deborah Kamal between May 2007 and September 2013 were included in the original audit. In the re-audit, three patients were excluded due to incomplete data. During routine clinical practice, clinicians were encouraged and asked to report the presence or absence of DHs, but it was left at their discretion to report it in the notes. For the re-audit, all clinical information from both eyes of all clinical examinations during the complete follow-up of all patients were categorised depending on the description of the fundus and DHs (fundus not described, fundus described but with no details of DHs, and fundus described with details about DHs), the acquisition of HRT (acquired or not), and the DH status (DH+, DH-, and DH clock hour location). The following postoperative visits were excluded from the analysis because these appointments were focused on the

postoperative care more than the regular evaluation of glaucoma: the first clinical examination after any laser procedure, the first month after cataract surgery, and the first three months after trabeculectomy. Between 2017-2018 the SLO images acquired with HRT3 (Heidelberg Engineering, Germany; version 3.0.60) on the same day as clinical examinations were retrospectively assessed by a single observer masked to trabeculectomy outcomes, clinical notes, and the DH status reported by clinicians. The HRT reflectance images were analysed with Heyex (version 1.6.2.0) which automatically aligns all the images and offers the option to flicker the baseline with the follow-up scans (the technique was extensively described in Chapter 2). For each image, the DH presence or absence and location were reported.

The clinical notes of patients in whom the DHs were first detected by HRT were reviewed to assess the effect of delaying the identification of the DH. The time between the detection of DHs by HRT and the detection by clinical examination was measured, and the clinical decision after the DH was finally identified by clinical examination was reported. In the group of patients in whom DHs were only detected by HRT, the time between the DH detection by HRT and further glaucoma surgery was analysed.

All patients/eyes with a DH detected either by HRT or clinical examination were considered DH+. The diagnostic performance of HRT and clinical examination were compared. Sensitivity, specificity, predictive values, likelihood ratios, and accuracy were calculated with Medcalc (Ostend, Belgium). All the remaining statistical analyses were performed with SPSS (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.)

3.3.4 Results

The surgical diaries reported 134 consecutive eyes of 101 patients from the NTG clinic that underwent trabeculectomy between May 2007 and September 2013. Three patients were excluded from the initial audit, one due to incomplete data and the other two because all follow-up after trabeculectomy was outside the UK. During the re-audit, one patient was identified as having secondary glaucoma,

and two eyes were identified as duplicated. For the present DH analysis, both eyes of the 97 included patients were investigated (Figure 25).

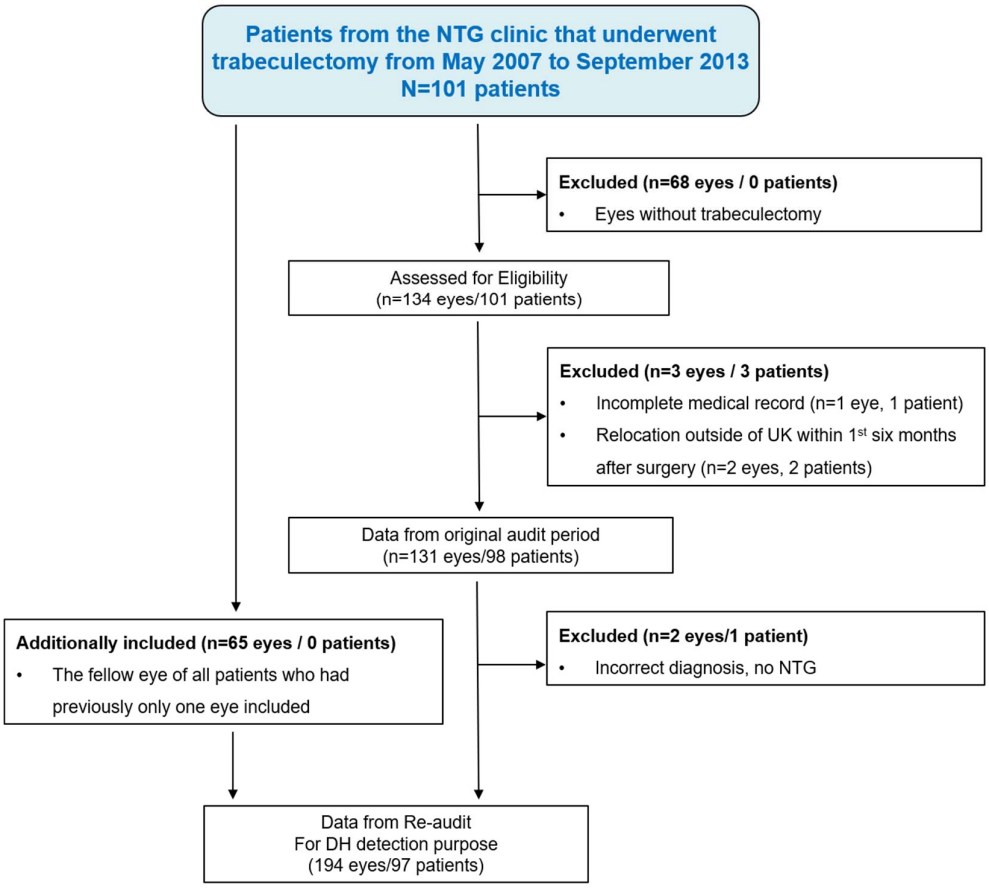

Figure 25 Flowchart of patients included in the detection of DHs analysis.

The 97 included patients had 3740 clinical examinations with data from both eyes reported (7480 eye examinations). In 994 visits (26.6%), the patients underwent HRT imaging and clinical examinations in both eyes on the same day. The mean (SD) number of visits per patient over the complete follow-up was 38.6 (12.2) visits during a follow-up time of 16.5 (5.2) years. Among the 7480 eye clinical examinations, 270 (3.6%) had no description of any characteristic of the optic nerve or the presence/absence of DHs, in 5508 (73.6%) some characteristics of the optic nerve were described, but the presence/absence of DHs was not described, and in 1702 (22.8%) the presence/absence of DHs was described. Over the complete follow-up period, 101 eyes (52.1%) of 69 patients (71.1%) had a DH detected in at least one clinical or HRT examination (see Figure 26). During

routine clinical examination, 77 eyes (39.7%) of 56 patients (57.7%) had at least one visit with a DH. The detection of DHs based on HRT identified at least one visit with a DH in 66 eyes (34.0%) of 49 patients (50.5%).

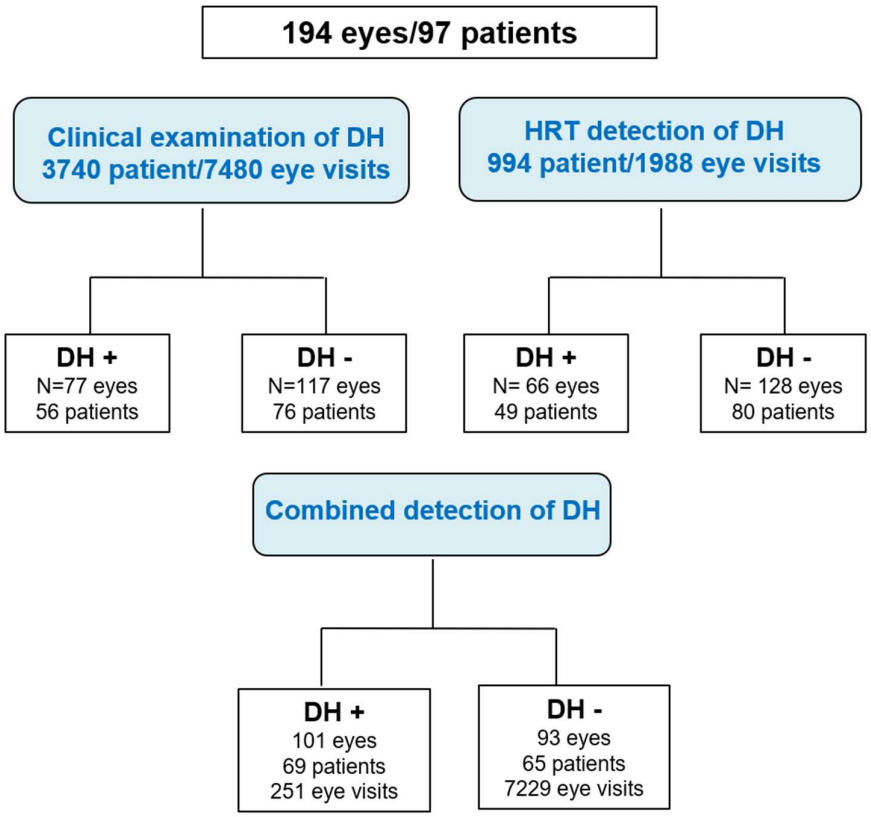

Figure 26 Distribution of DH+ and DH- patients, eyes, and visits across all follow-up.

Among the 7480 eye examinations, 251 (3.4%) had a DH identified by either method, 32 (0.4%) by HRT and clinical examination simultaneously, 84 (1.1%) only by HRT, and 135 (1.8%) only by clinical examination. However, in many of the days that patients had a clinical examination, an HRT was not acquired. Of the 994 visits (1988 eye visits) with both HRT imaging and clinical examination, 123 eye examinations (6.2%) had a DH identified by either method, 39 (2.0%) by clinical examination and 116 (5.8%) by HRT. Only 7 (0.4%) were detected by fundus examination alone, 32 (1.6%) by HRT and clinical examination simultaneously, and 84 (4.2%) only by HRT (Figure 27).

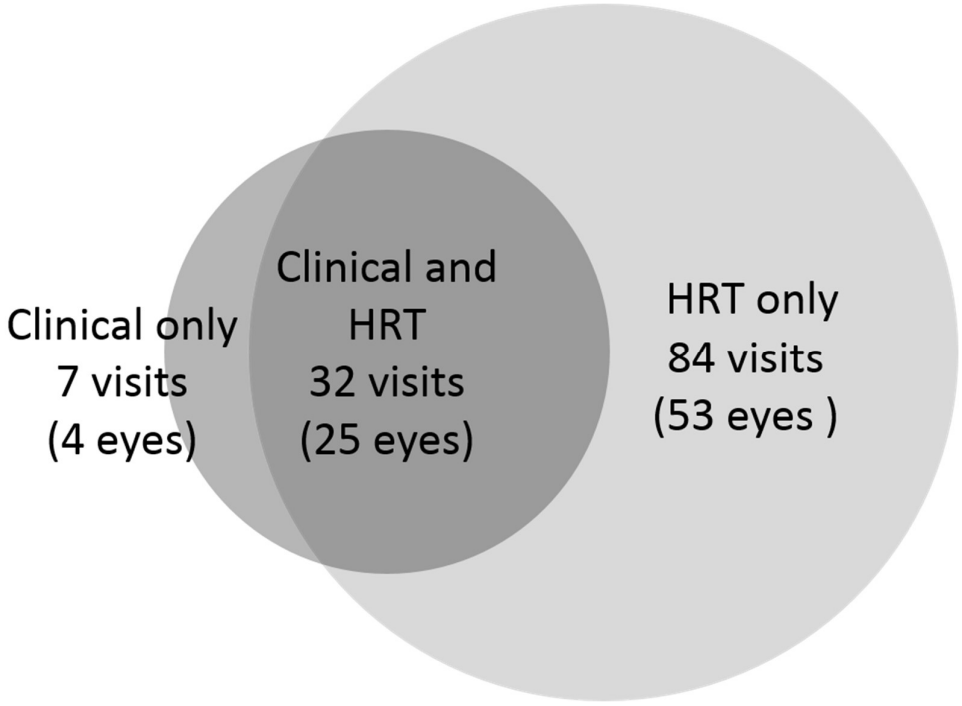

Figure 27 Venn diagram of DH detection methods during the visits in which patients had an HRT acquired on the same day as the clinical examination.

The number of eye visits with a missed diagnosis by clinical examination (DHs only detected in HRT images) was 2 (0.7%) when the fundus was not described, 63 (1.1%) when the fundus was described but with no comment about DHs, and 21 (1.2%) when the fundus was described with a comment about DHs. The Fisher's exact test p-value was 0.602 when the latter two groups were compared.

Table 20 contains the diagnostic performance of HRT and clinical examinations. For this analysis, only the 1988 visits in which patients had HRT imaging and clinical examination on the same day were included. A DH detected with either HRT, clinical examination or both was used as the reference standard. There was a statistically significant difference in the diagnostic performance of HRT compared to routine clinical examination that is depicted with the ROC curve of Figure 28. The AUC was 0.97 (95% CI 0.95 - 1.00; p=<0.001) for HRT and 0.66 (95% CI 0.60 - 0.72; p=<0.001) for the routine clinical examination.

Table 20 Diagnostic performance of HRT and clinical examination to detect the presence of DH in a real-world glaucoma clinic.

	HRT (95% CI)	Clinical Examination (95% CI)
Sensitivity	94.3% 88.6% to 97.7%	31.7% 23.6% to 40.7%
Negative likelihood ratio	0.06 0.03 to 0.12	0.68 0.61 to 0.77
Negative predictive value	99.6 % 99.2% to 99.8%	95.7 % 95.2% to 96.2%
Accuracy	99.7%	95.8% 94.8% to 96.6%

Specificity, positive likelihood ratio and positive predictive value were not reported because these results were almost perfect due to the DH definition that included all DHs described by either method.

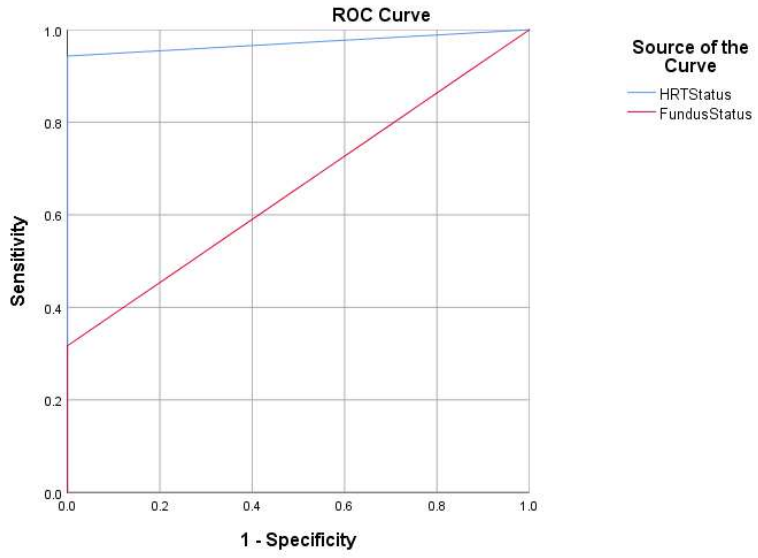

Figure 28 ROC curves of HRT and clinical examination to detect the presence of DH in a real-world glaucoma clinic.

The 84 visits (68% of all DH+ visits) in which HRT only detected the DHs (Figure 27) occurred in 53 eyes. Among these 53 eyes, ten had a DH identified by clinical examination later in the follow-up, but before the eye underwent a trabeculectomy, 33 later in the follow-up, but after the eye underwent a

trabeculectomy, and ten were never identified as DH+ by clinical examination. There was a median (IQR) delay between the HRT and clinical classification of eyes as DH+ of 2.2 years (0.4-3.3). In these patients, when the DH was finally detected clinically, the clinician decided to shorten the follow-up or escalate medical treatment in all the cases. The median (IQR) time between HRT detection of DHs and subsequent glaucoma surgery was 2.9 years (1.9-3.9).

3.3.5 Discussion

The implementation of an SLO-based imaging method to identify DHs increased the detection rate from 2.0% of visits with clinical examination alone to 5.8% with the HRT SLO. The sensitivity to detect DHs was 94.3% (95% CI 88.6% to 97.7%) for HRT and 31.7% (95% CI 23.6% to 40.7%) for clinical examination. The incorrect classification of patients or eyes as DH-, or the delay in detecting DHs in some of these eyes, may have influenced clinical decisions such as the escalation of treatment or recommendation of surgery.

Methods to improve DH detection have mainly focused on expanding the implementation of fundus photography (7) and optimising detection with consecutive photographs and flickering techniques (Table 12). However, the current trend in glaucoma imaging shows a tendency toward more OCT-based imaging and less fundus photography. The SLO images used to detect DHs in the current study were acquired with HRT, which, however, despite being the first non-photographic imaging instrument commercially available for glaucoma management, is no longer sold by the company for glaucoma purposes. However, the SLO technology used by the HRT is still built in many of the modern OCT instruments to optimise the images and help in the eye-tracking system (8).

Routine clinical examination by experienced glaucoma specialists missed 68% of the DHs; this is similar to the 84% missed in the Ocular Hypertension Treatment Study (OHTS) (4), 80% missed in the European Glaucoma Prevention Study (EGPS) (9), and higher than the 29% missed in the Early Manifest Glaucoma Trial (EMGT) (10). For clinical examination, there was no difference in the DH detection rate between visits in which the fundus was described or not described, described without mention of DHs, or described with the absence of DHs. Furthermore, the

percentage of visits with a DH missed by clinical examination was not significantly different between visits in which the presence or absence of a DH was described compared to visits in which only other characteristics of the optic nerve were described. Although a careful clinical examination and reporting of the presence/absence of a DH have been advocated to increase DH detection skills (7), the current study could not identify a benefit from describing the presence or absence of a DH. Electronic patient records could be tailored to include automated alarm systems that remind the clinician to describe specific characteristics of the examination, such as the presence or absence of DHs. Although these tools could be easily implemented in current electronic patient records, the present study did not identify a benefit in reporting the presence or absence of DHs. However, in the present study, the description of DHs' presence/absence was not randomized. It is possible that a randomised clinical trial that assessed the effect of an alarm system on the detection rate of DHs could identify a benefit from this strategy.

An alternative strategy to improve the detection rate of DHs could be the education of clinicians. A recent clinical trial randomized orthoptists to no extra training or an online educational programme to increase knowledge of features of the optic nerve in glaucoma (11). The orthoptists who received further training were more accurate during the optic disc photograph assessment, and there was a significant difference in the number of DHs detected. However, in routine clinical practice, most patients with glaucoma are seen by ophthalmologists or optometrists who are already experienced in detecting DHs. The benefit obtained by an educational programme that targeted orthoptists could not be easily extrapolated to routine clinical examinations by more experienced clinicians who are already experienced in detecting DHs.

The misclassification of an eye during multiple follow-up visits as DH- was detected in 53 eyes (84 visits) and this could have affected clinical management by incorrectly encouraging the clinicians to lower the patient's risk profile. In 43 eyes that later had a DH detected by clinical examination, the clinician changed the management every time a DH was detected, and the delay between HRT detection and clinical detection had a median (IQR) of 2.2 (0.4-3.3) years. Another group of patients (10) were wrongly classified as DH- and the clinician never re-

classified them as DH+; this group had a glaucoma surgery a median (IQR) of 2.9 (1.9-3.9) years later. It might be possible that patients who were wrongly classified as DH- could have had an escalation of medical treatment or a recommendation to proceed to surgery earlier if the DH status had been classified correctly the first time a DH was present.

An interesting finding from this cohort of NTG patients with long follow-up (mean 16.5 years) is the high cumulative incidence of eyes and patients with at least one visit with a DH. In 101 eyes (52.1%) of 69 patients (71.1%), a DH was detected in at least one clinical or HRT examination. The EMGT reported that, after a median follow-up of eight years, a DH was identified in approximately 55% of patients by clinical examination and fundus photography (12). Professor Kitazawa followed a group of NTG patients only with clinical examination and identified a DH in 20.5% of patients (3). The higher detection of DH+ patients in the current study is likely due to the implementation of an image-based method of detection that was not used in Kitazawa's publication and the long follow-up double that of the follow-up in the EMGT. However, even with regular examinations and the use of imaging techniques, the current cohort confirmed previous observations that DHs are not a universal characteristic of NTG patients but an independent biomarker present in some glaucoma patients that could represent a different phenotype of glaucoma (13).

The absence of fundus photography as the reference standard to compare the diagnostic performance of HRT and clinical examination is an important limitation of the present study. However, as extensively described in Chapter 2, our group had previously reported good performance of the HRT method to detect DHs (14). A related limitation is the pragmatic definition of the reference standard of DH+ based on the detection with HRT, clinical examination or both; these problems make it impossible to correctly analyse the test's specificity, positive likelihood ratio, and positive predictive value. Therefore, these results were not presented in Table 20. However, it is still valuable to highlight the differences in the negative likelihood ratios between HRT (0.06) and clinical examination (0.68). Despite not being a perfect diagnostic tool, HRT detection can significantly reduce the post-test probability of finding a DH when the HRT did not identify a DH compared to clinical examination. A limitation of the routine clinical examination reported in this

study is that the description of the presence/absence of DH was left to the clinicians' discretion. Although only two consultants supervised this clinic, many other fellows, optometrists and other clinicians assisted in the management of the patients. The use of data from routine clinical examinations over a long follow-up period reduced the standardization of the examinations, but it may better represent real-life clinical practice with multiple clinicians.

The results of the present study are difficult to be generalised to other populations because almost 70% of patients were white, and the frequency of DHs has been reported to be influenced by ethnicity (15). In addition, other patients with NTG might differ from the patients that attended the NTG clinic because it was a clinic in which patients with a more extreme phenotype of NTG weres referred for a more experienced examination in a tertiary eye care centre.

Finally, it is important to highlight that the HRT's SLO uses a laser of 670nm wavelength, which is different to the same manufacturer's (Heidelberg Engineering, Germany) Spectralis OCT which uses an SLO of 840nm. Future studies will have to assess the diagnostic ability to detect DHs of the SLO built into each of the commercially available OCT instruments. The widespread use of imaging in glaucoma and the experience gained with SLO technology make it promising to utilize them for DH detection. The detection of a possible DH could be part of the automatic measurements that are reported after a routine OCT scan. An OCT report that guides clinicians to a clock hour of the disc with a possible DH could significantly improve the capacity of clinicians to detect DHs and adjust clinical management at an earlier time.

To conclude, an SLO-based method to detect DHs using HRT images compared to routine clinical examination revealed a large number of undiagnosed DH visits. These patients could have benefited from earlier medical or surgical intervention if DHs had been detected earlier. The implementation of a non-photographic method to detect DHs is possible using the SLO technology built in many modern OCT instruments and could improve and speed up DH detection.

*Part of this work was presented in the World Glaucoma Congress 2019 and it is accessible as an abstract (16).

3.3.6 Bibliography

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Chapter 4 Risk factors for disc haemorrhages in the United Kingdom glaucoma treatment study (UKGTS).

3.4.1 Abstract

Purpose: To investigate risk factors associated with disc haemorrhages (DH) in the UKGTS.

Methods: Patients with newly diagnosed open angle glaucoma were included in the UKGTS for 11 visits during 24 months of follow-up. During the visits, the following were acquired: comorbidities, use of systemic drugs, questionnaires of medical conditions (migraine, Raynauds, claudication), laboratory test, visual field test, imaging scans (fundus photography, Heidelberg retina tomograph (HRT) and others) and different types of tonometry (Goldmann, ocular response analyzer and dynamic contour tonometer). Masked to treatment allocation and participant characteristics and using a flickering method between baseline and each follow-up scan, patients were classified as DH+ if a DH was identified in any eye at any visit. The frequency of DHs (percentage of DH+ visits among all visits with imaging) and the presence of bilateral DH+ (DHs identified in both eyes at the same or different visits) were analysed in DH+ participants. Uni- and multi-variable regressions were constructed to predict DH+ participants, bilateral DH+ participants, and percentage of DH+ visits.

Results: Among the 516 participants, 121 (26.2%) were DH+ and 18 (3.9%) bilateral DH+. Among the 121 DH+, the median (IQR) percentage of DH+ visits was 20 (12.5-44.0) %. In a multivariable analysis, female gender (OR 1.85, 95% CI 1.10-3.12; p=0.020), age (OR 1.04 per extra year, 95% CI 1.01-1.07; p=0.009), tea or coffee consumption (OR 1.16 per extra cup per week, 95% CI 1.05-1.28; p=0.003), and use of selective serotonin reuptake inhibitors (SSRI) (OR 3.66, 95% CI 1.00-13.35; p=0.049) were associated with higher risk of DH+, while use of calcium channel blockers (CCB) (OR 0.27, 95% CI 0.10-0.75; p=0.012), and refraction (OR 0.90 per + dioptre, 95% CI 0.80-0.98; p=0.013) were associated with lower risk. Aspirin use was associated with higher risk of bilateral DHs (OR 7.06, 95% CI 1.50-33.23; p=0.013). Current or previous smoking (β = -0.16, 95%

CI -0.27 to -0.06; p=0.003) and Raynaud's (β = -0.15, 95% CI -0.3 to -0.3; p=0.044) were associated with a lower percentage of DH+ visits.

Conclusions: The consumption of tea or coffee and the use of SSRIs have, for the first time, been found to be associated with DH occurrence. Variables that affect the risk to develop a DH over the study period differ from the variables associated with the frequency of DH+ visits or with bilateral DHs.

3.4.2 Introduction

The presence of a DH is an important clinical sign that is very often used by clinicians to guide clinical management (Chapter 1). The strong relationship between DHs and visual field progression makes them a useful biomarker to predict progression. In addition, animal models investigating microbleeds in the brain identified that blood in contact with neural tissue has been shown to induce glial activation and direct compression to the surrounding tissues (1). The glial activation in animal models of microbleeds extends to the limits of the area affected by the microbleed, with larger bleeding inducing a larger area of astrocyte activation (2). It is biologically plausible that DHs also have a direct deleterious effect on the optic nerve and surrounding tissue. If a contributing role of DHs in glaucomatous pathogenesis is later confirmed, then DHs could also be considered as a potential therapeutic target. It would be desirable to identify strategies that reduce the duration, number, frequency, and size of DHs. A reduction in the duration could reduce the magnitude of the deleterious effect of the blood in the RNFL, optic nerve microarchitecture and surrounding tissues. A reduction of the frequency of DHs over time would reduce the repetitive deleterious effect of DHs and its magnitude. A reduction in the size would diminish the amount of neural tissue affected. Even if the direct and negative effect of DHs in the optic nerve is not confirmed, a better understanding of the risk factors involved in the occurrence of DHs would be important because the association between DHs and visual field progression would continue to be valuable for clinicians to better stratify the risk of each patient to progress.

Multiple publications have explored ocular and systemic factors that increase the risk of the occurrence of DHs (3). However, the great variability in the duration of

visible DHs before they disappear make it very difficult to dichotomise patients as DH+ or DH-. Therefore, the best evidence available should be extracted from publications that systematically acquired images to identify DHs on a group of patients that were followed-up for a long period. Some glaucoma randomised clinical trials fulfil these characteristics and have published results on the risk factors for DHs (Table 21).

The Early Manifest Glaucoma Trial (EMGT) followed 255 patients for up to 11 years with fundus photography every six months and clinical examination every three months (4). A DH was identified in 55% of the participants in at least one eye and one visit with either clinical or photographic examination. The authors decided to analyse variables that predict the classification of a patient as DH+ twice. Firstly, for the participants who had a DH detected clinically and secondly, for the participants that had the DH detected by fundus photography. Gender was the variable most closely associated with DHs detected by fundus photography with a reduced risk in men, OR = 0.64; CI, 0.38–1.09; P=0.10. For DHs detected clinically, the following factors were associated with DHs: 1) OR = 0.74 per dioptre in the direction of hyperopia; CI, 0.63–0.88; P=0.0005, 2) Lower baseline IOP: OR = 0.92 per mmHg higher IOP; CI, 0.86–0.99; P=0.03, 3) Lower follow-up IOP: OR = 0.89 per mmHg higher IOP; CI, 0.82–0.97; P=0.01, 4) OR = 0.48 for male sex; CI, 0.26–0.90; P=0.022. The authors could not explain the discrepancy between the different factors associated with DHs detected clinically and photographically but mentioned that sex was the factor with the strongest association with both methods of identification. The investigators also analysed the frequency of DHs in the entire follow-up period. Between 57 and 67% of participants (67% clinically and 57% photographically) had more than two visits with a DH. A higher frequency of DH+ visits (percentage of DH+ visits) identified clinically or photographically was associated with lower baseline IOP (P=0.015), lower mean follow-up IOP (P=0.038), and current smoking (P=0.006).

The Collaborative Normal Tension Glaucoma Study group reported the identification of 23 patients with a DH at baseline among the 144 randomised participants (5). In a subsequent publication, they reported the “density of DHs” that referred to the number of DHs during the entire follow-up period divided between the total number of follow-up days (6). Unfortunately, the factors

associated with DHs at baseline or during the follow up ("density of DHs") were not reported. Interestingly, the authors decided to calculate the "density" accounting for follow-up days and not for the number of observations.

In the Low-pressure Glaucoma Treatment Study (LoGTS), 178 participants with NTG were randomised to either timolol or brimonidine (7). The participants were followed every four months clinically and with fundus photographs annually. Among the 178 participants, 127 completed a follow-up longer than 16 months and were included in the analysis of factors associated with DHs (8). The mean (SD) follow up was 40.6 months (12 months) and 15 patients (18 eyes) had a DH identified by either method. In the multivariable analysis, history of migraine, narrower neuroretinal rim at baseline, mean follow-up ocular perfusion pressure, and the use of systemic beta-blockers were significantly associated with a higher risk of developing a DH. There was no significant difference (p=0.33) between the number of eyes experiencing a DH between the brimonidine (5.2%) and timolol (8.6%) groups. However, in the multivariable analysis, randomisation to brimonidine approached significance (p=0.072) with a lower risk for DHs (Table 21).

The OHTS originally randomised 1636 participants with ocular hypertension between 24mmHg and 32mmHg to observation or treatment. The OHTS patients were followed-up every six months for clinical examination and annually for fundus photography (9). A more recent publication (10) reported factors associated with DHs on patients followed from 1994 to 2009. During this period, the median follow-up for the 1636 participants was 13 years. In the multivariable regression, older age, larger CDR, and higher IOP were associated with a higher risk of DH, while self-reported black race was associated with a lower risk. The authors did not analyse factors associated with multiple or bilateral DHs, or the frequency of DHs.

Table 21 Randomized clinical trials that reported risk factors for disc haemorrhages.

Study	Variable	Risk	(95% CI; p value)
EMGT (11) Presence*	Male	0.48	0.26-0.90; 0.022
	Refractive error in the direction of hyperopia	0.74	0.63-0.88; <.001
	Higher baseline IOP	0.92	0.86-0.99; 0.030
	Higher mean FU IOP	0.89	0.82-0.97; 0.010
EMGT (11) Frequency*	Smoking	NR	p= <0.000
	Lower baseline IOP	NR	p= 0.015
	Lower mean FU IOP	NR	p= 0.038
LoGTS (8)	Brimonidine	0.30	0.08-1.12; 0.072
	Systemic B-Blockers	5.59	1.12-27.78; 0.036
	Migraine	5.78	1.46-22.73; 0.012
	Lower mean systolic blood pressure	1.06	1.01-1.12; 0.020
	Narrower neuroretinal rim	2.91	1.01-8.40; 0.048
OHTS 8 years (12)	Older age	1.58	1.32-1.88; <.001
	Thicker central cornea	1.23	1.03-1.47; 0.024
	Larger Cup-disc-ratio	1.14	1.04-1.25; 0.005
	Greater VF PSD	2.51	1.32-4.78; 0.005
	History of heart disease	1.91	1.07-3.38; 0.028
	Family history glaucoma	1.59	1.07-2.38; 0.023
	History of smoking**	1.53	1.05-2.24; 0.027
OHTS 13 years (10)	Older age	1.39	1.20-1.61; <.001
	Larger Cup-disc-ratio	1.21	1.12-1.30; <.001
	Higher IOP	2.00	1.32-3.06; 0.001
	Black race	0.48	0.52-0.95; <.001

*Based on clinical examination.
** More than 100 cigarettes during life
NR = Not reported in the paper; only the p value was given.

Beyond randomised clinical trials, epidemiological studies (3) and other types of studies have identified interesting associations with DHs. Kim et al. (13), in NTG patients, identified systemic hypertension, wider IOP fluctuation, and the use of aspirin as risk factors for DHs. Poinoosawmy et al. (14) identified an association between abnormal glucose tolerance and DHs. Jonas et al. (15) identified an association between smaller neuroretinal rim and larger beta zone parapapillary atrophy (PPA) and DHs. In patients with unilateral DHs, a higher prevalence of PPA and a greater extent of the PPA has been associated with the eyes with DHs compared to the fellow eyes without DHs (16). The association between DHs and PPA is plausible considering that in the area closer to the optic disc of beta PPA, the choriocapillaris is closed; it could be possible that similar vascular mechanisms are involved in the pathogenesis of PPA and DHs.

Some systemic medications, such as calcium channel blockers (CCB), have been considered as treatment intervention complementary to IOP-lowering for some POAG patients in whom vascular dysregulation is considered to play an important role (17, 18). However, CCB and other systemic medications have also been associated with POAG. A recent database study identified, among all prescription drug classes, an association between presumed advanced or progressive glaucoma and the use of systemic beta-blockers and selective serotonin reuptake inhibitors (SSRIs) and CCBs (19). There are no similar studies with large sample sizes and hypothesis-independent designs that have identified associations between systemic medications and DHs.

The United Kingdom Glaucoma Treatment Study (UKGTS) was the first placebo randomised clinical trial that investigated VF deterioration in participants treated with a prostaglandin analogue. One of the secondary objectives of the UKGTS was to establish whether initial observation, rather than immediate treatment, was feasible for selected patients (20). To complete this objective, risk factors for VF progression have been identified, and the presence of DHs was identified as the most important risk factor (21, 22). The large number of ocular measurements, imaging modalities, and systemic variables that were collected to complete the secondary objectives of the study made the UKGTS database ideal to explore the association between these variables and the occurrence of DHs.

3.4.3 Methodology

The data used in this study of factors associated with DHs were acquired during the participant's visits while enrolled in the UKGTS. The 516 participants were enrolled between December 2006 and March 2010 for 11 visits over a 24-month period. The UKGTS followed the good clinical practice recommendations and the Declaration of Helsinki. The study was approved by Moorfields and Whittington Research Ethics Committee on June 1, 2006 (reference 09/H0721/56) and registered at the ISRCTN registry with the reference ISRCTN96423140. All patients signed written informed consent before the screening visit. An independent Data and Safety Monitoring Committee (DSMC) was appointed by the trial steering committee. Adverse events were monitored and reported to the operational DSMC at Moorfields Eye Hospital. Serious adverse events were

reported to the Medicines and Healthcare Products Regulatory Agency (20). The current study of factors associated with DHs in the UKGTS was a post-hoc exploratory analysis that was not part of the original primary or secondary objectives. The sample size of the UKGTS was calculated only for the primary objective and not for the current analysis of the data. However, most of the previous randomised clinical trials in glaucoma have similarly explored the factors associated with DHs due to the strong effect that DHs have on VF progression (Table 21). A post-hoc analysis of the power achieved in the UKGTS was performed with G*Power (version 3.1.9.7) for logistic regressions assuming a normal distribution and given an alpha of 0.05 and a sample size of 460 participants; a power over 0.8 was achieved for odd ratios below 0.75 or over 1.3 (23).

For the present study, DHs were identified based on imaging (SLO and photography) only and not based on the clinical examination. Participants were scheduled to have HRT images and fundus photographs acquired on all visits. An HRT image was acquired on 3,913 (94.2%) of all 4152 study visits; fundus photographs were obtained in 77% of the patient's visits. A patient was classified as DH+ if a DH was identified in any eye at any visit. The DH+ patients were further classified as bilateral DH+ if a DH was identified in both eyes at the same or at different visits. The frequency of DHs was defined as the percentage of DH+ visits among all visits when images were acquired. All possible classification options for UKGTS participants based on the detection of DHs are exemplified in Table 22.

Table 22 Classification options for UKGTS participants depending on disc haemorrhage status.

Study	Variable
No DH	No DH identified in either eye at any visit
Unilateral DH+	DHs detected in only one eye during one or multiple visits
Bilateral DH+	DHs detected in both eyes at the same or different visits
***Simultaneous Bilateral DH+**	DHs detected in both eyes at the same time in at least one visit
***Non-Simultaneous Bilateral DH+**	DHs detected in both eyes but at different visits

*These are subsets of bilateral DH+

All variables investigated as possibly associated with DHs were acquired during the trial visits following a predefined protocol (20). When a variable was measured per eye (such as IOP or axial length), the protocol of the UKGTS specified that the data from the eligible eye with the worst VF MD would be included for analysis. The following variables (measured per eye) were acquired and used for the present analysis; the unit of measurement and details about the methodology are in parenthesis:

1. Iris colour (continuous variable with a darker colouration with each increment from one. 1=blue, 2= blue/green, 3= hazel, 4= light brown, and 5= dark brown)
2. Blue iris colour (yes or no)
3. Number of visits with HRT acquired (visits)
4. Goldmann applanation tonometry IOP baseline, index eye (mmHg)
5. Goldmann applanation tonometry IOP baseline, fellow eye (mmHg)
6. Ocular pulse amplitude visit 1 (mmHg, measured with pascal dynamic contour tonometer (Ziemer Group, Port, Switzerland))
7. Supine IOP (mmHg, measured with Perkins tonometer)
8. The difference in IOP sitting-supine (mmHg)
9. Goldmann applanation tonometry IOP visit 1 after treatment (mmHg)
10. Percentage of IOP reduction at visit 1 (%)
11. Daytime phasing range (mmHg)
12. Axial length (mm, measured with IOL-Master Carl Zeiss Meditec Inc, Dublin, CA)
13. Central corneal thickness (CCT) (µm, measured with different ultrasound pachymeters)
14. Hysteresis (corneal hysteresis measured with ocular response analyzer ORA Reichert, Inc., Depew, NY)
15. Refractive error (dioptres)
16. Visual field mean deviation, mean at baseline (decibels, measured with Humphrey Field Analyzer II Carl Zeiss Meditec, Inc., Dublin, CA)

The following variables were measured per patient as the unit of measurement and details about the method are in parenthesis:

1. Left eye index eye (yes or no, if the left eye had the worst MD)
2. Both eyes are eligible (yes or no, if both eyes fulfilled the glaucoma eligibility criteria)
3. Randomisation to latanoprost (yes or no)
4. VF progression (yes or no, at a patient level irrespective of the eye that progressed as previously reported (24) and including four extra participants that progressed at 28 months from baseline)
5. Female (yes or no)
6. Current use of beta-blockers (yes or no)
7. Smoker previously (yes or no, of smoking at least one cigarette per day for a minimum of a year currently active or inactive)
8. Smoker now (yes or no, smoking at least one cigarette per day currently active)
9. Current use of any diabetic medications (yes or no)
10. Current use of any tricyclic antidepressants (yes or no)
11. Current use of any selective serotonin reuptake inhibitors (SSRIs) (yes or no)
12. Current use of any angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs) (yes or no)
13. Current use of any statins (yes or no)
14. Current use of aspirin, clopidogrel, or warfarin (yes or no)
15. Current use of aspirin (yes or no)
16. Current use of any calcium channel blockers (CCBs) (yes or no)
17. Self-reported diagnosis of diabetes (yes or no)
18. Self-reported diagnosis of systemic hypertension (yes or no)
19. Self-reported diagnosis of hypercholesterolemia (yes or no)
20. Self-reported diagnosis of asthma (yes or no)
21. Self-reported diagnosis of bronchitis (yes or no)
22. Self-reported diagnosis of heart attack (yes or no)
23. Self-reported diagnosis of stroke (yes or no)
24. Self-reported diagnosis of cancer (yes or no)
25. Self-reported negative past medical history (yes or no, when patients reported no previous diagnosis of any medical condition)
26. Self-reported previous diagnosis of anaemia (yes or no)
27. Self-reported history of blood loss (yes or no)

28. Self-reported diagnosis of history of blood transfusion (yes or no)
29. Self-reported diagnosis of sleep apnoea (yes or no)
30. Diagnosis of migraine with questionnaires (yes or no, based on the International Classification of Headache Disorders second edition criteria for migraine (25))
31. Diagnosis of Raynaud's phenomena with questionnaires (yes or no, based on the criteria for Raynaud's phenomena described by Wigley (26))
32. Diagnosis of claudication with questionnaires (yes or no, based on the Edinburgh claudication questionnaire (27))
33. Age (years)
34. BMI (weight in kg divided by the square of the height kg/m^2)
35. Vitamin B12 (ng/l)
36. Cholesterol (mmol/L)
37. HDL cholesterol (mmol/L)
38. LDL cholesterol (mmol/L)
39. Triglycerides (mmol/L)
40. Highly sensitive CRP (mg/L)
41. Homocysteine (µmol/L)
42. HbA1c (%)
43. Glucose (mmol/L)
44. Thyroxine T3 (pmol/L)
45. Thyroxine T4 (pmol/L)
46. Thyroid stimulating hormone (mIU/L)
47. Tea or coffee (cups per week)
48. Systolic blood pressure (mmHg, the mean of right and left arm at baseline)
49. Diastolic blood pressure (mmHg, the mean of right and left arm at baseline)
50. Difference systolic - diastolic blood pressure (mmHg, the mean systolic minus mean diastolic blood pressure)
51. Difference systolic left-right blood pressure (mmHg, systolic blood pressure in left minus right arm)
52. Difference diastolic left-right blood pressure (mmHg, diastolic blood pressure in left minus right arm)
53. Waist (cm)
54. Hips (cm)

55. Height (cm)
56. Weight (Kg)

The following variables were measured from images acquired during the baseline visit with the Heidelberg retina tomograph 3 (HRT 3; Heidelberg Engineering, Heidelberg, Germany; image acquisition software version 3.0.60, Heyex 1.6.2.0). The contour lines of the optic nerve head were delineated in the HRT images by two independent and experienced glaucoma specialists masked to the treatment arm, VF results and DH status. A third glaucoma specialist helped to reach a consensus when there was a discrepancy between graders. A different glaucoma specialist masked to the treatment arm, VF results and DH status measured the peripapillary area using the PPA Zone Analysis software (Heidelberg Engineering GmbH) (28). These variables use the eye with the worst MD as the unit of analysis; the unit of measurement and details about the method are in parenthesis:

1. Peripapillary atrophy (PPA) area baseline (mm^2)
2. Peripapillary atrophy circumferential extent at baseline (degrees)
3. Peripapillary atrophy radial extent at baseline (mm, from the centre of gravity of the optic nerve to the maximum radial extent of PPA)
4. Peripapillary atrophy maximum distance at baseline (mm, from the contour line of the optic nerve head to the maximum radial extent of PPA)
5. Peripapillary atrophy maximum distance radial at baseline (a ratio, PPA Max distance / radius between the centre of gravity of the optic nerve and the contour line of the optic nerve head)
6. Disc area baseline (mm^2)
7. Rim area baseline (mm^2)
8. Cup volume baseline (mm^3)
9. Maximum cup depth baseline (mm)
10. Retinal nerve fibres layer thickness baseline (mm)
11. Vertical cup/disk ratio baseline (ratio)

Previous reports of the UKGTS, such as the analysis of risk factors of visual field progression (21, 22), identified a different hazard of progressing between study sites. To account for this, multivariable analysis with frailty models (an extension

of the Cox proportional model) were used previously. In the current analysis of the risk factors for developing a DH, the effect of different sites on the risk of developing a DH was investigated with a univariable and multivariable logistic regression for the sites and the Omnibus test of model coefficients to assess the overall effect of introducing site as a covariate.

Biologically plausible variables, variables previously found associated with DHs, and all variables with a p value below 0.20 during the unadjusted logistic regression were further adjusted for the number of visits and later included in the final analysis. Automatic tools to explore useful predictor variables to build the multivariable models, such as the stepwise or best subsets, were not used. Instead, the final multivariable logistic regression model was constructed with all the statistically significant variables after adjustment, clinically relevant, and previously found associated with DHs. A p value lower than 0.05 was considered statistically significant. However, multiple variables were investigated and the results need to be interpreted considering the high risk of identifying statistical significance by chance. Due to the hypothesis-generating type of analysis of the present study, it was decided not to adjust for multiple testing and instead interpret the result in this context. All statistical analyses were performed using SPSS (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.).

3.4.4 Results

Five-hundred-and-sixteen patients signed consent for the UKGTS and were included in the study. Fifty-five patients (10.7%) did not attend any post-baseline visits (27 from the latanoprost group and 28 from the placebo group) and had only one visit analysed. Imaging (HRT or fundus photograph) was not acquired in 56 patients (10.9%) and the DHs status was impossible to define. The data from the remaining 460 participants (89.1%) were used to analyse risk factors for DHs. From a total of 4152 visits, the mean (SD) number of visits per participant was 8.1 (3.6) with a range from 1 to 15. The UKGTS had 11 visits scheduled in the protocol, but if a patient was identified with progression, a confirmation visit was required and if confirmed, an exit visit was added. Therefore, some patients attended multiple confirmation visits and had more than the 11 scheduled visits

in the protocol. One hundred and twenty-one participants (26.3%) developed a DH in any eye for at least one visit. Among the 4152 visits of all UKGTS participants, 356 (8.6%) were DH+, with a mean (SD) percentage of DH+ visits among all participants of 8.5% (19.7%). Among the 121 DH+ participants, the median (IQR) percentage of DH+ visits was 20% (12.5 - 44.0). Thirty-nine (7.6%) of the UKGTS participants had a DH detected at baseline. Eighteen (3.9%) participants had a DH identified in both eyes during follow-up; in 11 participants at the same visit and in 7 patients at different visits (see Table 23).

Table 23 Distribution of UKGTS participants UKGTS participants depending on disc haemorrhage status.

	Number of participants (%)
No DH	339 (73.7)
Unilateral DH+	103 (22.4%)
Bilateral DH+	18 (3.9%)
***Simultaneous Bilateral DH+**	11 (2.4%)
***Non-Simultaneous Bilateral DH+**	7 (1.5%)
*These are subsets of bilateral DH+	

The risk of developing a DH was not associated with the study site (Table 24). The Omnibus test for the model including UKGTS study site had a p value of 0.788

Table 24 Risk of developing DHs (expressed as the odds ratio) by UKGTS study site.

Site	Number of participants	OR (95% CI)	p value
Moorfields Eye Hospital	91 (17.6%)	Reference	0.794
Aberdeen Royal Infirmary	42 (8.1%)	1.49 (0.66-3.38)	0.335
Norfolk and Norwich University Hospital	83 (16.1%)	1.28 (0.64-2.53)	0.483
Birmingham Heartlands and Solihull	74 (14.3%)	0.71 (0.33-1.54)	0.385
Cheltenham General Hospital	24 (4.7%)	0.67 (0.21-2.17)	0.500
Bristol Eye Hospital	26 (5%)	1.48 (0.56-3.89)	0.425
Sunderland Eye Infirmary	32 (6.2%)	0.77 (0.28-2.12)	0.612
Hinchingbrooke Hospital	109 (21.1%)	0.99 (0.51-1.92)	0.981
Addenbrooke's Hospital	21 (4.1%)	0.78 (0.24-2.59)	0.690
West of England Eye Unit	2 (0.4%)	3.33 (0.2-55.61)	0.402
Huddersfield Royal Infirmary	12 (2.3%)	1.11 (0.28-4.48)	0.882

The univariable logistic regression analysis for the association between all variables assessed in the UKGTS and DH+ status is reported in Table 25.

Table 25 Factors associated with DH+ status (patient level) in the UKGTS. Univariable logistic regression.

				Univariable	
	Data N=	**Mean SD**	**Count %**	**OR (95% CI)**	**p value**
Left eye with worst MD and eligible	516	---	312 (60.5%)	1.09 (0.72-1.65)	0.696
Both eyes eligible	516	---	265 (51.4%)	0.95 (0.63-1.43)	0.812
Randomization to Latanoprost	516	---	258 (50.0%)	0.94 (0.62-1.41)	0.755
Number of visits	516	8.0 (3.6)	---	**1.15 (1.07-1.22)**	**<0.001**
VF progression	516	---	98 (19.0%)	**2.28 (1.42-3.66)**	**<0.001**
GAT IOP baseline	514	19.3 (4.8)	---	0.97 (0.93-1.02)	0.224
GAT IOP baseline fellow eye	514	19.1 (4.6)	---	0.98 (0.93-1.02)	0.309
Ocular Pulse Amplitude visit 1	420	2.5 (1.1)	---	0.95 (0.78-1.16)	0.626
Supine IOP	413	17.7 (5.2)	---	0.99 (0.95-1.03)	0.643
Difference in IOP Sitting-Supine	413	1.7 (4.9)	---	0.99 (0.94-1.03)	0.582
GAT IOP visit 1	456	16.6 (4.8)	---	0.98 (0.94-1.02)	0.326
Percentage IOP reduction	455	0.1 (0.2)	---	1.17 (0.44-3.13)	0.753
Phasing range	476	3.8 (2.2)	---	0.97 (0.89-1.07)	0.601
Axial length	457	24.1 (1.3)	---	1.11 (0.95-1.3)	0.203
CCT	464	541 (33.7)	---	1 (0.99-1)	0.271
Hysteresis	461	9.0 (2.0)	---	0.99 (0.89-1.1)	0.821
Refraction	515	-0.7 (2.9)	---	**0.92 (0.86-0.98)**	**0.015**
VF MD	516	-4.3 (3.4)	---	**0.96 (0.9-1.01)**	**0.126**

PPA area baseline	460	1.2 (0.8)	---	**1.18 (0.92-1.52)**	**0.193**
PPA degrees baseline	460	351.1 (40.0)	---	1 (1-1.01)	0.627
PPA Radial Extent baseline	460	0.3 (0.2)	---	1.49 (0.4-5.56)	0.551
PPA Max distance baseline	460	0.3 (0.2)	---	**1.96 (0.73-5.27)**	**0.184**
PPA Max distance radial baseline	460	0.4 (0.3)	---	1.48 (0.78-2.81)	0.234
Disc area baseline	460	2.0 (0.5)	---	**0.66 (0.42-1.05)**	**0.082**
Rim area baseline	460	1.0 (0.3)	---	0.96 (0.49-1.89)	0.910
Cup volume baseline	460	0.3 (0.2)	---	**0.26 (0.09-0.77)**	**0.015**
Maximum cup depth baseline	460	0.7 (0.2)	---	**0.39 (0.14-1.08)**	**0.070**
RNFL thickness baseline	460	0.2 (0.1)	---	0.53 (0.04-7.7)	0.643
Vertical cup/disk ratio baseline	460	0.7 (0.2)	---	2 (0.6-6.66)	0.258
Blue Iris	516	---	240 (46.5%)	1.59 (1.06-2.4)	**0.026**
Female	516	---	243 (47.1%)	2.01 (1.33-3.05)	**0.001**
Age	516	65.6 (10.6)	---	1.01 (0.99-1.03)	0.254
Beta blockers	516	---	64 (12.4%)	1 (0.54-1.85)	0.998
BMI	471	27.4 (4.8)	---	**0.95 (0.9-0.99)**	**0.027**
Smoker previously	515	---	255 (49.5%)	**0.74 (0.49-1.12)**	**0.151**
Smoker now	513	---	41 (8.0%)	1.38 (0.68-2.79)	0.373
Diabetic medications	516	---	31 (6.0%)	0.77 (0.31-1.93)	0.580
Tricyclic antidepressants	516	---	20 (3.9%)	1.42 (0.53-3.78)	0.483
Selective serotonin reuptake inhibitors (SSRIs)	516	---	16 (3.1%)	**3.42 (1.26-9.33)**	**0.016**
Angiotensin-converting enzyme (ACE) inhibitors/Angiotensin II receptor blockers (ARBs)	516	---	106 (20.5%)	0.82 (0.49-1.39)	0.463

Statins	516	---	143 (27.7%)	1.03 (0.65-1.61)	0.914
Aspirin, Clopidogrel, or Warfarin	516	---	105 (20.3%)	1.24 (0.76-2.03)	0.384
Aspirin	516	---	85 (16.5%)	1.01 (0.58-1.74)	0.985
Calcium channel blockers	516	---	64 (12.4%)	**0.3 (0.13-0.72)**	**0.007**
Diabetes	516	---	54 (10.5%)	0.72 (0.35-1.48)	0.368
Systemic hypertension	516	---	212 (41.1%)	**0.64 (0.42-0.98)**	**0.041**
Hypercholesterolemia	516	---	151 (29.3%)	0.88 (0.56-1.39)	0.582
Asthma	516	---	60 (11.6%)	0.99 (0.53-1.88)	0.982
Bronchitis	516	---	31 (6.0%)	1.15 (0.5-2.63)	0.750
Heart attack	516	---	28 (5.4%)	0.7 (0.26-1.87)	0.475
Stroke	516	---	17 (3.3%)	0.69 (0.2-2.45)	0.568
Cancer	516	---	45 (8.7%)	0.8 (0.37-1.71)	0.568
Negative past medical history	516	---	187 (36.2%)	1.27 (0.83-1.92)	0.266
Vitamin B12 ng/l	265	353.9 (149.9)	---	1 (1-1)	0.897
Cholesterol mmol/L	313	5.5 (1.3)	---	1.07 (0.89-1.29)	0.489
HDL Cholesterol mmol/L	304	1.6 (0.6)	---	1.18 (0.8-1.73)	0.410
LDL Cholesterol mmol/L	299	3.2 (1.1)	---	1.06 (0.85-1.32)	0.584
Triglycerides mmol/L	297	1.4 (0.7)	---	0.97 (0.67-1.42)	0.887
Highly Sensitive CRP mg/L	143	3.6 (3.7)	---	0.99 (0.89-1.09)	0.827
Homocysteine µmol/L	155	12.6 (10.9)	---	0.98 (0.94-1.02)	0.354
HbA1c %	278	5.7 (0.7)	---	0.89 (0.6-1.33)	0.578
Glucose mmol/L	274	5.6 (1.3)	---	0.94 (0.76-1.16)	0.544
Thyroxine T3 pmol/L	98	5.0 (1.7)	---	0.92 (0.63-1.33)	0.647
Thyroxine T4 pmol/L	217	14.5 (2.8)	---	0.96 (0.87-1.07)	0.475

Thyroid Stimulating Hormone mIU/L	287	2.4 (2.3)	---	**1.12 (1.01-1.25)**	**0.039**
Tea or Coffee cups per week	512	4.9 (2.6)	---	**1.12 (1.04-1.21)**	**0.002**
History of anaemia	511	---	48 (9.4%)	**2.15 (1.15-4.02)**	**0.016**
History of blood loss	511	---	43 (8.4%)	1.22 (0.8-1.85)	0.366
History of blood transfusion	511	---	53 (10.4%)	1.29 (0.85-1.98)	0.236
Sleep apnea	477	---	28 (5.9%)	0.56 (0.14-2.22)	0.414
Migraine	478	---	74 (15.5%)	0.87 (0.49-1.57)	0.646
Raynaud's	516	---	56 (10.9%)	**1.8 (0.99-3.27)**	**0.052**
Claudication	516	---	53 (10.3%)	1.07 (0.55-2.07)	0.845
Systolic blood pressure	473	135.6 (19.8)	---	1.01 (1-1.02)	0.302
Dyastolic blood pressure	473	80.7 (10.8)	---	1.01 (0.99-1.03)	0.225
Difference Syst-Diast blood pressure	471	54.9 (15.1)	---	1 (0.99-1.02)	0.637
Difference systolic left- right blood pressure	470	-0.9 (8.7)	---	1 (0.97-1.02)	0.900
Difference dyastolic left- right blood pressure	470	-1 (7.7)	---	0.99 (0.96-1.02)	0.531
Waist	472	97.9 (14.7)	---	**0.98 (0.96-0.99)**	**0.001**
Hips	472	107.7 (13.6)	---	**0.97 (0.96-0.99)**	**0.010**
Height	473	168.7 (9.5)	---	**0.98 (0.96-1)**	**0.055**
Weight	471	78.0 (15.3)	---	**0.98 (0.97-0.99)**	**0.005**

P values and OR are bold when the p value is <0.2

If a variable was biologically associated with another such as BMI and weight with an R^2=0.663, only the one with the higher statistical significance was included in the multivariable analysis. For this reason, the following variables, although significant in the univariable analysis, were excluded from the multivariable analysis:

1. PPA at baseline was removed to keep only PPA Max distance at baseline
2. Maximum cup depth baseline was removed to keep cup volume at baseline
3. Hips and waist were removed to include BMI that was correlated with both and with weight in a single variable
4. Thyroid-stimulating hormone levels were not included because only around 50% of participants had results available
5. Height and weight were removed to include BMI that contains the relationship between both

Visual field progression was not included in the analysis because it was not defined until the last visit of each participant. Therefore, it cannot be considered a baseline predictor for the future detection of DHs.

The multivariable model included data from the 439 participants (326 DH- and 113 DH+) in whom all variables were reported (Table 26).

Table 26 Multivariable analysis of variables associated with DH+ status in the UKGTS.

Variables in 439 UKGTS participants	OR (95% CI)	p value
Number of visits	1.05 (1.00-1.14)	0.315
Female	**1.85 (1.10-3.12)**	**0.020**
Age	**1.04 (1.01-1.07)**	**0.009**
Refraction	**0.90 (0.80-0.98)**	**0.013**
IOP at baseline	0.98 (0.93-1.03)	0.469
MD at baseline	0.95 (0.89-1.02)	0.156
PPA Maximum Distance	0.74 (0.21-2.58)	0.633
Disc area (mm2)	0.78 (0.39-1.56)	0.479
Cup volume (mm3)	0.40 (0.09-1.79)	0.231
Blue Iris	1.44 (0.89-2.34)	0.140
BMI	0.96 (0.90-1.01)	0.104
Previous smoker	0.88 (0.54-1.44)	0.615
Cups of tea/coffee per week	**1.16 (1.05-1.28)**	**0.003**
Beta-blockers systemic	0.90 (0.39-2.11)	0.814
SSRI	**3.66 (1.00-13.35)**	**0.049**
Aspirin/Clopidogrel/Warfarin	1.59 (0.82-3.10)	0.171
CCB	**0.25 (0.09-0.68)**	**0.007**
Systemic hypertension	0.96 (0.51-1.80)	0.886
History of anaemia	2.09 (0.87-4.99)	0.098
Migraine	0.60 (0.29-1.24)	0.164
Raynaud's	1.33 (0.64-2.78)	0.448

P values and OR are bold when the p value is <0.05

Among the 64 participants who were using CCB, 56 (87.5%) were on the dihydropyridine (DHP) type of CCB, in the remaining participants, the type was not specified, and no participants declared using non-DHPs. Among the 64 users of CCB, 6 (9.4%) developed a DH compared to 115 (25.4%) in the non-users of CCB. The two tail Fisher's exact test comparing the proportion of DHs between CCB and non-CCB users reported a p=0.004. Sixteen participants were on treatment with SSRI, 50% developed a DH compared to 22.6% in non-SSRI users. The two tail Fisher's exact test comparing the proportion of DHs between CCB and non-CCB users reported a p=0.017. The 243 females analysed for this study represented 47.1% of all UKGTS participants but 60.3% of all DH+ participants. Seventy-three (30%) of females developed a DH during the UKGTS

compared to 48 (17.6%) of males (p=0.001). The mean (95% CI) number of cups of tea or coffee consumed per week was 4.7 (4.5-4.9) for DH- and 5.5 (5.0-6.1) for DH+ participants (p=0.007). With a mean difference in cups in the DH+ group of +0.9 (95% CI 0.3-1.4).

Two independent extra multivariable models (with the same n=439) were constructed with the same variables included in Table 26 but excluding IOP at baseline to include two other IOP related variables in the worst eye that have been previously considered to be potentially associated with DHs. Percentage of IOP reduction had an OR 1.28 (95% CI 0.40-4.09; p=0.68). GAT IOP fluctuation during baseline phasing had an OR 1.00 (95% CI 0.90-1.11; p=0.99). The other variables that were statistically significant in the model described in Table 26 remained statistically significant in the three models that included the two alternative IOP-related variables.

Fewer than 300 participants underwent laboratory tests, such as glycated haemoglobin (HbA1c%, 278 participants) and thyroid-stimulating hormone (TSH, 287 participants). However, they were separately included in extra multivariable models because TSH was statistically significant in the univariable analysis, and glucose intolerance was previously reported to be associated with DHs and is related to glycated haemoglobin. None of the lab test variables were associated with DHs in the multivariable analysis; glycated haemoglobin had an OR=0.88 (95% CI 0.53-1.46; p=0.623), and TSH had an OR=1.08 (95% CI 0.97-1.21; p=0.175).

In the multivariable model, systemic hypertension (HTN) was not statistically significantly associated with DH+ status and all the other systemic blood pressure related variables were also not statistically significantly associated in the univariable analysis. However, systolic blood pressure has been associated with DHs, and therefore an extra multivariable model excluding HTN was constructed. The mean of both arms systolic blood pressure at baseline was included and had an OR=1.01 (95% CI 0.99-1.02; p=0.243).

In the multivariable analysis, optic nerve related parameters were included (PPA maximum distance, disc area, and cup volume) and were not statistically

significantly associated with DH+ status. However, CDR and neuroretinal rim area have been associated with DHs in previous publications and were included in an independent multivariable analysis that excluded the cup volume. Vertical CDR in the worst eye, measured with HRT at baseline, had an OR=2.28 (95%CI 0.53-9.84; p=0.271). Rim area measured with HRT had an OR=1.36 (95%CI 0.60-3.10; p=0.464).

The same variables used to construct the multivariable model (Table 26) were used to investigate factors associated with Bilateral DH+ status (18 participants). The multivariable model investigating the association with Bilateral DH+ status included 16 participants with the variables investigated (Table 27). Only the use of aspirin, clopidogrel or warfarin was associated with bilateral DH+ status with an OR=7.06 (1.50-33.23; p=0.013). None of the participants with bilateral DH+ status used CCB compared to 6 (5%) of the participants with a DH+ status and 56 (10.9%) of the no DH status.

Table 27 Multivariable analysis of variables associated with Bilateral DH+ status in the UKGTS.

Variables in 16 UKGTS participants with DH+ BE	OR (95% CI)	p value
Number of visits	1.20 (0.93-1.55)	0.167
Female	1.36 (0.29-6.40)	0.694
Age	1.07 (0.99-1.16)	0.093
Refraction	0.91 (0.74-1.13)	0.390
IOP at baseline	1.01 (0.87-1.17)	0.919
MD at baseline	1.09 (0.88-1.35)	0.452
PPA Maximum Distance	1.75 (0.12-25.78)	0.685
Disc area (mm2)	2.22 (0.38-12.78)	0.374
Cup volume (mm3)	0.85 (0.03-29.33)	0.928
Blue Iris	1.65 (0.49-5.58)	0.424
BMI	0.85 (0.72-1.00)	0.054
Previous smoker	0.32 (0.08-1.33)	0.116
Cups of tea/coffee per week	1.04 (0.79-1.36)	0.785
Beta-blockers systemic	0.87 (0.08-9.49)	0.908
SSRI	2.45 (0.12-52.18)	0.567
Aspirin/Clopidogrel/Warfarin	**7.06 (1.50-33.23)**	**0.013**
CCB	0 (0-0)	0.997
Systemic hypertension	0.14 (0.02-1.10)	0.061
History of anaemia	0.99 (0.13-7.18)	0.986
Migraine	2.10 (0.43-10.31)	0.363
Raynaud's	0.27 (0.02-3.23)	0.299
P values and OR are bold when the p value is <0.05		

The same variables used in the models to investigate DH+ and bilateral DH+ status were further used to evaluate the association with the number of DH+ visits (in either eye, see Table 28) that DH+ participants (121 participants) had during the study.

Table 28 Number of DH+ visit in all UKGTS participants.

Number of DH+ visits*	Number (%)
0	339 (73.7%)
1	49 (10.7%)
2	27 (5.9%)
3	10 (2.2%)
4	13 (2.8%)
5	7 (1.5%)
6	4 (0.9%)
7	2 (0.4%)
8	2 (0.4%)
9	2 (0.4%)
10	2 (0.4%)
11	3 (0.7%)

*The total number of DH+ visits each participant had in either eye.

This analysis was performed using 1) the total number of DH+ visits during a participant´s series of visits and 2) the percentage of DH+ visits per participant. For the analysis with the total number of DHs, the variable “number of visits” was included in the multivariable analysis to adjust for the number of observations (Table 29). In comparison, in the analysis that used the percentage of DH+ visits, the number of visits was excluded from the multivariable analysis (Table 30).

Table 29 Multivariable analysis of variables associated with the number of visits with DHs in the UKGTS.

Variables in 121 UKGTS participants with DHs	Unstandardized Coefficients β (95% CI)	p value
Number of visits	**0.25 (0.06 to 0.43)**	**0.009**
Female	-0.26 (-1.49 to 0.98)	0.680
Age	-0.01 (-0.08 to 0.05)	0.671
Refraction	0.11 (-0.07 to 0.29)	0.243
IOP at baseline	-0.05 (-0.17 to 0.08)	0.465
MD at baseline	0.05 (-0.1 to 0.21)	0.485
PPA Maximum Distance	1.47 (-1.73 to 4.68)	0.364
Disc area (mm2)	-0.82 (-2.56 to 0.91)	0.349
Cup volume (mm3)	0.48 (-3.06 to 4.02)	0.789
Blue Iris	-0.04 (-1.08 to 0.99)	0.935
BMI	-0.10 (-0.23 to 0.22)	0.104
Previous smoker	**-1.42 (-2.45 to -0.38)**	**0.008**
Cups of tea/coffee per week	-0.02 (-0.19 to 0.15)	0.815
Beta-blockers systemic	-0.14 (-1.95 to 1.67)	0.875
SSRI	1.62 (-0.59 to 3.82)	0.149
Aspirin/Clopidogrel/Warfarin	0.42 (-0.96 to 1.81)	0.544
CCB	-1.06 (-3.61 to 1.49)	0.411
Systemic hypertension	0.01 (-1.43 to 1.45)	0.989
History of anaemia	-0.18 (-1.71 to 1.35)	0.815
Migraine	0.31 (-1.23 to 1.85)	0.688
Raynaud's	-1.02 (-2.49 to 0.46)	0.174

P values and OR are bold when the p value is <0.05

Table 30 Multivariable analysis of variables associated with the percentage of DH+ visits in the UKGTS.

Variables in 121 UKGTS participants with DHs	Unstandardized Coefficients β (95% CI)	p value
Female	-0.05 (-0.18 to 0.07)	0.408
Age	0 (-0.01 to 0.01)	0.747
Refraction	0.01 (-0.01 to 0.03)	0.255
IOP at baseline	-0.01 (-0.02 to 0.01)	0.368
MD at baseline	0.01 (-0.01 to 0.02)	0.254
PPA Maximum Distance	0.2 (-0.13 to 0.54)	0.223
Disc area (mm2)	-0.13 (-0.31 to 0.05)	0.152
Cup volume (mm3)	0.14 (-0.23 to 0.5)	0.460
Blue Iris	0 (-0.1 to 0.11)	0.933
BMI	-0.85 (-2.13 to 0.44)	0.194
Previous smoker	**-0.16 (-0.27 to -0.06)**	**0.003**
Cups of tea/coffee per week	-0.01 (-0.02 to 0.01)	0.531
Beta-blockers systemic	-0.02 (-0.21 to 0.17)	0.825
SSRI	0.21 (-0.02 to 0.44)	0.068
Aspirin/Clopidogrel/Warfarin	0 (-0.14 to 0.14)	0.963
CCB	-0.14 (-0.4 to 0.12)	0.281
Systemic hypertension	0.01 (-0.14 to 0.16)	0.901
History of anaemia	0.02 (-0.13 to 0.18)	0.772
Migraine	0.05 (-0.11 to 0.21)	0.542
Raynaud's	**-0.15 (-0.3 to 0)**	**0.044**

P values and OR are bold when the p value is <0.05

Participants who had smoked more than one cigarette daily per year had fewer DH+ visits in both multivariable models. In a univariable analysis using the percentage of DH+ visits as the dependent variable, participants who smoked had a lower percentage of DH+ visits (β= -0.11; 95% CI=- 0.21 to -0.02; p=0.024). Current smokers with DHs during the UKGTS (12 participants) were not associated with a lower number of DH+ visits.

The 53 (43.8%) participants who had smoked and developed DHs during the study had a mean (SD) of 0.27 (0.21) visits with a DH detected compared to the participants who had never smoked who had a mean (SD) of 0.39 (0.30) visits with a DH. The participants who had smoked had a lower percentage of DH+ visits with a mean difference of -0.12 DH+ visits (95%CI -0.22 to -0.03; p= 0.014).

3.4.5 Discussion

Variables that affected the risk of participants in the UKGTS to develop a DH over the study period differed from the variables associated with the number of DH+ visits or with participants with DHs in both eyes. The variables associated with DH+ status seem to generate a vulnerability in a patient to develop DHs but not to increase their frequency or the presence in both eyes. In a patient vulnerable to develop DHs, it seems possible that other factors affect the frequency of DH+ visits and the risk of bilateral DHs. However, the analysis of the bilateral DH+ status and the frequency/number of DH+ visits included a smaller number of participants and the results need to be considered an exploratory analysis. The interaction of a group of variables that affect the risk of developing DHs and a different group that affects its frequency or laterality is in favour with the conceptualization of DHs as a possible marker of a DH+ glaucoma endotype. The variables that are associated with the presence of DHs are involved in the development of the DH+ endotype, while the variables that increase the frequency of DHs or the laterality only modify the vulnerability to bleed that is present in the DH+ endotype of POAG patients. It is widely accepted that glaucoma is a complex disease (29) similar to diabetes (30), asthma (31), osteoarthritis (32), and many other common diseases. Complex diseases are characterized by a great number of genetic factors that interact with the environment to produce a disease with clinical heterogeneity. Individual risk of having glaucoma is affected by multiple genes (33) and the disease affects similar patients very differently, with the majority progressing very slowly and a small group progressing rapidly to visual impairment. Research in asthma or osteoarthritis has led clinicians and scientists to categorise patients suffering from these diseases into different phenotypes and endotypes (34). Phenotypes are the observable characteristics that are caused by the interaction of genetic susceptibility with the environment. Some phenotypes might also represent a specific pathophysiologic mechanism of the disease, and in some areas, such as asthma research, they are called endotypes. DH+ glaucoma patients are a different phenotype of glaucoma patients and could also represent a different endotype in which vessels are more susceptible to bleed and cause DHs. The possible DH+ endotype is also complex and multifactorial like glaucoma with some patients bleeding rarely and others frequently and bilaterally.

The variables identified in the EMGT to be associated with the presence of DHs were not completely confirmed in the UKGTS. Two novel variables, the consumption of tea or coffee and SSRI use, were for the first time identified as being associated with developing DHs. The UKGTS was designed to be similar to the EMGT to facilitate comparisons (20). However, the length of the study was very different, 24 months for the UKGTS and a median of 8 years in the EMGT. For a variable like DHs, which depends on the number of observations and the length of follow up, the proportion of DH+ participants was expected to be different. In the EMGT and UKGTS, 55% and 23% of the participants developed a DH during follow-up. Other important differences in the UKGTS were the inclusion of double the number of participants, more imaging, systemic, and laboratory variables. Therefore, variables not statistically significant in the EMGT and other novel variables were expected to be found in the UKGTS. Refractive error was significantly associated with DH+ participants in both trials with an OR of 0.74 in the EMGT and 0.89 in the UKGTS. Although the axial length (AL) was not significantly associated with DH development, the direction of the association was the same as that of refractive error (a longer AL, which is more common in myopes, was associated with DHs). Although these trials identified similar results, a recent Japanese longitudinal cohort of 90 healthy untreated NTG patients identified the opposite results in 34 DH+ participants (35). The Japanese cohort followed participants with fundus photographs every six months and identified a 35.5% cumulative probability of identifying DH+ participants at five years. The spherical refraction was associated with DHs but in the opposite direction to the EMGT and UKGTS with a HR = 1.18 (95% CI 1.04–1.32; p=0.018). Thus the effect of refraction may vary with ethnicity, NTG, or the association with other comorbidities. It is also possible that with increasing myopia, the effect of each dioptre on the risk of DHs differs compared to emmetropia or hyperopia. The Japanese cohort had a mean (SD) spherical refraction of -3.7 (2.9) compared to the UKGTS with -0.7 (2.9).

In the EMGT, males had a lower risk of DHs with an OR = 0.48 (95% CI, 0.26-0.90; p=0.022) and in the UKGTS sex was also statistically significant, with a tendency in the same direction as in the EMGT with an OR for females = 1.85 (95% CI, 1.10-3.12; p=0.020). A possible association of female hormones variation and glaucoma has been reported in cross-sectional (36) and longitudinal

(37) epidemiological studies. How female hormones affect the risk of glaucoma could be explained by their effect on collagen formation, vascular regulation, trabecular meshwork outflow facility via nitric oxide, among other mechanisms (37). The relationship between female hormones and DHs could be more closely related than with POAG due to the presence of oestrogen receptors in vascular wall endothelial cells. Oestrogens upregulate the activity and expression of the endothelial isoform of nitric oxide synthase with the consequent increase in nitric oxide activity vasodilation and vasoprotective effect (38). Before menopause, 17 β-oestradiol (E2) produced by the ovary is the main oestrogen. After menopause, the level of oestrogens reduces to the level of men, and the main source of oestrogen is estrone produced peripherally by adipose tissue. The reduction of oestrogens after menopause might leave the vascular walls of women more vulnerable to bleed in comparison to men who have more constant levels of oestrogens derived from peripheral fat and from the local conversion of testosterone. In support of this hypothesis, in the univariable logistic regression, BMI, waist, weight and hips were all associated with a lower risk of DHs. Greater BMI, waist or hips are associated with greater peripheral adipose tissue which produces oestrogens that could improve vasculature regulation and eventually reduce the risk of DHs.

Contrary to the EMGT, baseline IOP, and mean follow up IOP were not associated with DHs in the UKGTS (Table 26); this was an interesting finding because none of the IOP-related variables was associated with DHs in the UKGTS. The OHTS identified the opposite effect of IOP on the risk of developing DHs with higher IOP being a risk in the OHTS and a protective factor in the EMGT. The contrasting difference between the OHTS and EMGT could be due to the large baseline IOP differences in these trials with 20.6 mmHg in the EMGT (39) and 24.9 mmHg in the OHTS (40). However, it could also be related to the exclusion of baseline DHs in the OHTS or a differential effect of IOP on the risk of DHs depending on the baseline IOP. Patients in the low range of IOP who develop glaucoma may represent a group with more vascular abnormalities. On the other hand, OHT patients in the high range of IOP (with no confirmed glaucoma at baseline, as in the OHTS) with DHs could represent a subgroup of patients with a greater chance of progressing to glaucoma and therefore increasing their risk of DHs for already having glaucoma. Multiple publications without the methodological rigour of an

RCT have identified lower IOP as a risk factor for DHs among patients who already have glaucoma. Possible explanations for the association of low IOP with DHs are based on the more frequent occurrence of systemic vascular abnormalities in NTG patients (migraine, Raynaud's phenomenon, among others) that may also be present in the eye causing vascular dysregulation in the peripapillary region, leading to DHs. An alternative and more mechanical possibility is related to the lower resistance to bleeding given the lower IOP in NTG patients (41, 42). The lower resistance to bleeding would also make these DHs last for longer and extend to a larger area; these factors would make DHs more likely to be detected. On the contrary, it is possible that the level of IOP in POAG patients is not associated with DHs. The findings of the OHTS could represent a bias in the selection of patients with no DHs at baseline who only developed DHs after converting to POAG, which happened more frequently in patients with higher IOP. In the EMGT, the association between lower baseline IOP and DHs, although statistically significant, was only marginally significant with a 95% CI of 0.86 to 0.99, and was not confirmed in the UKGTS with more frequent imaging and the inclusion of twice the number of participants.

In the UKGTS, none of the variables associated with a DH+ status were associated with a higher number or percentage of DH+ visits. Among all variables analysed, only participants that ever smoked (active or inactive) had a lower number or frequency of DH+ visits. Only in the analysis that used frequency of DHs instead of the number of DH+ visits, the presence of Raynaud's phenomena was marginally significant. In the EMGT, smoking, lower baseline IOP and lower mean follow up IOP were associated with a higher frequency of DHs, but only the p value was reported; unfortunately, the magnitude of this association was not mentioned. In contrast to the UKGTS, and in support of the EMGT, a single report from the OHTS identified a positive association between those who had ever smoked more than 100 cigarettes and DH+ status (10). However, this was reported only in the eight-year report and was not further confirmed in any previous or following reports. It is interesting that the OHTS and the EMGT also reported an association between smoking and DHs, although the direction of the effect was in the opposite direction of the UKGTS.

The specific question that was asked to the UKGTS participants was “Have you ever smoked as much as one cigarette a day for as long as a year?; this question did not separate between active and ex-smokers. Among the 255 participants that responded yes, 41 were active smokers while the remaining 214 were ex-smokers. When the 41 active smokers were investigated independently, there was no association with DH+ status or number of DH+ visits. Therefore, it is possible that an unknown and unmeasured factor that is common in ex-smokers, who make up the majority of this group, is responsible for the association with the number or frequency of DH+ visits and not a direct effect of smoking on reducing the number of DH+ visits. For instance, it is possible that ‘ever smoked’ represents a more health-conscious group of individuals who smoked more than 100 cigarettes but quit smoking because they were more conscious of the risk; this group of mostly ex-smokers might represent individuals with a current healthier lifestyle which could explain the lower risk of DHs. An alternative explanation is that participants who quit smoking were less sensitive to nicotine addiction and the mechanism involved in the reduced sensitivity to nicotine is also involved in an unknown way to a vascular pathway that could make DHs appear less frequently. It is well established that smoking dependence varies among people and is possibly related to the inter-individual differences in the effect of nicotine (43). Another alternative possibility is that ‘ever smoked’ represents a group of patients who were more susceptible to become daily smokers. The UKGTS participants, with a mean age of 66 years, lived during a period in Great Britain when the prevalence of smoking was above 50% of the population (44). A large majority of this generation of people tried at least once in their lifetime a cigarette, but not all became daily smokers, as required in the ‘ever smoked’ definition of the UKGTS. It has been reported that two-thirds of people who tried at least one cigarette become daily smokers (45). The UKGTS participants who responded yes to the ‘ever smoked’ question represent these two-thirds, and they may represent a susceptibility to nicotine that in an unknown way could be related to a pathway that makes this patient develop DHs less frequently. Finally, a direct effect of smoking on DHs frequency is also possible and could be related to the effect of smoking on blood flow, which has been confirmed in human eyes of habitual smokers who showed an increase in the blood flow at the optic nerve head and choroid; these changes were mainly induced by increasing the linear velocity of flow, pulse rate, and blood pressure (46). However, the mechanisms

that increase blood flow are not fully understood and interact with the well-known vasoconstriction that smoking produces in vessels of different diameters and capillary networks such as those in the retina (47). One of the mechanisms involved in the vasoconstriction induced by smoking is related to the impaired vasodilation to nitric oxide caused by a reduced nitric oxide generation and diminished activity of the endothelial nitric oxide synthase (48). Overall, it is impossible to differentiate between a direct effect of smoking on the reduced number/frequency of DH+ visits from a confounding factor that makes this group of UKGTS participants less vulnerable to bleed frequently. Moreover, even if there is a direct effect of smoking on the optic nerve, it would be difficult to identify the exact chemical compound and its mechanisms of action. Because the combustion of cigarettes delivers thousands of active compounds that could act on unknown pathways to make peripapillary and optic nerve head vessels less likely to bleed frequently.

The association of 'ever smoked' with fewer/less frequency DH+ visits supports the results presented in Chapter 6, which identified 'ever smoked' as protective of visual field deterioration (HR 0.59; 95% CI 0.37 - 0.93; p=0.040). Unexpectedly, 'ever smoked' was not associated with the DH+ status. The effect of 'ever smoked' on reducing visual field deterioration could be interpreted as a positive effect of reducing the frequency/number of DH+ visits. However, the present study only identified an association and not a causation. An alternative hypothesis is that the reduction in the frequency/number of DH+ visits identified in the 'ever smoked' group may be secondary to the association of this group with the slower VF deterioration identified in the 'ever smoked' group. This would imply that DHs appear only as a consequence of glaucomatous progression and not that DHs are part of the causes that actively promote glaucomatous progression. An unpublished analysis of the association between 'ever smoked' and VF deterioration identified, in a subgroup analysis, that the reduction of VF deterioration was statistically significant only in the DH+ participants and not in the DH-. In addition, if the Cox regression multivariable analysis previously published (which identified a protective effect of 'ever smoked') includes the interaction between 'ever smoked' and DH+ status, 'ever smoked' (on its own) is no longer associated with a lower risk of VF deterioration. It seems that patients who are vulnerable to developing DHs had fewer DHs and slower VF deterioration

if they had ‘ever smoked’. Moreover, patients without DHs had no association between ‘ever smoked’ and VF deterioration.

The association of ‘ever smoked’ only with the frequency/number of DH+ visits (and not on DH+ status) is plausible based on the findings reported in Chapter 6, in which there was a tendency toward a higher number/frequency of DH+ visits in DH+ participants to further increase their risk of VF deterioration. Although all the results reported from the UKGTS tend toward supporting the concept that a higher frequency/number of DH+ visits further increases the risk of VF progression, the number of participants for the analysis was smaller (only the 121 DH+ participants), the results failed to achieve statistical significance, and previous reports have identified conflicting results on the effect of recurring DHs and VF progression, compared to patients with a single DH (49, 50). It is possible that the DH+ endotype is what increases the risk of VF progression, irrespective of the frequency/number of DH+ visits. To support this alternative hypothesis, the direct effect of blood on the neural tissue that is supported by animal models of stroke (1) would have to be clinically insignificant. The amount of blood, or the time it is present, in the peripapillary tissue would have no clinical impact and would not affect the visual fields. In this hypothesis, a patient's vulnerability to bleed at least once is what increases the risk of visual field progression. It would probably represent a broader abnormality of different mechanisms that increases the risk of VF progression independently from the direct effect of the blood in the optic nerve.

The association of tea or coffee consumption with DH+ status is novel, although tea and coffee consumption has been previously associated with other aspects of glaucoma. The Blue Mountains eye study identified an association between coffee consumption and higher IOP (51). A small experimental study that prescribed caffeinated or decaffeinated coffee to NTG and OHT patients using a crossover method supported the association between coffee consumption and higher IOP (52). A larger RCT confirmed a statistically significant effect of one cup of coffee (237ml with 182mg of caffeine) on IOP of around 1 mmHg but concluded that it was not likely to be clinically significant (53). Contrary to these publications, a Japanese cohort study of 9,850 individuals (the Nagahama study), identified a lower IOP in participants without glaucoma with higher coffee

consumption (54). Individuals who consumed coffee more than or equal to three times a day had an IOP 0.4 mmHg lower (95% CI, 0.2 - 0.5) than individuals who consumed coffee less than once a day. In that study, there was no association between coffee consumption and POAG. An epidemiological study from the USA which surveyed health and nutrition of 1678 participants with a glaucoma prevalence of 5.1% identified a lower risk of glaucoma only among patients who consumed at least a daily cup of hot tea with an OR=0.26 (95% CI 0.09 to 0.72; p=0.004) (55). There was no association between glaucoma and cold tea, coffee or other caffeinated beverages in the same study. Two different epidemiological studies, the nurses' health study (78,977 women) and the health professionals follow-up study (41,202 men) investigated possible risk factors for exfoliation glaucoma and exfoliation syndrome. Three-hundred-and-sixty cases were identified with exfoliation, and there was an association between heavy caffeine consumption (of more than three cups a day) and exfoliation glaucoma or syndrome with an RR= 1.66 (95% CI, 1.09-2.54; p= 0.02) (56). The overall problem of research on caffeine or tea consumption is the large widespread use of these psychoactive substances in multiple products and the high risk of identifying associations just by chance. Irrespective of the complexities of research on caffeine consumption, it is an important research question because even a small effect of this substance on a health parameter could have an important effect on the population due to its widespread consumption. For instance, in the United Kingdom, adults over 40 years old consume, on average, 2.2 cups of coffee per day (57), and 80% consume more than one cup of tea per day with a mean of 5 cups per day among daily consumers (58). In addition, caffeine consumption has been associated with either a lower or higher risk of multiple diseases. For instance, in stroke research, there is conflicting evidence on the effect of caffeine on ischaemic or haemorrhagic stroke (59). Some studies have identified an association between caffeine consumption and a lower risk of ischaemic stroke in women (60) while others have identified an increased risk in haemorrhagic stroke (61). Furthermore, coffee and tea consumption have been identified to increase CSF pressure; for that reason, they have been considered an initial approach to treat symptomatic intracranial hypotension (62). Even in glaucoma, caffeine has been considered as a possible therapeutic strategy to increase CSF pressure and improve the balance of forces affecting the optic nerve head biomechanics (63). A Korean epidemiologic study that separated

caffeine contained in medicines from the caffeine consumed in coffee identified an increased risk of haemorrhagic stroke among patients who consumed caffeine-containing medicines with an OR = 2.23 (95% CI, 1.41-3.69) (61). The risk of haemorrhagic stroke did not increase in coffee consumers but, on the contrary, increased in patients who never consumed coffee with an adjusted OR = 2.95 (95% CI, 1.45-5.98). It is possible that caffeine increases the risk of central nervous system bleeding and simultaneously reduces the risk of stroke by a different component of the coffee or tea. A reduced risk of cardiovascular events and total mortality has been associated with consumption of tea (64). A similar situation might occur in glaucoma, with caffeine increasing the risk of DHs but other components in the coffee/tea reducing the overall risk of developing glaucoma or glaucoma progression.

The LoGTS identified migraine, systolic blood pressure, systemic beta-blockers, and smaller neuroretinal rim area as associated with DH+ status (8). In the UKGTS, none of these variables were associated with DHs in the univariable analysis. Because previous publications, such as the LoGTS, that found associations of these factors with DHs, they were included in the multivariable model. However, they were found not to be associated with DHs in our data. Beta-blockers were of special interest due to their effect on lowering IOP (65) and reducing the risk of developing POAG (66) – a finding identified in various epidemiological studies; beta-blockers also modify multiple cardiovascular parameters. In the UKGTS, systemic beta-blockers were used in 64 participants (12.4%) but were not associated with DHs in a univariable analysis or after adjusting for age, gender, HTN, or in the complete final multivariable analysis. Comparing the results of the UKGTS and LoGTS is difficult due to the differences in the inclusion criteria, with the UKGTS enrolling participants with IOP up to 29 mmHg, and around half of them having high tension POAG compared to only NTG participants in the LoGTS. Another difference is that almost 30% of the LoGTS participants withdrew from the study compared to only 15% for the present report of the UKGTS. Irrespective of the methodological differences, it is also biologically plausible that some factors associated with DHs have a differential effect between patients with high and normal pressure glaucoma.

The OHTS reported at eight (10) and thirteen years of mean follow up different variables associated with DHs (10, 12). The last report identified greater age, larger cup disc ratio, higher IOP and non-black race as associated with DH+ status. Only older age and larger cup disc ratio were identified to increase the risk of DHs in both reports. None of these factors were confirmed in the UKGTS, except for older age which was associated with DH+ status. The association of older age and non-black race with DHs further supports the idea that mechanical deformation of the optic nerve is not enough for developing haemorrhages. Black patients tend to have POAG at a younger age and with very high IOP, but DHs were rarely seen in these patients. At even younger ages, children with glaucoma usually have very high IOP and great deformation of the optic nerve head, but DHs have not been reported in children. Other phenomena associated with ageing must be involved in the pathogenesis of DHs. The OHTS investigators suggested that a history of smoking and heart disease, which may be associated with greater age, would support DHs' vascular pathogenesis (10). The association of ageing and DHs could also be related to the overall ageing process, which affects mitochondrial function (67), cardiovascular function (68), endothelial function (69), fat distribution (70) and many other processes that, by multiple pathways, could increase the risk of developing a DH. Some authors have even proposed the concept of vascular ageing phenotypes (71), which could also explain why only some older patients develop DHs.

A novel association between the use of SSRIs and CCB with DH+ status was identified in the UKGTS. These very different medication groups have two similarities; they have a very wide range of clinical applications and are very frequently prescribed. The SSRIs and other antidepressant medications are becoming more frequently prescribed and, in NHS England, there were more than 70 million prescriptions during 2018 (72, 73). CCB also remain regularly prescribed worldwide and, as an example, amlodipine is the fourth most frequently prescribed drug in the USA with 87 million prescriptions during 2018 (74). The association of these groups of medication with glaucoma is not new. Khawaja et al. analysed a database of 6,130 POAG patients who required a glaucoma procedure and compared them with 30,650 controls with no glaucoma and identified an increased risk of glaucoma in users of CCB with an OR = 1.26 (95% CI 1.18 -1.35; $p=1.8 \times 10^{-11}$) and reduced risk in users of SSRIs with an OR

= 0.70 (95% CI 0.64 - 0.76; p= 1 x 10^{-15}) (19). It is remarkable that in the UKGTS these medications were associated with the *opposite* effect on DHs. In the UKGTS, SSRIs increased the risk of DH+ status more than threefold while they were associated with a lower risk of POAG that required a glaucoma procedure in the Khawaja study. A possible explanation to accept both results is that the overall effect of SSRIs on all subjects is to decrease their risk of developing POAG that requires an intervention. However, SSRIs increase the risk of DHs in patients with already established POAG, which might be explained by different mechanisms involved in the risk of developing glaucoma and developing DHs. It might also be possible that SSRIs' effect is not generalizable to all individuals and a subgroup could be vulnerable to an increased risk of POAG as well as an increased risk of DHs as identified in the UKGTS. However, if POAG requiring a glaucoma procedure is closer to POAG progression than to POAG development, the higher risk of DHs would be more difficult to explain. The association of depression with DHs was investigated in a Korean retrospective case-control study (75) that did not identify an association between DHs and depression (classified as a total score higher than 13 using the self-reported Beck's Depression Inventory-II). However, certain differences in personality have been associated with primary vascular dysregulation that is associated with DHs (76) and the Korean case-control study (75) identified DHs to be associated with a six-fold increase in the risk of anxiety (classified as a total score higher than 9 using the self-reported Beck's Anxiety Inventory). It could be possible that the association of SSRIs and DHs is the result of confounding with depression, however, other anti-depressive drugs, such as tricyclic antidepressants, were not associated with DHs. Various case reports have mentioned a possible risk of angle-closure and increased IOP after starting SSRIs, and the change in IOP could be related to the risk of DHs (77). However, in the UKGTS, (which included only open angle glaucoma based on gonioscopy (20)) neither IOP fluctuation nor any other IOP-related variable was associated with DHs. A direct effect of the serotoninergic pathways on the risk of glaucoma and DHs is possible, but the current evidence suggests SSRIs promote retinal ganglion cell function and potentially reduce the excitotoxic damage induced by ocular hypertension (78).

Participants on treatment with CCBs had a much lower risk of developing a DH compared to non-users (OR = 0.25, 95% CI 0.09-0.68; p=0.007). Most of the CCB

users were on the dihydropyridine (DHP) type of CCB (such as amlodipine or nifedipine). The DHP group of CCBs, compared to the non- dihydropyridine (such as verapamil or diltiazem), has a stronger systemic vascular effect, with vasodilation of arteries and the presentation of common side effects related to the vasodilation such as systemic hypotension and reflex tachycardia (79). The non-dihydropyridine type of CCBs are more selective for action on the myocardium, have a less clinically significant effect on the peripheral vascular system and are less likely to cause systemic side effects such as hypotension or reflex tachycardia. The vasodilation effect of the DHP CCBs has made these drugs potential therapeutic agents for diseases with peripheral vasoconstriction, such as Raynaud's phenomenon (80). The vasodilation effect also led ophthalmologists to consider CCBs as a potential therapeutic intervention for POAG patients with a more "vascular" phenotype that progressed despite low IOP (17). However, the effect of CCBs on patients with established POAG has been controversial, with some advocating a possible positive effect against abnormal vascular regulation at the optic nerve head and others concerned for a possible reduction in the perfusion pressure associated with the reduction in systemic blood pressure. In Japan, a small clinical trial randomised 33 NTG patients to receive placebo or the CCB nilvadipine (18). In this trial, three (18%) participants of the nilvadipine group had a DH during the three years of follow up, while 7 (44%) participants from the placebo group had DH. Although there was a tendency toward more DHs in the placebo group, the difference was not statistically significant ($p=0.10$), and the small number of participants makes interpretation of the results very difficult. A different prospective Japanese study randomized 28 NTG patients to Brovincamine (CCB) or placebo and identified a possible VF improvement in some NTG patients (81). A retrospective Japanese publication investigated risk factors for VF progression in NTG patients and identified a possible favourable effect of CCBs (82). These prospective and retrospective clinical publications are supported by basic research which shows an increase in the optic nerve blood flow (83), a neuroprotective effect to retinal cells (84, 85), a reduction in the extracellular matrix response induced by mechanical strain in lamina cribosa cells (86), and reduced the N-methyl-D-aspartate receptor (NMDA-receptor) mediated neurotoxicity in retinal ganglion cells (87). Over the years, these publications have led many clinicians to suggest the use of CCBs in POAG, primarily for NTG (88). However, the relationship

between CCBs and glaucoma is very difficult to untangle from the relationship between POAG and systemic hypertension (HTN); this complex interaction gets even more complex when DHs, hypotension induced by medications, and other pleiotropic effects of hypertension medications are analysed (89). Although it is possible that the improvement in blood pressure levels is responsible for the reduction in the risk of DHs it is unlikely because the association of self-reported HTN and DHs was only marginally clinically significant in the univariable analysis (OR= 0.64; p=0.041) and it was no longer significant after adjustment in the multivariable analysis (OR= 0.96; p=0.886). In addition, the most commonly prescribed group of medications for HTN, the ACE inhibitors, were not associated with DHs. Furthermore, none of the blood pressure related variables (systolic, diastolic, differential between arms, mean arterial pressure) were associated with DHs. It is also possible that the effect of CCB on DHs is related to the reduction in blood pressure and the control of asymptomatic arrhythmias. It has been suggested by different and independent groups that DHs are caused by a similar vascular event as retinal vein occlusion (90, 91). If that were the case, the improvement of the arrhythmias and HTN frequently associated with RVO would improve the vascular dysfunction in patients vulnerable to developing DHs, and therefore, reduce the risk of developing DH+ status (92, 93). An alternative mechanism that could be involved in the relationship between CCBs and DHs is the effect of these drugs on platelet function. The CCBs can inhibit changes in platelet shape, reduce aggregation induced by adenosine-5'-diphosphate (ADP) (94), and reduce the effect of clopidogrel on platelets by inhibiting the cytochrome P 450 enzyme (CYP3A4) (95). It is possible that these changes in aggregation and shape could have led patients to be less likely to develop DHs. An alternative pathway by which CCBs could affect DHs is by improving endothelial function. Previous in vitro and in vivo studies have identified CCBs to have the ability to increase the activity of the enzyme nitric oxide synthase (eNOS) (96) and protect the endothelium from free radicals by scavenging O_2^- (97). In support of the positive effect of CCBs on endothelial function, clinical trials in cardiology have shown improved coronary endothelial function in patients randomized to nifedipine (98). These potential positive effects of CCBs (99) in patients with established POAG are in contrast with the results of Khawaja et al. (19) which identified CCB as the drug class most strongly associated with the risk of developing POAG that requires a procedure (19). The contrasting result between

Khawaja et al. and the current study from the UKGTS could be explained by the different outcome measures; risk of developing POAG requiring a procedure in Khawaja's publication (19) and risk of DHs in patients with newly diagnosed open angle glaucoma in the current study. Although unlikely, both associations could be true, CCB users without glaucoma may have an increased risk of developing POAG in the future and when the glaucoma is already present, CCB may reduce their risk of developing DHs. An alternative explanation is that CCBs have a deleterious effect on patients with POAG but simultaneously, in a different pathway, reduce the risk of having DHs. This situation would lead patients more likely to have DHs, such as NTG patients, to benefit more from CCBs while patients with high tension glaucoma with a lower probability of developing DHs to benefit less or even have a negative effect using CCBs. Also, POAG patients requiring a procedure probably represent those who have deteriorated. Patients with deterioration could represent a subset of patients who are more susceptible to the adverse effects of CCBs, such as systemic hypotension.

Central corneal thickness was not associated with DHs in the UKGTS in contrast to the eight-year report of the OHTS which identified an increased risk of DHs in patients with thinner corneas (12). The different findings might be related to the different inclusion criteria between the OHTS and the UKGTS. The OHTS recruited patients based on high GAT IOP compared to the UKGTS, which did not have an IOP minimum level to recruit participants. The use of GAT IOP in the OHTS as an inclusion criterion, irrespective of the CCT, lead to the inclusion of subjects with thicker corneas with 'pseudo OHT' who had little or no risk of glaucoma and, therefore, of DHs. On the contrary, OHTS participants with thinner CCT were more likely to progress to POAG and subsequently were also more likely to develop DHs. The absence of a high GAT IOP inclusion criterion in the UKGTS explains the lack of association between CCT and DHs in the UKGTS.

An abnormal glucose tolerance was previously described in a case-control study of patients with and without DHs (14) to be a risk factor for DHs. In the UKGTS, a glucose tolerance test was not performed, but glucose and glycosylated haemoglobin (HbA1c) tests were performed and were not associated with DHs. These glucose measurements were performed only on one of the study visits. If the relationship between glucose and DHs requires a temporal association, the

ideal analysis would be in participants with the DH status assessed in a visit in which a glucose test was also performed. In the UKGTS, a small group of participants developed a DH on the visit of the glucose test, but there was no association between these participant's glucose levels and the risk of DHs.

History of anaemia was reported in 48 participants and it was associated with a two-fold increase in the risk of DH+ status in the univariable analysis; it was not statistically significant in the final multivariable analysis, but there was a trend toward being significant with an OR = 2.01 (p=0.098). Previous publications in adults and children have identified a possible association between anaemia and reduced RNFL measured with OCT (100-102). The association between 'history of anaemia' and the later DH+ status is difficult to interpret if the factors that triggered the previous episode of anaemia are no longer active. However, if some of the multiple factors that can produce anaemia are still present, they could be affecting the microcirculation of the optic nerve head and increase the risk of developing DHs. Other diseases associated with hypoxia such as obstructive sleep apnoea syndrome (OSAS) have been reported to be associated with a higher risk of POAG (103) through a probable increase in endothelin-1, which has also been associated with NTG (104) and vascular dysregulation (76) with disc haemorrhages.

Finally, the factors that were associated with the classification of participants as DH+, or with the frequency/number of DH+ visits, were not identified as risk factors for presenting DHs in both eyes. For the small group of 16 participants with bilateral DHs, the use of blood-thinning drugs (aspirin, clopidogrel, or warfarin) was the only variable significantly associated with a 7-fold increase in the risk of bilateral DHs. A population-based study in Sweden identified platelet aggregation inhibitors for the first time as a risk factor for DHs (105); this was later confirmed in a longitudinal cohort in Canada (106) and a retrospective Korean study (13). The latter retrospective study investigated 281 patients with NTG (with 113 cases with DHs) and identified aspirin as a risk factor for DH+ status, but not for the recurrence of DHs. Unfortunately, the authors did not investigate patients with bilateral DHs. Users of blood-thinning drugs are more likely to present with other cardiovascular diseases that could be confounding the association with DHs. It is also possible that these drugs increase the risk of DH+ by their direct

effect on platelet aggregation, although this was not confirmed in the UKGTS. An alternative option is that patients that are vulnerable to DHs develop larger DHs that take longer to disappear; this could make these larger DHs remain in the peripapillary area for a longer period to be detected by a clinician in one or both eyes, which is consistent with the findings in the UKGTS of more bilateral DHs in users of blood-thinning drugs.

To conclude, variables that affect the risk of participants in the UKGTS to develop a DH over the study period differ from the variables associated with the frequency/number of DH+ visits or with the occurrence of DHs in both eyes. The consumption of tea or coffee and the use of SSRIs have, for the first time, been found to be associated with DH occurrence.

*Part of this work was accepted for a paper presentation in ARVO 2020 and is accessible as an abstract (107).

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Chapter 5 A probabilistic approach to exploring a possible systemic pathophysiological mechanism for disc haemorrhages.

3.5.1 Abstract

Purpose: To investigate if bilateral and simultaneous disc haemorrhages (DH) in the United Kingdom glaucoma treatment study (UKGTS) occurred by chance.

Methods: Patients with newly diagnosed open angle glaucoma were included in the UKGTS for 11 visits (24 months) in which the DH status was defined per eye, patient and visit. The following probabilities of detecting a DH were calculated: unconditional or observed [P(A)], joint [P(A∩B)], and conditional [P(A|B)]. The observed probability of simultaneous DHs in both eyes (BE) was compared to the calculated probability assuming each eye to be independent P(A∩B)=P(A)*P(B). It was hypothesised that DHs in BE could be higher than calculated because BE might bleed simultaneously due to a systemic pathophysiological mechanism. Alternatively, if DHs in one eye are independent of the fellow eye at the time of bleeding, the observed and calculated probabilities of DHs in BE would be the same.

Results: 488 (94.6%) patients from the UKGTS had at least one visit with imaging of the optic nerve and were included in this analysis. A DH in either eye on at least one visit was identified in 121 (24.9%) participants; 77 (15.9%) participants with at least one DH in the right eye (RE), and 61 (12.6%) in the left eye (LE). DHs in BE were identified in 18 (3.7%) participants; 11 (2.3%) had a DH on at least one visit in BE simultaneously, and 7 (1.4%) had a DH in BE but at different (non-simultaneous) visits. At a visit level, 5.2% of the visits had a DH in the RE and 4.2% in the LE. Assuming that each eye was independent, the observed probability of participants with DHs in BE simultaneously was 3.7% compared to the 2% predicted (p=0.109), while the probability of a visit with DHs in BE simultaneously was 0.9% compared to the 0.2% predicted (p<0.001). The probability of a participant having a DH at any visit during follow-up increased up to eight times when the fellow eye previously had had a DH. 80% of the

participants with a DH during the complete follow up were categorized as DH+ during the first five visits.

Conclusions: Dependency between eyes was confirmed based on the increased risk of detecting a DH in the fellow eye of a patient with a previous DH. In addition, there was also a possible dependency on the time of bleeding between eyes based on the higher than expected frequency of bilateral and simultaneous DHs.

3.5.2 Introduction

The controversies around the clinical management of patients with DHs go hand in hand with the controversies over their origin. There is a controversy between an arterial (1) or venous origin (2), an association with primary vascular dysregulation (3) or fibrous glial scar formation (4), or the frequently described vascular (5) and mechanical (6) theories that were originally used to describe the pathogenesis of glaucoma. The vascular theory proposes that a primary vascular factor is involved (either locally or systemically), while the mechanical theory proposes that structural changes at the level of the lamina cribrosa cause the mechanical rupture of small blood vessels.

There are clinical characteristics, such as RNFL defects (7-9) and lamina cribosa disinsertion (10), that could support a biomechanical mechanism due to the deformation of the topography of the RNFL surface or lamina cribosa that could stretch vessels, damage its wall, and cause the bleeding. However, RNFL defects and lamina cribosa disinsertion could also support a vascular mechanism in which an abnormal vasculature fails to provide nutrients to a focal sector of the optic nerve resulting in characteristic RNFL defects or LC disinsertion. It would be expected that, if an inherited or systemic biomechanical susceptibility is influencing the appearance of DHs in one eye, that same susceptibility would also be present in the fellow eye and result in DHs in both eyes. However, in this situation, DHs would not be expected to appear in both eyes simultaneously, except by chance. On the contrary, a vascular theory that includes characteristics such as abnormal vascular autoregulation, systemic hypotension, altered levels of vasoactive substances (such as endothelin-1 or nitric oxide), vasospasm, or systemic medications, would be systemically present in all vessels of a patient

and manifest clinically in different parts of the body in which the microcirculation is more vulnerable, such as the simultaneous presentation of DHs in both eyes (3). Considering these two alternative hypotheses, the identification of DHs in BE simultaneously could be better explained by a vascular theory with a possible systemic effect on all microcirculation of the body (11).

Since the early years after the rediscovery of DHs by Stephen Drance (12), investigators have attempted to better understand their role in POAG and possible pathogenesis. Different characteristics of DHs have been analysed, such as the incidence of DHs, their recurrence, and the probability of identifying them. Kitazawa (13) followed 70 patients with NTG with a clinical examination every four weeks to identify the incidence per visit and the cumulative incidence of DHs over a mean (range) follow up time of 15.7 months (6-32 months) that was completed in 58 patients (82.1%). He concluded that NTG patients are from either a group of patients with recurrent DHs or from a group with few or no DHs. Unfortunately, all the data that were collected on how DHs appeared on each visit were not used to construct the probabilities of identifying DHs on a patient, eye, and visit level. A few years earlier than Kitazawa's paper, Krakau (14) reported the probability of identifying at a random visit a DH in a patient that would later be classified as DH+ over multiple follow up visits. He concluded that DHs are part of the glaucomatous damage of the optic nerve in most of the patients, but that DHs tend to appear in 'wet' and 'dry' periods referring to episodes with frequent or infrequent DHs. This novel approach of using probabilities to analyse the occurrence of DHs and explain the natural history of DHs was developed assuming independence between the eyes concerning the moment in time when DHs appeared. He identified a probability of finding at least one DH in a random examination of 26%, and then constructed conditional probabilities given a previous DH to calculate the undetected DHs. Unfortunately, no details were published about why DHs are more commonly detected in the fellow eye of a patient with a previous DH or why DHs are occasionally seen in both eyes simultaneously. Krakau later published a stochastic model of the probability of the occurrence of DHs in an attempt to explain the similarities between DHs and retinal vein occlusion (15). He concluded that DHs are not detected in many patients not because they do not occur, but because during the visits to the clinic just by chance, a DH was not present. In a final publication by Krakau using observed and predicted

probabilities, he identified an 80% cumulative incidence of DHs over a long follow up using previously unpublished and longitudinal data (16). He concluded by proposing that all glaucomatous optic neuropathies are preceded by DHs but accepted that it is impossible to fully confirm or falsify the hypothesis that all glaucoma patients develop DHs and that all DHs end up developing glaucomatous damage.

The implementation of probability analysis on clinical and epidemiological data helped Krakau to understand the role of DHs as precursors of POAG. Irrespective of the validity of his conclusions, the use of probabilities was a useful approach to get a deeper understanding of the pathophysiological role of DHs in patients with POAG. The heated debate of whether DHs and POAG are caused by a mechanical or vascular mechanism could be explored with a probability approach. If it is assumed that a vascular mechanism will affect all vessels in the body systemically and a mechanical mechanism will be less likely to have a systemic effect, then it can be assumed that in the mechanical theory each eye is more independent from the fellow eye (although not completely independent) in comparison to the vascular theory in which both eyes would be expected to be affected by a systemic mechanism simultaneously.

We investigated the series of visits from the UKGTS to identify the pattern of DH appearance in each participant and hypothesised that the higher than predicted presence of simultaneous bilateral DHs supported a possible systemic vascular pathophysiological mechanism. In the context of these clinical trial data, we evaluated whether the incidence of simultaneous bilateral DHs occurred by chance. We also explored the frequency of DHs in the images acquired in UKGTS at an eye, participant, and visit level, and calculated conditional probabilities for the occurrence of DHs.

3.5.3 Methodology

This probabilistic analysis used data acquired during the scheduled visits of the UKGTS. The details of how images were acquired and processed to identify the presence or absence of DHs have been described in Chapter 2. The data from the study were analysed in a multilevel fashion that included a patient level, eye

level, and visit level. Each of the participants was categorized depending on the characteristics of DHs during the study visits as follows:

a) No disc haemorrhage in either eye at any visit
b) Disc haemorrhage in the right eye in at least one visit
c) Disc haemorrhage in the left eye in at least one visit
d) Disc haemorrhage in both eyes but never at the same visit
e) Disc haemorrhage in both eyes simultaneously in at least one visit

Patients in categories b) and c) are termed unilaterals, while those in categories d) and e) are termed non-simultaneous bilaterals and simultaneous bilaterals, respectively. At a participant analysis level, participants in category a) are labelled DH- while any of b), c), d), or e) would make a participant DH+. An example of all the possible categories over the 11 scheduled visits of the UKGTS at a participant level is shown in Table 31.

Table 31 Categories of UKGTS participants depending on DHs

Categories	Visits										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
a) No DH in either eye at any visit											
b) DH in RE in at least one visit	♦RE				♦RE						
c) DH in LE in at least one visit			♦LE						♦LE		
d) DH in BE, not at the same visit					♦LE		♦RE				
e) DH in BE, at the same visit		♦BE				♦BE				♦BE	

DH = disc haemorrhages, RE = right eye, LE = Left eye, BE = both eyes, ♦ = visit with a DH.

In probability theory, the probabilities of an event can be described in three forms:

a) Marginal or unconditional probabilities
b) Joint probabilities
c) Conditional probabilities

The probabilities (marginal or unconditional probabilities) of an event occurring (in this case an eye/patient/visit to have a DH identified) is defined as the frequency of that event based on previous observations (in this case the series of

visits of the UKGTS participants); this can be formulated as P(A) where P is the probabilities of the event A. This marginal or unconditional probability can also be described as the observed probability or observed frequency of an event. The joint probabilities refer to the probability of the event of interest A occurring simultaneously as the event of interest B; this can be formulated as P(A∩B) where P is the probability of one event of interest A (DH in the right eye) occurring simultaneously (∩) as the event of interest B (DH in the left eye). Conditional probabilities are different because they are a measure of the probability of an event occurring given that another event has occurred; this can be formulated as P(A│B) where P is the probabilities of the event of interest (A) given that (which is expressed as │) another event (B) has occurred. The conditional probabilities are exactly the same as the unconditional probabilities if the other event (B) has no influence on the probability of the event of interest (A). In this case, it can be assumed that event A and event B are independent. On the contrary, the conditional probabilities will be different from the unconditional probabilities when the other event (B) has an influence on the event of interest (A). In the current analysis, it is assumed that a systemic pathophysiological mechanism behind DHs will affect both eyes and, therefore, the probability of having a DH in one eye will be affected by the probability of a DH event in the fellow eye.

In the current multilevel analysis of patients from the UKGTS, each participant and each visit had an outcome for right and left eye, and the intersection of those outcomes constructed a third outcome which is DHs in both eyes as illustrated in Figure 29. The probability of a participant having the event of interest (DH) in both eyes simultaneously can be calculated based on either of two assumptions. First, assuming that both eyes are independent, which would support a more local hypothesis for the pathogenesis of DHs that affect each optic nerve irrespective of the events occurring in the fellow eye. Second, assuming that both eyes are affected by the same factor that makes both eyes more vulnerable to bleed simultaneously, which would support a systemic hypothesis for the pathogenesis of DHs. The first and local hypothesis can be formulated as P(A∩B)=P(A)*P(B) where the probability (P) of having the event of interest A and B at the same time is equal to the probabilities (P) of the event of interest A multiplied by the event of interest B. The second and systemic hypothesis can be formulated as P(A∩B)=P(A)*P(B│A) where the probability (P) of having the event of interest A

and B at the same time is equal to the probability (P) of the event of interest A multiplied by the probability of the event B, given that the event A has occurred. The main difference between these formulae is that the first assumes that both eyes are independent while the second constructs the probability considering the fellow eye's probability. Therefore, for the mechanical theory, given that each eye is assumed to be independent of the fellow eye in the time of bleeding, the probability of a simultaneous bilateral DH would be better described by the first formula. Whereas, according to the vascular theory, the probability of a DH in one eye is affected by the status of the fellow eye, and thus, the probability of simultaneous bleeding would be better calculated using the second formula.

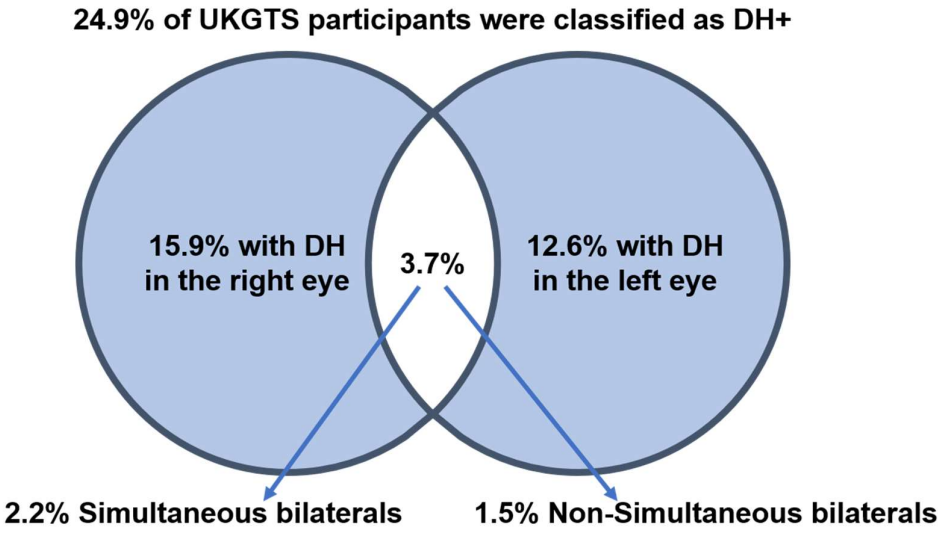

Figure 29 Categorization of patients from the UKGTS depending on the disc haemorrhages.

The UKGTS participants' visit series also enabled the calculation of the number of visits required to categorize a participant as DH+ and to report the number of extra DH+ participants that each extra examination helped to identify.

All analyses were done using R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria) and Python version 3.7.0 (Python Software Foundation. Available at http://www.python.org). Statistical significance for the

difference between the conditional probabilities predicted from different subgroups of participants and the observed probability of bilateral simultaneous DHs was calculated using a two-proportions z-test for equality of proportions with continuity correction.

3.5.4 Results

Among the 516 participants of the UKGTS, 488 (94.6%) had at least one visit with imaging of the optic nerve and were included for this analysis. In 77% of the patient's visits, the DHs were identified in both fundus photographs and HRT images. In the remaining 23% of visits, DHs were identified only from HRT images due to the absence of fundus photography at the same visit (as described in detail in Chapter 2). One hundred and twenty-one participants (24.9%) had a DH in either eye on at least one visit. Seventy-seven participants (15.9%) had at least one visit with a DH in the right eye (RE) and 61 (12.6%) in the left eye (LE). Eighteen participants (3.7%) had at least one visit with a DH in the RE and at least one in the LE (bilaterals). Among these bilateral participants, 11 (2.3%) had a DH on at least one visit in both eyes simultaneously, and 7 (1.4%) had a DH in BE but at different (non-simultaneous) visits (Figure 29). At a visit level, the 488 participants analysed attended 4151 visits with a mean of 8.5 visits per participant. In 357 (8.6%) visits, a participant was noted to have a DH in either eye (DH+ visit), and 3794 (91.4%) were DH-. The four possible categories of DH state, at a participant level, and the number of visits in which the participant had that state, are reported in Table 32 with the mean and percentage of DH+ visits on each category.

Table 32 Number of visits with DHs for each category of UKGTS participants.

	Participants	Visits with DH in RE	Visits with DH in LE	Visits with DH in any eye	Mean (%) number of DH+ visits*
No DH	366	0	0	0	0 (0%)
DH+ Unilateral	104	140	111	251	2.4 (28%)
DH+ BE Non-Simultaneous	7	16	15	31	4.4 (52%)
DH+ BE Simultaneous	11	60	51	75	6.8 (81%)

*% of DH+ visits among each of the categories of UKGTS participants.

After implementing the formula that assumed that each eye was independent, $P(A \cap B)=P(A)*P(B)$, the predicted joint probability of participants having DHs in BE (simultaneous or not simultaneous) was 0.020 (2.0%); this is the product of the probability of DHs in the RE 0.159 (15.9%) multiplied by the probability of DHs in the LE 0.126 (12.6%). In comparison, the observed marginal probability of participants with DH+ in BE was 0.037 (3.7%), with a p value of 0.109 for the difference between the calculated (predicted joint probability) and the observed marginal probability.

The same formula that was used at a participant level, and assumed independence between eyes, was applied at a visit level in all UKGTS participants and then in three subgroups. Among the 488 participants included in the analysis (who attended 4151 visits), 5.2% of the visits had a DH in the RE and 4.2% in the LE. The joint probability of identifying a visit with DHs in BE simultaneously was 0.2%. The observed frequency of visits with DHs in BE simultaneously was 0.9% with a p value <0.0001 for the difference between the calculated (predicted joint probability) and the observed marginal probability. The joint probability of identifying a visit with DHs in BE simultaneously was 1.9%, 15.8%, and 23.9% among the 111 DH+ unilaterals and non-simultaneous bilaterals, the 18 simultaneous and non-simultaneous bilaterals, and the 11 simultaneous bilaterals, respectively. The observed frequency of visits with simultaneous DHs in BE among the simultaneous bilaterals was 31.9%. Table 33 summarizes the joint or predicted probabilities and compares them with the marginal probabilities or observed frequencies of DHs with the p value for the differences in the three subgroups.

Table 33 Frequency of simultaneous bilateral DH+ visits. Differences between the predicted (joint) and observed (marginal) probabilities.

	Participants	Joint or predicted	Marginal or observed	p value
All included participants	488	0.2%	0.9%	<0.0001
Unilaterals and non-simultaneous bilaterals	111	1.9%	0%	0.007*
All bilaterals	18	15.8%	0%	0.003*
Only simultaneous bilaterals	11	23.9%	31.9%	0.207

***** These p values are in comparison to observed probabilities in simultaneous bilaterals.

Conditional probabilities were analysed at a visit level for each eye to assess the effect of a previous RE or LE DH on the fellow eye. The probability of a left eye having a DH at any visit during follow-up increased from an observed or marginal probability of 4.2% to a predicted conditional probability of 38.8% at any future visit when the right eye previously had a DH and from 5.2% to 35.6% for the right eye when the left eye previously had a DH (Table 34).

Table 34 Observed or marginal probabilities (first and second row) for a RE or LE to have a DH at a visit level and conditional probabilities (third and fourth row) given that the fellow eye had had a DH at any previous visit.

	Probabilities
P (RE=DH+)	5.2%
P (LE=DH+)	4.2%
P (RE DH+ | LE DH+)	35.6%
P (LE DH+ | RE DH+)	38.8%
P (visit DH+RE ∩ visit DH+LE) 'predicted'	0.2%
P (visit DH+RE ∩ visit DH+LE) 'observed'	0.9%*

*p=<0.0001

Eighty per cent of the UKGTS participants with a DH during the complete follow up were categorized as DH+ during the first five visits. The cumulative observed or marginal probabilities of identifying a participant as DH+ in the UKGTS are reported in Figure 30, which also depicts Krarau's probabilistic analysis of 1983 (14) superimposed in the same figure for comparison. The current and Krakau's cumulative probability curves have a Spearman correlation coefficient of 0.981 (p= <.0001).

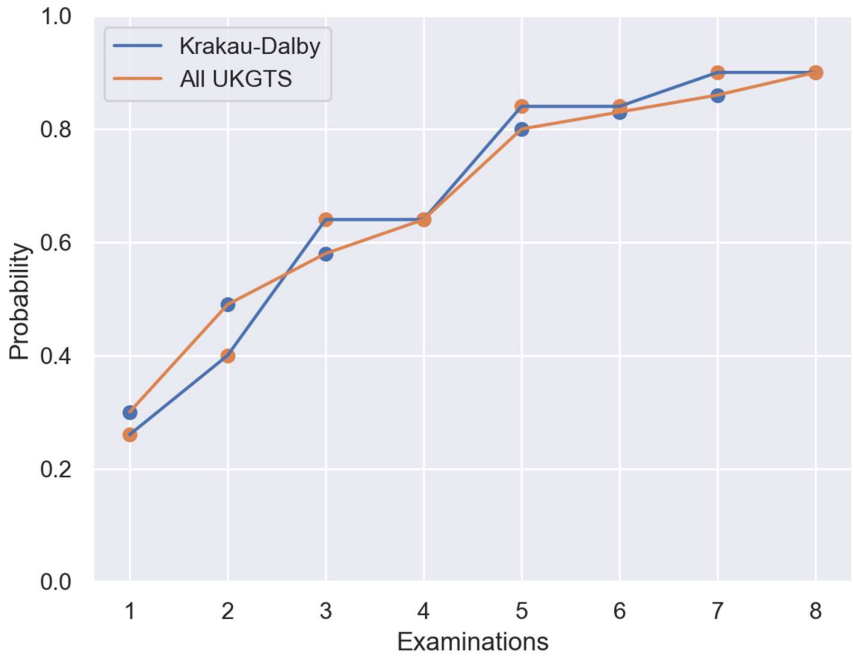

Figure 30 Cumulative probabilities of identifying UKGTS participants as DH+ during the study examinations. Krakau's are superimposed (14).

3.5.5 Discussion

The pattern of DHs appearance in the UKGTS participants' visit series supports a possible systemic pathophysiological mechanism for DHs. The observed frequency of visits with DHs in BE simultaneously was four times higher than the joint probability predicted from the analysis of each eye independently and assuming independence of the eyes. However, simultaneous bilateral DH+ participants had more frequent DHs (Table 31), in itself increasing the probability of identifying a DH in both eyes at the same time. Despite this, there was still an excess of observed (31.9%) over predicted (23.9%) visits with simultaneous bilateral DHs. The difference did not reach statistical significance, but only 11 participants were simultaneous bilateral DH+. The higher than predicted frequency of DHs in BE in the present study supports the well-known correlation between eyes of the same person that has been extensively described in ophthalmic publications about the epidemiology of disease, anatomical variations

and measurements, surgical outcomes, among many other areas (17, 18). However, the results of the current analysis expand this to correlation in time. It seems that the mechanism that increases the risk of DHs in both eyes also increases the risk of developing these bleedings simultaneously in both eyes. These findings support a systemic vulnerability to develop DHs in both eyes that acts simultaneously to make both eyes be more likely to have a DH detected at the same visit.

The analysis of the complete series of visits of all UKGTS participants identified a pattern in which the conditional probabilities of a participant to have a DH at any visit during follow-up increased up to eight times when the fellow eye previously had had a DH. The strong influence of detecting a DH in one eye on the fellow eye further supports the correlation between eyes. However, this finding does not require the development of the DHs at the same time and supports only a generalized vulnerability in both eyes to bleed, acting in both optic nerves independently of the time of bleeding.

The generalized vulnerability to bleed, the higher number of simultaneous bilateral DHs, and the absence of DHs in more than 70% of participants, despite repeated imaging over two years, support the concept of a 'bleeder' and a 'non-bleeder' phenotype. The differentiation of two populations of patients with POAG, 'bleeders' and 'non-bleeders', was previously suggested (13, 19). Others attempted to reject the hypothesis because of the high risk of misclassifying patients as DH- and the occasional detection of DHs in patients that over many years were classified as DH- (14, 16). In support of the division of patients as bleeders and non-bleeders, clinical trials with long follow up and carefully curated data have identified DHs only in a subgroup of patients spanning from OHT to established POAG. The thirteen-year follow-up report of the OHTS only identified DHs in 10.3% of the 1636 participants (20). Although it is important to note that, as all OHTS patients who had a DH during the screening and baseline visits were excluded from the study, this criterion could have resulted in the selection of a population that was less likely to develop frequent DHs during the follow-up. The EMGT, with six-monthly fundus photography and clinical examinations every three months, only identified DHs in half of the participants over a median follow up of 8 years (21). The longitudinal cohort with frequent examination in Kitazawa's

publication from 1986, in which NTG patients were followed every month with clinical examination for a mean of 15.7 months, identified DHs in only 24.8% of the 58 patients (13). A recent Australian longitudinal study followed POAG patients every three months for a mean of 5.3 years and only identified DHs in 45% of the participants (22). It seems clear from the current data and these previous publications that DHs are a common but not universal clinical sign of glaucoma. From the pattern of DH appearance in different patients, it seems that DH+ patients are not from a homogenous category but a rather heterogeneous group that looks more like a spectrum of grey than a black and white categorization. A large group of patients will not develop DHs at all, some patients have few and mostly unilateral DHs, while a small group have very frequent and more bilateral DHs. The 'bleeder' endotype seems to influence more the risk of patients to have DHs than the detection of manifest glaucoma (by the trial definition). For instance, in Chapter 6, it will be shown that 16% of DH+ eyes did not meet the glaucoma eligibility criteria in the UKGTS and 67% of participants with a DH in BE had only one eye that met the eligibility criteria for the trial.

The strong influence that DHs in one eye have on the risk of developing DHs in the fellow eye, in addition to the identification of a larger than predicted number of DHs in both eyes simultaneously, supports a mechanism of DH pathogenesis that involves a systemic factor that makes both eyes strongly correlated and sometimes more likely to bleed simultaneously than just by chance. Mechanical theories (23, 24) based on the stretching that vessels suffer secondary to the deformation of the optic nerve could explain the correlation between eyes but not the simultaneous appearance of DHs. The correlation between eyes could be explained by a generalized vulnerability of the structural material of the optic nerve head that makes some individuals more or less likely to suffer from glaucomatous damage after the insult of IOP, vascular abnormalities, mitochondrial dysfunction, or other factors. However, this vulnerability would not explain the simultaneous bilateral DHs. An example of a mechanical mechanism that affects both eyes simultaneously is the elevation of IOP in both eyes sufficient to disturb the balance of the optic nerve head and lead to a DH. The water drinking test has been extensively studied by Susana; in summary, it consists of assessing the IOP for an hour after a patient consumes one litre of water (25). The mechanism in which this 'challenging test' increases IOP is not fully

understood, but possible mechanisms are the increase in the episcleral venous pressure and the expansion of the choroid secondary to the change in the osmotic pressure in the blood vessels that consequently increases the IOP in patients with an abnormal outflow pathway that is not able to compensate for the increase in ocular volume (26). The 'challenging test' provides the possibility of a simultaneous change in IOP that could lead to bilateral and simultaneous DHs. Alternative explanations include every-day and common mechanisms that increase the IOP temporarily, such as playing musical instruments, some yoga positions, coughing, squeezing the lids, rubbing of the eyes, or any Valsalva manoeuvre that can increase the IOP in BE up to 90mmHg without producing DHs (27). In addition, diseases associated with increased rubbing of the eyes, such as keratoconus (28), and atopic dermatitis (29), have been associated with a higher risk of glaucoma but not with an increased risk of DHs. Furthermore, IOP-related variables were not associated with an increased risk of DHs in the present study (as described in Chapter 4). Alternatively, a vascular mechanism that affects the blood flow, oxygenation levels, vascular regulation, or any variable that reduces the optic nerve perfusion would explain the correlation between eyes and the occurrence of simultaneous DHs. Any substance that is delivered to the optic nerves via the bloodstream could also explain simultaneous DHs. Altered levels of ET-1, MMP-9, or nitric oxide have been previously postulated (3) as possible factors related to the pathogenesis of DHs, but many other hormones or molecules could be involved.

The UKGTS participants who were categorized as DH+ during the complete follow up tended to be classified as DH+ during the first examinations. Eighty per cent of the participants had the first DH detected during the first five visits. The clustering of the detection of DH+ participants over the first examinations is most likely related to different DH presentation patterns among DH+ participants. A small group of the DH+ patients tended to have bilateral DHs and very frequently, the largest group tended to have unilateral and repeated DHs, and a small group had DHs rarely (21). The cumulative DH incidence identified in the UKGTS was very similar to what was reported by Krakau, as depicted in Figure 30. The remarkable similarities between Krakau's publication and the UKGTS reflect similarities in the characteristics of the participants. Krakau's data came from the Dalby population survey with participants ranging from 55 to 70 years, early

glaucomatous VF damage and a mean IOP of 21 mmHg (14). These participants' characteristics are similar to the UKGTS participants who had a mean age of 66 years, early glaucomatous VF damage (median baseline MD for all eligible eyes was -2.9 dB) and a mean baseline IOP of 20 mmHg (30). Other longitudinal studies in NTG (13), glaucoma suspects, and high tension POAG (19) have also reported similar clustering of the identification of DH+ patients during the first examinations. It is interesting that among these longitudinal studies, the identification of a DH was better when fundus photography was implemented. Under these circumstances, it seems important for newly diagnosed POAG patients to have frequent (preferentially aided with imaging to detect DHs) examinations early during the follow up to enable the correct classification of patients as DH+ or DH- and, therefore, to better individualize the risk calculation of patients for progression and potential visual disability due to glaucoma. The previous and current guidelines of the European Glaucoma Society (31, 32) highlight the importance of the first two years of follow up to assess visual field progression “Ideally, all newly diagnosed glaucoma patients should be tested with SAP three times per year during the first two years after diagnosis”. Ideally, this recommendation would be complemented with fundus photography or a careful optic nerve examination. If this were the case, a large proportion of patients could be rapidly categorized as DH+ or DH- and the risk stratification and treatment plan adjusted accordingly.

Some of the assumptions made in this work result in important limitations, particularly the use of the term ‘simultaneous’ to refer to DHs that are seen in both eyes at the same visit, assuming that they bled at the same time. These ‘simultaneous’ DHs represent DHs that persisted long enough to be observed in both eyes at the same visit but could have appeared at different time points between the study visits. Kitazawa (13) identified a mean duration of DHs of 10.6 weeks; this implies that the two eyes of ‘simultaneous bilateral DH+’ UKGTS participants could have bled up to two months apart. With the spacing of the UKGTS scheduled visits, it is impossible to know if DHs occurred exactly on the same day. Another limitation is the method to detect DHs, which was only based on HRT in 23% of the visits. Although this technique has reported good levels of agreement and accuracy to detect DHs (33), it may underestimate the total number of DHs by missing DHs that are only located in the cup or rim with no

extension to the RNFL; this would have misclassified some patients as DH- while they were DH+ but with a DH in the cup or rim that was undetected by the HRT. In Kitazawa's paper, many patients had DHs that lasted less than ten weeks. In the UKGTS the average time between visits was 10 weeks, and it is likely that we did not observe all DHs that occurred and that some participants were wrongly misclassified as DH-. Another limitation that is particularly important for the present study that investigated the pattern of DH appearance in both eyes is that only about 50% of UKGTS participants had manifest glaucoma in both eyes and this likely affected the observed frequency of DHs in both eyes. However, only ~16% of DH+ eyes did not meet the eligibility criteria for the trial (see Chapter 6). Finally, the conditional probabilities constructed to predict future DHs in patients with previous DHs in the fellow eye used the observed frequencies of DHs in the UKGTS, which present an imbalance between the categories DH+ and DH- (24.9% DH+ vs 75.1% DH-) that could lead to over or under prediction of the probabilities. The present results need to be interpreted considering the characteristics of the UKGTS population, which might not be generalizable to patients with glaucoma at a more severe stage of glaucomatous damage, higher levels of baseline IOP, or from different ethnicities. The occurrence of DHs has been reported to be less frequent in patients with a more advanced stage of glaucoma (34), higher IOP (35) and self-reported black race (20).

To conclude, there was a strong correlation in the risk of developing DHs between eyes in participants enrolled in the UKGTS. The categorization of patients as DH+ seems to be an oversimplification of the phenomena because this category is composed of a heterogeneous group of patients: a large group with frequent but mostly unilateral DHs, and two small groups, one with only one DH, and another with very frequent and more bilateral DHs.

*Georgios Lazaridis (PhD student at UCL department of computer science) collaborated in the design of this chapter, the computation of the conditional probabilities, and in the making of Figure 30. He also computed a Variable length Markov chain model and probabilistic suffix trees to better analyse the sequential data that is not presented in this chapter but was presented as a paper presentation in ARVO 2019 and is accessible as an abstract (36).

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Chapter 6 Disc haemorrhages in the United Kingdom glaucoma treatment study (UKGTS) and their impact on visual field deterioration.

3.6.1 Abstract

Purpose: To evaluate disc haemorrhages (DH) in the United Kingdom glaucoma treatment study (UKGTS) and their impact on visual field (VF) deterioration.

Methods: Patients with newly diagnosed open angle glaucoma were included in the UKGTS for 11 visits (24 months) to investigate the effect of latanoprost on VF deterioration. The primary outcome was time to VF deterioration defined with the aid of the guided progression analysis (GPA). The presence of disc haemorrhages (DH) was defined based on a flickering method between baseline and each follow-up Heidelberg retina tomograph (HRT) scan masked to treatment allocation and VF outcomes. DH+ status was defined per patient as the ever presence of a DH in at least one eye. Univariable and multivariable Cox regression models were constructed with VF deterioration as the dependent variable; the multivariable models were adjusted for participant's site, randomization to latanoprost, both eyes being eligible, IOP at baseline in the worst eye, sex, history of heart attack, and smoking.

Results: 460 (89.1%) participants had imaging of the optic nerve and VF data and were included in the present analysis. The cumulative incidence of DH+ status over the mean (SD) of 8.9 (2.9) visits with imaging of the optic nerve was 26.3% (121/460); 72 (59.5%) had more than one DH+ visit (recurrence or rebleeding). 117 (16.7%) of the eligible eyes had a DH compared to 22 (10%) of the non-eligible eyes (p=0.014). 85 (18.5%) of the eyes with the worse MD had a DH compared to 54 (11.7%) of the eyes with the better MD (p=0.004). 36 (29.8%) of the participants categorized as DH+ had VF deterioration compared to 62 (18.3%) of the DH- participants (p=0.008). In the multivariable Cox regression analysis, the adjusted HR for participants categorized as DH+ was 1.67 (95% CI 1.09 – 2.56; p=0.02), for the number of DH+ visits was 1.25 (95% CI 1.12 – 1.38;

p<0.001), and for participants with DHs in the first visit was 2.13 (95% CI 1.16 – 3.90; p=0.01).

Conclusions: DHs were more common in patients with manifest glaucoma and with the worst VF, but DHs were also detected in eyes that did not fulfil the trial definition of glaucoma; this supports the idea that DHs could precede in some patients the detection of glaucoma. The detection of DHs is strongly associated with VF deterioration.

3.6.2 Introduction

Identifying a DH during routine clinical practice is an important finding that makes most ophthalmologists reconsider the risk of visual field deterioration. The high importance that clinicians put on DHs (see Chapter 1) has led researchers to observe carefully and describe in detail all their morphological characteristics and associations using observational studies as well as clinical trials. For instance, the location of DHs has consistently been reported to appear more commonly in the inferotemporal and superotemporal sectors in close proximity to the areas of more frequent RNFL and neuroretinal rim deformation.

There are conflicting reports on the proportion of patients with OAG who develop DHs throughout the disease. Some have even speculated that they could be present in all patients (1). The conflicting evidence among these reports is in part due to differences in: 1) methods of detection, 2) length of follow-up, 3) number of examinations, 4) level of IOP, 5) types of glaucoma, and 6) type of medical or surgical treatment for IOP reduction. Despite these differences, most of the publications seem to be consistent with the idea that DHs are not a universal feature of glaucomatous damage (2, 3). Data from major glaucoma randomized clinical trials overcome many of the limitations of other study designs because the detection of DHs is based on a systematic method, with frequent examinations, usually based on imaging, the length of follow-up is predetermined, the follow-up time involves multiple visits, and a predefined level of IOP or IOP-lowering treatment is usually defined in the protocol.

Although it is accepted that patients with a new or a previous DH have a higher risk of VF deterioration (4), the proportion of patients who will develop VF deterioration, and when, is still debated. The factors that lead only a group of patients with DHs to develop VF deterioration are still unknown. The EMGT, CNTG, OHTS and other studies that identified a higher risk of VF deterioration in participants with DHs (see 2.1.21) have also reported characteristics of the DHs, such as location, duration, frequency/number of DH+ visits or recurrence, without identifying characteristics that could clearly divide the DH+ patients who will develop VF deterioration from those who will remain stable over time. This chapter will analyse the association of DHs with VF deterioration and multiple characteristics of the DHs that could differentiate the DH+ participants who develop VF deterioration.

3.6.3 Methodology

The UKGTS methodology (5), baseline characteristics (6), primary outcome results (7), and risk factors for VF deterioration (8), have been explained in detail. The present analysis will follow previous publications from the UKGTS that used the participant as the unit of analysis for VF deterioration, the selection of the worst eye outcomes when BE were eligible, and the same VF endpoint criteria. Visual fields were acquired with Humphrey Field Analyser Mark II (or II-i) using the Swedish interactive threshold algorithm standard 24–2 programme (Carl Zeiss Meditec, Dublin, CA, USA). VF deterioration was determined with the aid of the Guided Progression Analysis II-i software (version 5.1.1) when at least three visual field locations (contiguous or not) were worse than the baseline (defined with two VFs) at the 5% levels in four reliable VFs. The four VFs had to be two pairs of consecutive VFs with 'possible deterioration' identified in both pairs. The locations with 'possible deterioration' in the first and second pair were not required to be identical. For the survival analysis, time to event (visual field deterioration) was defined as the time from baseline to the fourth VF with confirmed deterioration. From the 516 randomized participants, only 461 were included in the previous publications from the UKGTS (7) because the remainder did not attend follow-up visits. For the present study, 461 participants with at least one visit with imaging of the optic nerve (fundus photography or HRT) were

included. DHs were detected using HRT and fundus photography, as described in Chapter 2.

A participant was categorized as DH+ when a DH was identified in at least one eye for at least one visit. The cumulative or overall incidence of DH+ participants was defined as the percentage of participants who were categorized as DH+, considering all available visits with imaging of the optic nerve (9, 10). The prevalence of DHs was defined as the total number of DH+ participants on every single visit divided by the total number of participants with imaging of the optic nerve on the same visit. The mean prevalence of DHs was the mean of the prevalence of DHs of all UKGTS visits. To define the clock-hour location of DHs, the LEs were transformed into RE format.

In a group of 50 randomly chosen DH+ participants, a random visit and eye with a unique DH was selected. In these eyes, the following characteristics were measured: the area of the DH (measured in mm^2), its angular extent (measured in degrees of the circumference of the optic nerve border, previously delineated in the HRT by two independent glaucoma specialists experienced in delineating the border of the optic nerve in HRT scans), and the association with RNFL defects detected in the reflectivity image of the HRT (measured as a binary category) (11, 12). The characteristics of the DHs were assessed on the reflectivity image produced by the Heidelberg retina tomograph 3 (HRT 3; Heidelberg Engineering, Heidelberg, Germany; image acquisition software version 3.0.60, Heyex 1.6.2.0) and measured by an experienced ophthalmologist, using the PPA Zone Analysis software (Heidelberg Engineering GmbH) (13). The PPA analysis software automatically corrects for magnification utilizing the keratometric values inputted during the acquisition of the scans and calculating the refractive error at the plane of the optic nerve. Due to the telecentric characteristics of the HRT optical system, there are no magnification errors induced by varying the distance between the lens of the HRT and the eye, as is common with fundus photography (14). The optic disc size measurements acquired with HRT have been compared to those acquired by fundus photography corrected by different methods to compensate for magnification, and although the correction based on axial length is the ideal method, the HRT method is very similar to the methods based on axial length (15).

A univariable and multivariable Cox regression analysis was performed with VF deterioration as the dependent variable. The multivariable analysis was adjusted for the study site due to the previously-identified association with VF deterioration (7). The independent variables included in the multivariable analysis were selected as in a previous publication from the UKGTS group (8) and these variables were the following: randomization to latanoprost, both eyes eligible, IOP at baseline (in the worst eye), sex (male as constant), DH+ status at the first visit (around one month after treatment was started), history of heart attack, and smoking (defined as an affirmative response to the question "have you ever smoked as much as one cigarette a day for as long as a year?"). In the previously published multivariable analysis (8), the 'DH+ status at first visit' variable was preferred over other variables related to DHs to avoid including follow-up variables as predictors in a model with only baseline variables. However, for the current analysis that was focused primarily on DHs, multiple multivariable models were constructed to include different variables related to DHs and avoid the collinearity of including all in the same model. The other variables related to DHs investigated were: DH+ status (a DH in any eye at any visit), DH+ status at the first visit, bilateral DH+ status (a DH in the right and left eye in the same or different visits), percentage of DH+ visits (the number of DH+ visit in one or both eyes divided by the total number of visits with HRT imaging multiplied by 100), and the number of DH+ visits (this variable was always adjusted to the total number of visits of each participant). All the variables related to DHs that included data obtained after the first visit were included in the Cox regression analysis as time-dependent covariates. Multiple subgroup analyses were performed to compare differences in the proportion of participants with VF deterioration. For categorical and continuous variables, the Pearson Chi-square test and the independent samples Student's t-tests were used, respectively. The MD progression rate was compared between DH+ and DH- participants using the one-way ANOVA test and the independent samples Student's t-tests. All statistical analyses were performed using SPSS (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.).

3.6.4 Results

Among the 516 participants of the UKGTS, 488 (94.6%) had at least one visit with imaging of the optic nerve and were included in the description of the DH characteristics. For the VF analysis of the present study, 460 (89.1%) participants were included. Only one patient was excluded from the 461 participants previously included in the report of the primary outcome from the UKGTS (7); the excluded participant did not have imaging of the optic nerve. In 77% of the patient's visits, the DHs were identified in both fundus photographs and HRT images. In the remaining 23% of visits, DHs were identified from only HRT images, due to the absence of fundus photography.

The cumulative incidence of DH+ status over the mean (SD) of 8.9 (2.9) visits with imaging of the optic nerve was 26.3% (121/460) when only patients with imaging and a VF outcome were analysed. When all randomized participants were considered, the cumulative incidence was 23.5% (121/516). At the first visit (between one and two months after randomization), 38 of the 460 (8.3%) analysed participants had a DH. At an eye level analysis, 78 (17%) REs and 61 (13.3%) LEs were categorized as DH+. At a visit level, the mean (SD) prevalence of DH+ status at each visit was 8.5% (0.9%); Figure 31 depicts the prevalence at each visit.

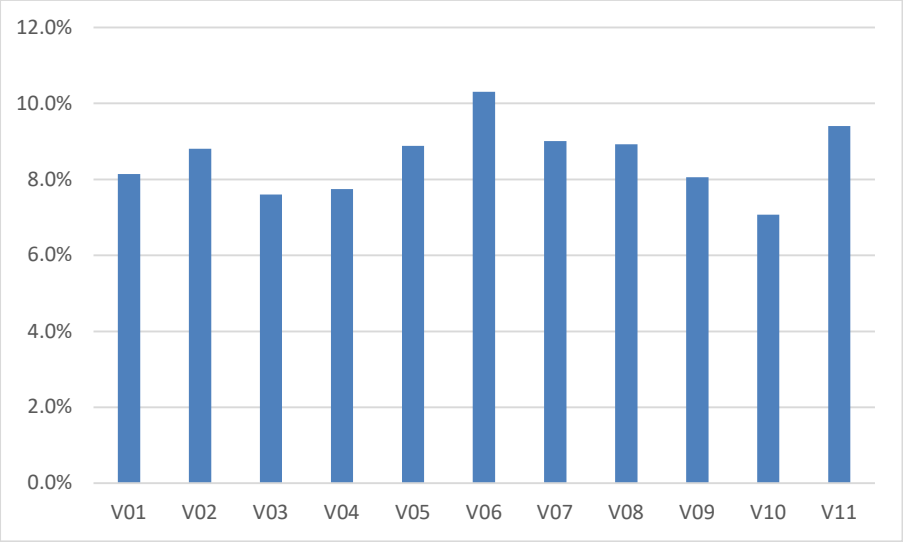

Figure 31 Prevalence of DH+ status in scheduled visits of the UKGTS.

At an eye level, the average (SD) prevalence at each visit of DHs in the RE and LE was 5.5% (0.9%) and 4.6% (0.6%), Figure 32. Among the 121 DH+ participants, the mean (SD) percentage of DH+ visits was 33.1% (26.8%). Among the 121 DH+ participants, 72 (59.5%) had more than one DH+ visit (recurrence or rebleeding); Figure 33 shows the percentage of participants who had from one to eleven DH+ visits. The mean (SD) number of DH+ visits among the DH+ participants was 2.9 (2.5). At an eye level, 139 eyes had a DH in 382 visits. In 34 (9.4%) of the DH+ visits, the participant had a DH identified in BE (bilateral simultaneous DHs). In relation to DHs in more than one visit, 47 (60.3%) of the REs and 33 (54.1%) of LEs had more than one visit with a DH (recurrence or rebleeding). Figure 34 shows the number of REs and LEs that had from one to eleven DH+ visits. The mean (SD, median, IQR) number of DH+ visits was 2.8 (2.4, 2.0, 1.0 – 3.3) for RE and 2.9 (2.7, 2.0, 1.0 – 4.0) for LE. In 30 visits (in 16 eyes) among the 382 DH+ visits (7.9%), considering each eye independently, there was more than one DH in the same eye. Eleven eyes had two DHs, four had three DHs, and one had four DHs.

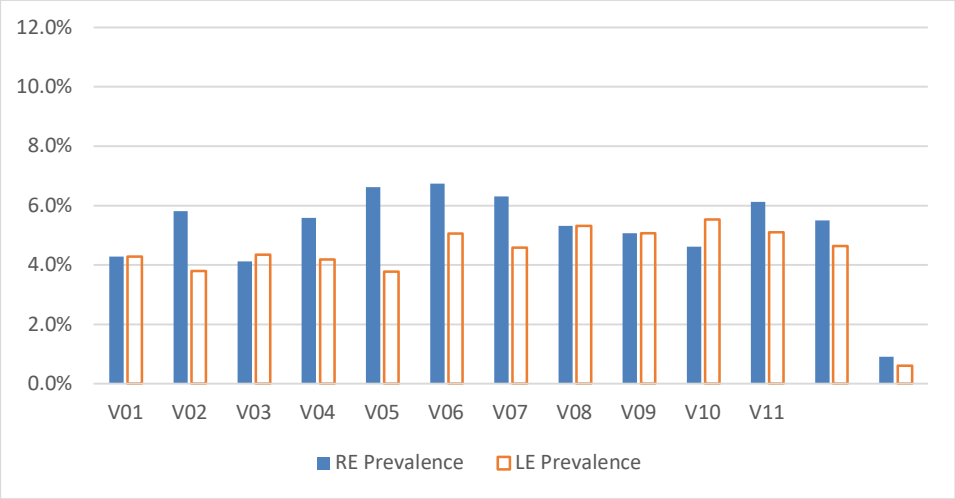

Figure 32 Prevalence of disc haemorrhages in each eye at the UKGTS scheduled visits.

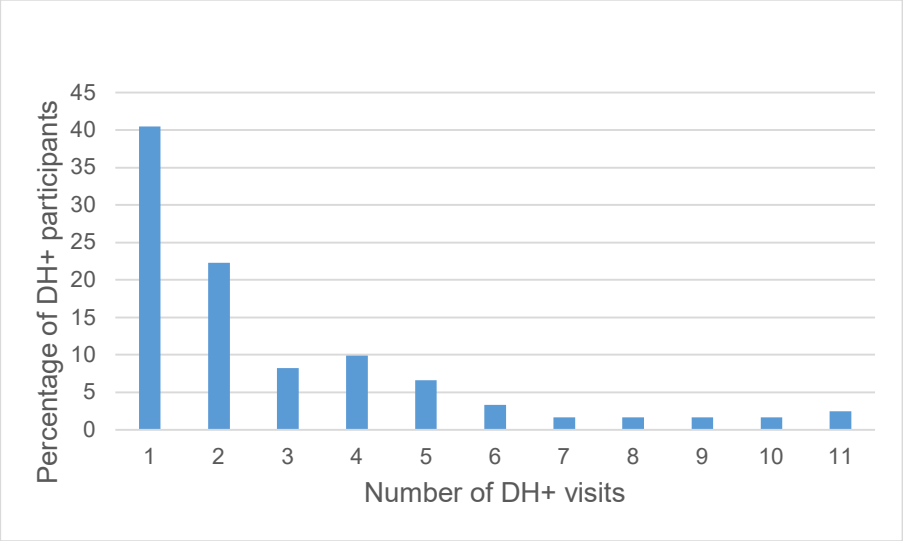

Figure 33 Percentage of DH+ participants in the UKGTS who had from one to eleven DH+ visits.

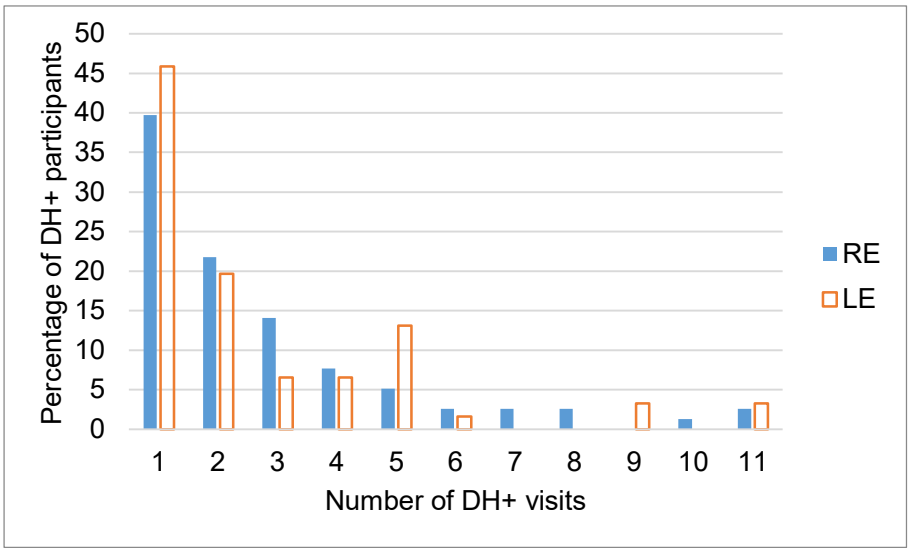

Figure 34 Percentage of right and left eyes of DH+ participants in the UKGTS who had from one to eleven DH+ visits.

Among the 460 analysed participants, 18 (3.9%) had a DH in the right or left eye at the same or different visits ('bilaterals'); 11 had at least one visit with DHs in BE simultaneously, and seven never had a DH in BE simultaneously. The remaining 103 DH+ participants had a DH in only one eye ('unilaterals'), 60 (13%) in the RE and 43 (9.3%) in the LE.

Some of these DHs appeared in eyes that did not satisfy the UKGTS definition of OAG or were not eligible due to poor quality VFs or imaging. Two hundred and twenty-one participants (48%) had only one eye eligible. Overall, 336 (73%) of the RE and 363 (78.9%) of the LE fulfilled the study definition of OAG and were eligible for the study. Among the 139 DH+ eyes, 86 (61.9%) had a DH in an eligible eye with the worst MD, 22 (15.8%) in an eye that was not eligible, and 31 (22.3%) in an eligible eye that had the better MD. One hundred and seventeen (16.7%) of the eligible eyes had DHs compared to 22 (10%) of the non-eligible eyes (p=0.014). Eighty-five (18.5%) of the eyes with the worse MD had a DH compared to 54 (11.7%) of the eyes with the better MD (p=0.004). At a patient level, 61 of the 239 (25.5%) participants with BE eligible had a DH in either eye and at any visit compared to 59 of the 221 (26.7%) of the participants with only one eye eligible (p=0.775). Also, at a patient level, 6 of the 239 (2.5%) participants with BE eligible had a DH in BE during the study visits compared to 12 of the 221

(5.4%) of the participants with only one eye eligible (p=0.107). Among the 221 non-eligible eyes, 21 (9.5%) had VF deterioration. Six (27.3%) DH+ participants had VF deterioration among the 22 not eligible DH+ participants, while 15 (7.5%) DH- participants had VF deterioration among the 199 not eligible DH- participants (p=0.035).

At the first visit with a DH, of the 139 eyes categorized as DH+ (from 121 participants), 86 DHs (57%) appeared in the inferotemporal quadrant. The superotemporal quadrant was the second most common with 34 DHs (23%). Figure 35 depicts the percentage of DHs on each clock hour, considering DHs in the left eye as mirrored to the RE. Eleven eyes had more than one DH during the same visit, and in these cases, all the locations were considered for the count of DHs per clock hour.

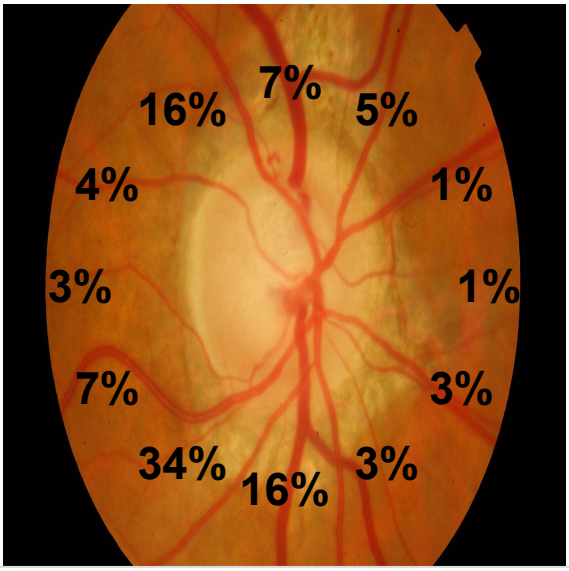

Figure 35 Percentage of DHs per clock hour.

The optic nerve characteristics planned to be measured with the HRT in the group of 50 randomly selected participants were successfully measured. The median (IQR) area of the DHs was 0.11 mm² (0.04 - 0.13), and the median angular extent

was 17 degrees (11 - 21). The DHs were next to a RNFL defect (identified by HRT reflective image) in 52% of the analysed participants.

Concerning VF deterioration, 36 (29.8%) of the participants categorized as DH+ had VF deterioration compared to 62 (18.3%) of the DH- participants (p=0.008). For context, 61 (26.9%) of the participants randomized to placebo had VF deterioration compared to 37 (15.9%) of the participants randomized to latanoprost (p=0.004). Fourteen (36.8%) of the participants with a DH at the first visit progressed compared to 84 (19.9%) of the DH- participants at the first visit (p=0.015). Eight (44.4%) of the 18 participants with a DH in BE at either the same or different visits had VF deterioration compared to 90 (20.4%) of the 442 participants who were either DH- or DH+ but never had a DH in BE (p=0.014). The mean percentage (SD) of DH+ visits was 14% (24%) in the participants with VF deterioration compared to 7% (18%) in the participants without VF deterioration (p=0.003). The same analysis performed only in the 121 DH+ participants identified a mean percentage (SD) of DH+ visits of 38% (26%) in the participants with VF deterioration compared to 31% (27%) in the participants without VF deterioration (p=0.240). The average MD progression rate in the worst eye of all participants in the UKGTS was -0.20dB/year. There was no statistically significant difference between the MD progression rate in DH+ participants (-0.31dB/year) and DH- participants (-0.16dB/year) (p=0.341).

In a univariable Cox regression analysis, DH+ participants (n=121) had a HR of 1.57 (95% CI 1.04 – 2.37; p= 0.03) for VF deterioration, and each extra DH+ visit had a HR of 1.18 (95% CI 1.08 – 1.29; p<0.001) for VF deterioration. Participants categorized as DH+ at the first visit (n=38) had a HR of 1.94 (95% CI 1.10 – 3.42; p= 0.02) for VF deterioration. Participants categorized with bilateral DHs at any visit (n=18) had a HR of 1.93 (95% CI 0.94 – 3.99; p=0.075) for VF deterioration. The percentage of DH+ visits among all participants had a HR of 1.012 (95% CI 1.004 – 1.020; p=0.003) for VF deterioration per additional percent higher.

All variables related to DHs that were included in different multivariable Cox regression models were associated with VF deterioration after adjustment for participant's site, randomization to latanoprost, BE being eligible, IOP at baseline in the worst eye, sex, history of heart attack, and smoking (Figure 36). From the

multivariable Cox regression analysis, the adjusted HR for participants categorized as DH+ was 1.67 (95% CI 1.09 – 2.56; p=0.02), for the number of DH+ visits was 1.25 (95% CI 1.12 – 1.38; p<0.001), for participants with DHs in the first visit was 2.13 (95% CI 1.16 – 3.90; p=0.01) and for participants with bilateral DHs was 2.08 (95% CI 0.97 – 4.45; p= 0.06). The effect of DHs in BE on the risk of VF deterioration was compared to the risk of the unilateral DH+ status. In this analysis of only DH+ participants, DHs in BE had an increased risk of VF deterioration compared to unilateral DHs, but it was not statistically significant with a HR of 1.44 (95% CI 0.66 – 3.17; p=0.364).

To facilitate comparison with the EMGT, we also performed a Cox regression analysis with the percentage of DH+ visits as a predictor of VF deterioration. In the univariable analysis, for every per cent higher of DH+ visits, the risk of VF deterioration increased with a HR of 1.012 (95% CI 1.004 – 1.020; p=0.003). In the multivariable analysis, for every per cent higher of DH+ visits, the risk of VF deterioration increased with a HR of 1.016 (95% CI 1.007 – 1.025; p<0.001).

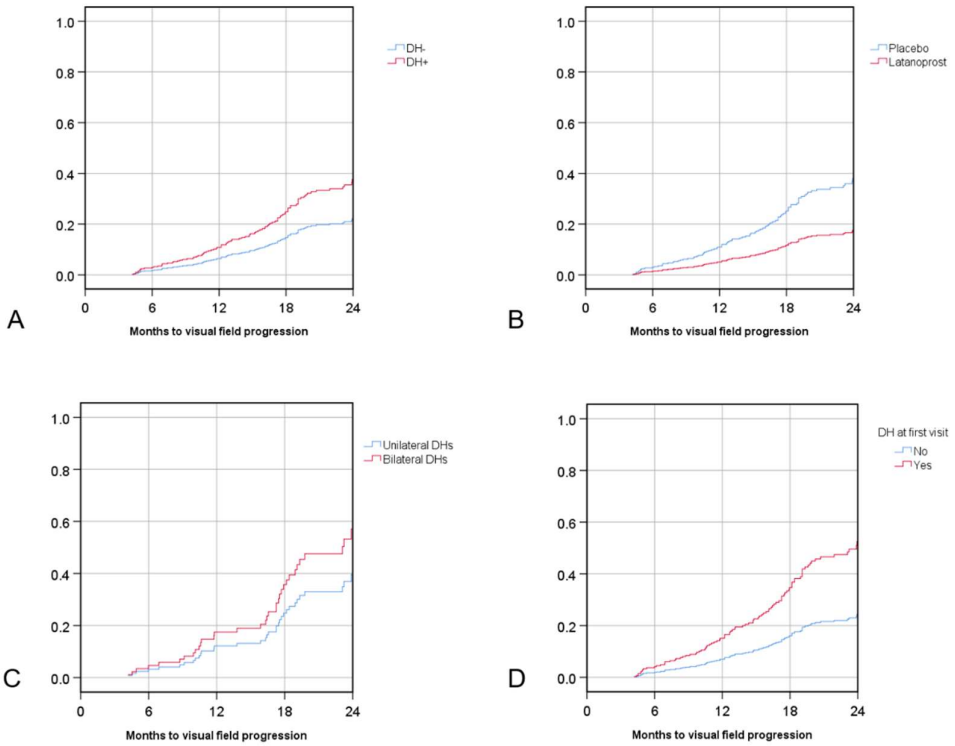

Figure 36 Kaplan-Meier failure estimates for visual field deterioration comparing different binary variables related to DHs and treatment. A) participants with a DH on at least one study visit vs no DH, B) between treatment groups, C) unilateral vs bilateral DH+ status at any visit, and D) participants DH+ vs DH- at the first visit.

3.6.5 Discussion

The presence of at least one DH during follow-up was associated with a substantially increased risk of VF deterioration in the UKGTS. The absence of DHs represented a relative risk reduction of VF deterioration of 38.6% (absolute risk reduction of 11.4%). The participants categorized as DH+ had a 67% increased risk of VF deterioration. Although the sample size was smaller when only DH+ participants were analysed, and the results were not statistically significant, there was a tendency toward a higher risk of VF deterioration in all variables that reflects more bleeding episodes (bilateral DHs, recurrence of DHs, number/frequency of DH+ visits). Compared to participants categorized as DH-, there was a 25% increased risk of VF deterioration for every extra DH+ visit.

The risk of VF deterioration in UKGTS participants with DH+ status (at least one eye at one visit with a DH) increased in comparison to patients with DH- status with a HR of 1.67 (95% CI 1.09 – 2.56; p=0.020). These results would be ideally compared with the EMGT, which shares a similar methodology, primary outcome, and baseline characteristics of the participants (3, 16). However, the EMGT investigators did not publish the effect of the DH status on the risk of VF deterioration. Instead, the EMGT investigators reported the effect of the per cent of DH+ visits on VF deterioration. To facilitate a comparison between the trials, a similar analysis was performed in the UKGTS, and the results were almost identical. Each additional percent higher of DH+ visits increased 2% the risk of VF deterioration in the EMGT (HR=1.02, 95% CI 1.01 – 1.03; p= 0.001) (17) and UKGTS (HR=1.02, 95% CI 1.01 – 1.03; p= <0.001). The striking similarities in this analysis would be expected, considering the similarities between the trials. However, there were some differences between the trials that need to be considered when interpreting the results:

1. The intervention in the treated arm (latanoprost in the UKGTS and argon laser trabeculoplasty plus betaxolol in the EMGT)
2. The number of study visits (mean of 8.9 in the UKGTS and median of 28 in the EMGT)
3. The method of DH detection (HRT-based in all cases of the UKGTS and a combination of clinical examination and fundus photography in the EMGT)
4. The percentage of DH+ participants (26.3% in the UKGTS and 55% in the EMGT)
5. The percentage of participants with exfoliation glaucoma (0.5% in the UKGTS and 9% in the EMGT).

Although the difference in the therapy used in the treated groups to lower the IOP is important, the effect of the interventions on reducing VF deterioration was very similar in both treated arms compared to the placebo o no treatment group (41% in the UKGTS and 45% in the EMGT). The number of study visits and the different percentage of DH+ participants between studies could have had a more important effect in the results of the trials. The DH+ participants in the UKGTS were detected over a shorter period, with more frequent visits, and exclusively based

on imaging. The accuracy in the UKGTS to dichotomize participants as DH+ and DH- based on the ever presence of a DH was strengthened by the frequent visits with imaging. In comparison, in the EMGT participants were classified as DH+ based on clinical examination (every 3 months) and fundus photography (every 6 months) but over a longer period which increased the chances of identifying the presence of DHs and VF deterioration. In most participants it is very likely that the longer follow-up of the EMGT but with sparser fundus photography probably cancelled each other out and produced a detection accuracy that might have been similar to that in the UKGTS. The difference in the percentage of participants with exfoliation glaucoma might also be considered carefully when interpreting the results of these trials. In the exfoliation group of the EMGT, 83% of the participants had VF deterioration, independently of having a DH. The presence of exfoliation was the strongest risk factor for VF deterioration in the final EMGT multivariable regression (HR=2.22; 95% CI 1.31-3.74; p=0.003). The almost total absence of exfoliation glaucoma in the UKGTS makes the risk of VF deterioration in UKGTS participants lower. As a consequence, the DH- participants in the UKGTS would have a lower risk of VF deterioration when compared to the DH- participants in the EMGT (who could have had exfoliation).

The higher risk of VF deterioration of participants with DH+ status at baseline compared to DH+ status during any follow-up visits further supports the concept that VF progression tends to occur close in time to the time of the bleeding (24 months in the UKGTS). On the contrary, the EMGT detected double the number of DH+ participants compared to the UKGTS but over a follow-up period which was more than four times longer and with single VF examinations (compared to the UKGTS with two VFs on the same visit to increase the clustering of examinations) every three months. A DH+ participant in the EMGT would have required a much longer time in the study to have the required number of VF examinations to identify VF deterioration. In addition, it is probable that the few participants who had a DH identified in the EMGT during the last trial visits did not have enough VFs to identify possible VF deterioration accurately. Furthermore, the six-month interval between visits with fundus photography in the EMGT makes the early misclassification of participants as DH- more likely than in the bimonthly examinations of the UKGTS.

The association of DH+ status at baseline and VF deterioration was also analysed in the CNTGS (18, 19) with 22 participants with a baseline DH. The authors identified 12 (55%) DH+ participants with VF deterioration and calculated an increased risk of VF deterioration with an OR of 2.72 (95% CI 1.39 – 5.32; p=0.004). In the UKGTS, the participants who were DH+ at baseline had an increased risk of VF deterioration with a HR of 2.13 (95% CI 1.16 – 3.90; p=0.014). Although the results were very similar, the slightly stronger association of DHs with VF deterioration in the CNTGS could be explained by the exclusive inclusion of NTG patients with a mean baseline IOP of 16.1mmHg compared to the UKGTS with a mean IOP of 20.1mmHg and with only about 50% of the participants having NTG. It is possible that the arguable association of vascular abnormalities with VF progression in NTG patients (20, 21) increases the susceptibility of these patients to develop VF deterioration when a DH is present. However, an alternative explanation is that the longer follow-up in the CNTGS, which lasted up to six years compared to the two years in the UKGTS, offered more time from the baseline DHs for progression.

The 18 participants of the UKGTS with DHs in BE had a tendency toward a higher risk of VF deterioration, despite their small number, which was close to being significant with a HR of 2.08 (95% CI 0.97 – 4.45; p= 0.06). However, it was not significant when participants with unilateral DHs were compared with participants with bilateral DHs. The previous clinical trials in glaucoma did not investigate specifically participants with DHs in BE. However, Seol et al. published a retrospective study that investigated the difference in VF deterioration between patients with DHs in one or both eyes (22). The authors measured VF deterioration in only one randomly selected eye per patient and identified a higher risk of VF deterioration in patients with unilateral DHs in comparison to patients with bilateral DHs with a HR of 2.6 for unilateral DHs. Seol et al. findings are in contrast to the current results that did not identify a statistically significant difference in the risk of VF deterioration between participants with unilateral and bilateral DHs. In addition, there was a tendency toward a higher risk of VF deterioration in participants with DHs in BE when analysed in the complete sample of UKGTS participants (Figure 36 C). The difference between Seol's and the UKGTS results seems to arise from the unit of analysis used in these studies. In the UKGTS, the endpoint of VF deterioration was assessed per patient

compared to Seol's et al. publication in which a randomly selected eye from the patients with bilateral DHs was selected. The randomly selected eye had a 50% chance of being the better eye of a patient, in comparison to the patients with unilateral DHs who always had the eye with DHs selected; patients with unilateral DHs tend to bleed in the worst eye, as seen in the UKGTS in which the eyes with the worse MD were more likely to develop DHs in comparison to the eyes with the better MD (18.5% of eyes with the worse MD had a DH compared to 11.7% of eyes with the better MD).

Almost 60% of the DH+ participants in the UKGTS had more than one visit with a DH (recurrence or rebleeding). In the EMGT, around 65% of the DH+ eyes had a rebleeding (23). The very similar results in both trials, despite the very different length and number of study visits, could be unexpected. Nevertheless, it seems to be related to the more frequent imaging in the UKGTS that made the identification of repeated DHs more likely despite the shorter length of the study. The similar number of recurrences in both clinical trials despite the shorter follow up of the UKGTS also supports the idea that DHs tend to appear in regular periods of time instead of random clusters. If DHs appeared in clusters of episodes, the longer follow-up of the EMGT, but with less frequent examinations compared to the UKGTS, would have missed many of the recurrences clustered around a DH+ visit. On the contrary, if DHs appear at regular intervals, the same proportion of recurrence would be expected to be detected by either a study with a longer follow-up but with fewer examinations (similar to the EMGT) or a study with a shorter follow-up but with more frequent examinations (similar to the UKGTS). The results of the UKGTS are in favour of a local or systemic factor that triggers regular and frequent disturbances of the optic nerve microcirculation that produces recurrent DHs.

Although the association between VF deterioration and a greater number of DHs in the same patient or eye could be considered logical, it has been the subject of a heated debate in previous retrospective studies. Ishida et al. (24) identified an increased risk of VF deterioration in NTG patients with rebleeding, while Kim and Park (25) and Beaufort et al. (26) found no differences between eyes with and without recurrent DHs. Park et al. reinvestigated whether the location of recurrent DHs is important; the authors identified a positive association between DHs

recurring at different locations and VF deterioration (27). The authors named these recurrent DHs which appear in different locations as 'migrating' DHs and identified a faster VF deterioration (-1.07dB/year) compared to recurrent DHs at the same location (-0.32dB/year) or non-recurrent DHs (-0.31dB/year). These findings have been recently replicated by An et al. in a different group of patients with different ethnicity (28). A possible reason for the conflicting evidence could be the unit of analysis. Some authors have investigated rebleeding of the same eye while others have investigated the number of DH+ visits per patient. In addition, the endpoint of VF deterioration between different publications has been variously assessed per eye or per patient and based on event-analysis or trend-analysis. An alternative to the binary variable of recurrent DHs is the use of continuous variables such as the total number of DH+ visits or the percentage of DH+ visits among the total number of follow-up visits. The former is useful because it is easy to understand by clinicians (by every extra visit with a DH the risk of VF deterioration increases by X amount). However, it has to be adjusted for the total number of visits either by including the number of visits in a regression analysis or by using the percentage of DH+ visits as the unit of analysis. The risk of using the percentage of DH+ visits is the limit of the unit from 0 to 100, which strictly speaking, is not an ideal continuous variable. The best data with which to compare the results of the UKGTS are the data from the EMGT which share very similar participant demographics and methodology. However, different publications arising from data of the EMGT have used different units to measure the frequency of DHs. In one of the reports, the authors identified a difference in the frequency of DH+ visits depending on the method of detection, 9.2% based on clinical forms and 12.5% based on photographs (acquired every six months) (3). In another report, the percentage of DH+ visits increased the risk of VF deterioration with a HR of 1.02 (95% CI 1.01-1.03; p=0.001), but only DHs detected clinically were included (16). In the UKGTS, the percentage of DH+ visits increased the risk of VF deterioration with a HR of 1.016 (95% CI 1.007 – 1.025; p<0.001). The difference in the method of DH detection is important because, in the EMGT, 29% of the DHs detected by fundus photography were missed by clinical examination. Nevertheless, the results are very similar, and it seems biologically plausible that the more frequent the DHs, the more likely the patient's VF will deteriorate.

The cumulative incidence of DHs in the UKGTS was 26.3%. Publications with different lengths of follow-up, frequency of examinations, treatments, and examination methods (based on imaging or clinical examination) have reported very different cumulative incidences. A report by Kitazawa, using only clinical examination, identified in a cohort of 58 NTG patients followed monthly for a mean of 15.7 months a cumulative incidence of 43.1% DH+ patients (9). The EMGT identified 55% of the participants as DH+ but with a much longer follow-up (median of 8 years) and detection of DHs based on fundus photography every six months or clinical examination every three months (23). With an even longer follow-up, the OHTS followed 1618 participants for a median of 13 years and identified a 10.4% cumulative incidence of DH+ participants (29). Considering these results, the cumulative incidence of the UKGTS seems to be in the expected range considering the shorter follow-up but more frequent visits with imaging. Irrespective of the exact cumulative incidence of DH, the present and previous publications seem to support the concept that DHs, although a common sign of glaucoma, are not a universal characteristic of all patients. The mean prevalence of DHs was 8.5% which is slightly lower than found in the EMGT (12% based on fundus photography and 9.2% on clinical examination) (3) but in the range of Kitazawa's(9) publication of between 5 to 13%. At an eye level, there were slightly more right eyes categorized as DH+ compared to left eyes, but the difference was not statistically significant. A difference in the risk of having DHs between RE and LE has not been previously reported, but in NTG, some reports identified that the LE is affected first with glaucomatous VF defects and with a worse MD compared to the right eye (30, 31). If there is a difference in the risk of DHs between the right and left eye, it would be expected that the LE would be more likely affected to support the previous publications that identified more cases with glaucoma in the LE and with more advanced disease. However, although not statistically significant, there was a tendency toward more DHs in the RE. If NTG is indeed more common in the LE, this higher risk is unlikely to be related to DHs because they tend to be equally distributed in BE or slightly more common in the RE.

The presence of DHs in BE was identified in 18 participants (14.9%), 11 with at least one visit with simultaneous bilateral DHs and 7 with DHs in BE but at different visits. At a visit level, simultaneous bilateral DHs were detected in 9.4%

of all the DH+ visits. The percentage of bilateral and simultaneous DHs has been assessed in previous studies. A retrospective cohort of 770 PACG patients, which was followed for a mean of 9 years, identified at least one DH in 44 (5.7%) patients and among them, 14 (32%) were in BE at the same or at a different visit (32). In an earlier retrospective cohort of OAG, Shihab identified bilateral DHs in 20% of the patients, but in only one patient were the DHs simultaneous (33). In a population-based screening project of 14,779 Japanese participants, a DH was identified in 88 (0.6%) participants, and among them, four (4.5%) had simultaneous and bilateral DHs (34). A Polish cohort of NTG patients were followed every three months for at least 18 months and a DH was identified in 81 (29.5%) participants, and among them, bilateral DHs were identified in 13 (15.5%) of the participants (the authors did not report if any patient had bilateral and simultaneous DHs) (35). The results of the UKGTS are similar to some of the previous publications, the percentage of DH+ visits with bilateral DHs was 9.4% in the UKGTS and 4.5% in the Japanese population-based screening project. The percentage of patients with DHs in BE (simultaneous or not-simultaneous) was 14.9% in the UKGTS, 15.5% in the Polish cohort of NTG patients, and 20% in Shihab's early publication of OAG. The cohort of PACG patients had the longest follow-up and the highest percentage of bilateral DHs (32% simultaneous or non-simultaneous). The high percentage of bilateral DHs could be due to the very long follow-up, although it could also be related to the smaller proportion of DH+ participants (5.7%) in the cohort that could represent a different phenotype of DH+ patients compared to POAG. The pathogenesis of the glaucomatous neuropathy in PACG is most of the time related to the elevation of IOP that is caused by the trabecular dysfunction associated with the iridotrabecular contact. The much lower number of DH+ patients in PACG compared to POAG further supports the idea that DHs are minimally or not involved in the glaucomatous neuropathy of PACG. Therefore, the small group of patients with DHs in the PACG cohort (5.7%) could represent a more extreme phenotype of DH+ patients with frequent DHs visits and more prone to develop bilateral DHs. These patients, just by chance, may have happened to also have an angle-closure configuration. The glaucomatous neuropathy of these patients may have developed secondary to the angle-closure related IOP elevation or due to the axonal death possibly associated with DHs.

Most of the DHs were identified in the poles of the optic nerve, in particular in the inferotemporal area (57%), which is consistent with multiple previous publications (1, 28, 36, 37). The DHs were also localized in half of the participants next to a recognizable RNFL defect which is in the range that has been summarized in previous publications (1, 36, 37). The localization of DHs in areas where the glaucomatous neuropathy most commonly produces deformation of the optic nerve and next to zones of damaged tissue (RNFL defects) is consistent with the strong association of DHs with active glaucomatous damage (measured as VF deterioration). In 7.9% of the eyes with a DH, the eye had more than one DH at the same visit; these cases are intriguing, but their small number makes it impossible to identify associations with other variables. Although the size of DHs and the extent of the rim that is involved is variable between patients, it is always limited to a small sector of the optic nerve. The size of the DHs had a narrow range from 0.04 to 0.13 mm² as well as the area of the rim involved that ranged from 11 to 22 degrees. The limited size of DHs is consistent with the idea that the peripapillary tissues are limiting the blood from spreading all around the peripapillary RNFL. Although it has been suggested that lower IOP might facilitate the bleeding in patients susceptible to develop DHs (38, 39), there was no relationship between the area or angular extent of the DHs and the baseline IOP in our analysis. Measurements of the size and angular extent of DHs have been previously reported in a retrospective Korean publication that measured the area, length, and angular extent of DHs (38) using fundus photography. The authors used the size and boundaries of the optic disc measured by the Cirrus SD-OCT as a measurement reference to calculate the size of the DH that was identified in a digital red-free RNFL fundus photograph. The magnification induced by the optics of the eye and the SD-OCT camera were corrected using the axial length of the eyes and camera characteristics. The DH area had a median (IQR) of 0.11 mm² (0.04 - 0.13) in the UKGTS and a mean (SD) of 0.072 (0.041) in the Korean study. The angular extent had a median (IQR) of 17 (11 - 22) degrees in the UKGTS and a mean (SD) of 12 (7) in the Korean study. In contrast to the UKGTS, the Korean study identified a larger area of DHs in NTG patients compared to HTG. In a multivariable analysis adjusted for aspirin use, age, and CDR, the authors also found an association between the area of DHs and lower baseline IOP (β= -0.26; p=0.01). The larger area of DHs in the UKGTS compared to the Korean study could be related to differences in the method of measurement. The

authors used Photoshop software to manually align the SD-OCT boundaries of the optic nerve with the red-free fundus photography. There is a high risk of error in this method because the border of the optic nerve is differently defined by the OCT and the fundus photography (40). In an attempt to make both borders match during the manual alignment, the authors could have inadvertently induced an over or underestimation of the size of the discs and DHs. Alternatively, the larger area of the DHs in the UKGTS could be related to the method of DH detection, based on HRT that could have missed smaller DHs. The difference in the association between IOP and the area of DHs could be explained by the retrospective nature of the Korean study in which clinicians could have been biased to assess more carefully clinically and with imaging for DHs in the NTG patients that are known to have more DHs. The characteristics of the populations could also explain the difference, besides the different ethnicity, the Korean study only enrolled patients from a tertiary referral centre compared to the UKGTS in which patients were newly diagnosed POAG referred by local optometrists. The Korean patients could represent a different phenotype of NTG patients in whom the DH size is more affected by IOP and tend to be smaller in size. An alternative explanation for the detection of larger DHs in the UKGTS could be that HRT detects the erythrocytes that produce the red colouration seen in fundus photography and also detects the plasma that extends further away. Although this has never been published, it is biologically plausible that the 670nm wavelength laser of the HRT could detect other characteristics of a DH that are undetected by conventional fundus photography. Figure 37 is an example of one participant from the UKGTS with a large area in the superotemporal region that changes in reflectivity next to a DH in one visit.

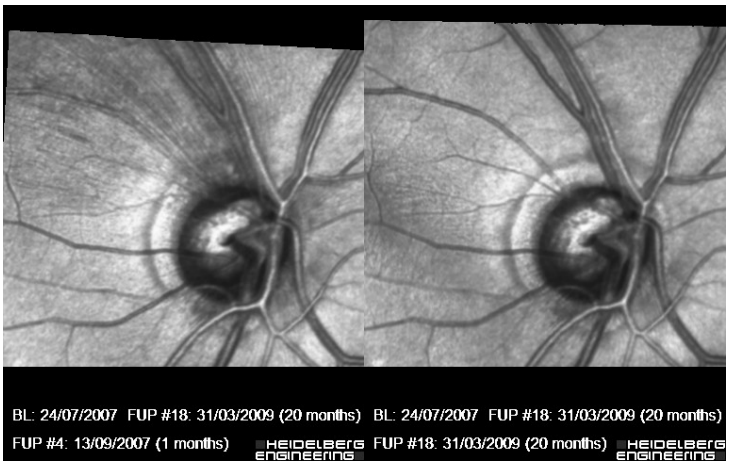

Figure 37 Example of a participant from the UKGTS with an unexpectedly large area with a change in the reflectivity in the superotemporal region of the image acquired one month after baseline (left) that is no longer visible in all following visits (right is the last visit).

Independently of the methods used to measure the size of DHs, it is very clear that DHs tend to vary in area, length, and angular extent between patients. There is conflicting evidence of possible factors that could be associated with these characteristics of the DHs. However, there could be important clinical reasons to investigate further possible ocular or systemic mechanisms that might be involved in reducing the bleeding or enhancing its clearance. If the duration or the extent of DHs is involved in a direct and deleterious effect on the axons of the RNFL, then it would be important to understand the mechanisms that make some DHs remain smaller and be cleared faster.

Among the DH+ participants in the UKGTS, 15.8% had a DH identified in the eye without glaucoma which was not eligible for the trial. Half of the UKGTS participants had only one eye eligible for the trial, although many of these non-eligible eyes had subtle abnormalities in the VF. These minor changes in VFs did not satisfy the UKGTS definition of glaucomatous VF loss which required two or more contiguous points with $P < 0.01$ loss or greater, or three or more contiguous points with $P < 0.05$ loss or greater, or a 10-dB difference across the nasal horizontal midline at two or more adjacent points in the total deviation plot. There are three possible reasons for the identification of DHs in eyes that did not fulfil the trial definition of OAG. Firstly, DHs are normally identified in population-based

studies in eyes without glaucoma in ranges from 0.4% to 1.4% (41, 42). Secondly, non-eligible eyes have OAG, but the definition was too stringent to include patients with the earliest stages of glaucoma. Thirdly, non-eligible eyes with DHs did not have glaucoma yet but are at a very high risk of developing glaucoma because the fellow eye has glaucoma, and the non-eligible eye had a DH which has been associated with an increased risk of conversion to glaucoma (43). If time allowed it, these eyes would be considered more likely to develop glaucomatous neuropathy and VFs abnormalities that would satisfy the trial definition of OAG.

The DHs were more frequently detected in eligible eyes with the worst MD followed by eligible eyes with the best MD, and least frequently in non-eligible eyes. These data support the idea that DHs are increasingly more frequent as the severity increases from glaucoma suspects/normal eyes to the eyes with the worst MD. However, the exclusion criteria of the UKGTS did not allow eyes with severe glaucoma with an MD worse than -16 dB in the worst eye. Previous publications that included patients with glaucomatous damage over all the stages of the diseases have identified the absence of DHs in eyes with very severe structural loss of the neuroretinal rim (39) as well as severe loss of VFs (44). It seems possible that the detection of DHs over the natural history of OAG is better characterised by an inverted V curve distribution. In this model of distribution, subjects without visible glaucomatous neuropathy rarely develop DHs, then the frequency increases as the severity progress, and then in more severe glaucomatous neuropathy the identification of DHs again becomes infrequent. The presence of DHs in eyes with undetectable manifestations of glaucoma (normal optic nerve and visual fields) which later develop unequivocal glaucoma has influenced many ophthalmologists to consider the possibility that DHs might trigger glaucomatous neuropathy in some patients. Since Professor Stephen Drance re-described DHs in 1969-1970 (45, 46), a heated debate has arisen around the role of DHs in the pathogenesis of glaucomatous neuropathy. Some have been strong proponents of DHs as ‘forerunners’ of glaucoma, as Krakau (43) described them, while many others have suggested the opposite and considered DHs only as a consequence of the mechanical deformation induced by the glaucomatous neuropathy (47). The identification in the UKGTS of DHs in eyes which did not satisfy the definition of glaucoma but later developed VFs

deterioration, further supports the idea that DHs could precede manifest OAG. Similar results, but in OHT patients, were identified in the OHTS. The thirteen years follow-up report identified the presence of DHs in 15.8% of participants before 'conversion' to OAG (29). However, most of the participants with a DH did not progress to OAG despite a very long follow-up. An alternative possibility is that DHs do not precede glaucoma and the 'normal eyes' with DHs already have glaucoma but the glaucoma was not identifiable with the diagnostic methods utilized. In this clinical scenario, the DHs detected in 'normal eyes' appear as a result of active but undetectable glaucoma.

The association of DH+ status with VF deterioration that has been replicated in different clinical trials and observational studies needs to be interpreted cautiously. It is important to avoid considering this association as a confirmation of causality. For instance, it is theoretically possible to consider that the presence of blood from a DH in the optic nerve head and peripapillary RNFL layer may trigger the glaucomatous damage via glial cell activation or other mechanisms which could be similar to what has been described for microbleedings in the brain cortex (48). It is also possible that DHs are only an epiphenomenon of progressive damage to the ONH that manifest as bleeding of the capillaries that surround the areas of active glaucomatous damage.

The most important limitation of the comparisons made between the UKGTS and the EMGT and other studies is the method of DH detection that was based only on HRT without fundus photography in 23% of the UKGTS visits. However, as described in Chapter 2, the HRT-based method has been compared with fundus photography with similar results. Another limitation is the time between study visits that was shorter in the UKGTS (around two months). More DHs could have been detected compared to other studies with longer intervals between study visits. In the UKGTS, it is less likely that participants categorized as DH- had an undetected DH between visits. Other particular characteristics to consider from the UKGTS is the predominantly white ethnic origin of participants, the inclusion of only mild and moderate OAG, and the restriction of IOP to below 30 mmHg which makes the generalization of the current findings more difficult for populations with different ethnicities, stages of OAG, or levels of IOP. A limitation of the VF analysis is the lack of agreement between the association of DHs and

the event-based or trend-based VF outcomes. There was a strong association between an event of VF deterioration and DHs, but there was no association with the VFs average MD rates of progression. A similar lack of agreement has been previously described (49) and in our analysis of DHs it could be related to the large proportion of participants with focal glaucomatous damage that could have been undetected using global parameters such as the MD. However, it is in contrast with An et al. who identified an association between DH+ status and progression rates (28); this makes it difficult to generalize the VF results for clinicians who regularly base their assessment of VF deterioration on an event-based analysis rather than a trend-based analysis.

To conclude, the current study confirmed that DHs tend to appear in areas of ongoing glaucomatous damage and are strongly associated with VF deterioration. The identification of DHs seems to precede manifest OAG and increase in frequency as the glaucomatous damage progresses.

*The association between DHs and VF deterioration in the UKGTS was previously published as a paper presentation by Panayiota Founti in ARVO 2017 (50) and later published in Ophthalmology (8).

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Chapter 7 The effect of latanoprost on patients with DHs. Reduction in the frequency of disc haemorrhages and visual field deterioration.

3.7.1 Abstract

Purpose: to investigate the effect of latanoprost on reducing the frequency of disc haemorrhages (DH) and visual field (VF) deterioration.

Methods: The UKGTS included patients with newly diagnosed open angle glaucoma that were randomised to placebo (258) or latanoprost (258) and followed up for 11 visits (24 months) to investigate the effect of latanoprost on visual field (VF) deterioration. Time to VF deterioration was defined with the aid of the guided progression analysis (GPA). The presence of DHs was defined per patient, eye and visit based on a flickering method between baseline and each follow-up Heidelberg retina tomograph (HRT) scan masked to treatment allocation and VF outcomes. The effect of treatment on VF deterioration was analyzed using Cox proportional hazard models with a subgroup analysis comparing the effect of treatment on the survival curves of participants with and without DHs. The proportion of DH+ participants and DH+ visits was compared between treatment arms.

Results: Among the 516 participants, 457 (88.6%) were included in the present analysis. A DH was detected in 59 (25.4%) participants in the latanoprost arm and 62 (28%) in the placebo arm (p=0.607). The mean (SD) percentage of DH+ visits was 8.7 (20.7) % in the latanoprost arm and 8.5 (18.6) % in the placebo arm (p=0.915). Treatment with latanoprost reduced the risk of VF deterioration in the multivariable Cox model with a HR of 0.44 (95% CI 0.29-0.67; p=<0.001); this effect was maintained in the DH- subgroup with a HR of 0.47 (95% CI 0.27-0.80; p=0.005) and in the DH+ subgroup with a HR of 0.35 (95% CI 0.16 -0.76; p=0.008).

Conclusions: The risk of VF deterioration associated with glaucoma can be significantly reduced with latanoprost in patients with or without DHs. Patients

with DHs who were once considered to have a reduced benefit of lowering IOP should be treated with latanoprost. Despite the strong benefit of reducing VF deterioration, the number of DH+ patients or DH+ visits was not reduced with latanoprost.

3.7.2 Introduction

Disc haemorrhages have been consistently associated with a higher risk of VF progression (1, 2); therefore, clinicians have considered them as a sign or indicator (3) that patients require escalation of their treatment (4). However, this clinical rationale assumes that the VF or structural deterioration that has been detected in patients with DHs will respond to IOP-lowering treatment similarly as in patients without DHs.

The positive effect of an intervention to reduce VF deterioration can be measured as the decrease in the rate of VF deterioration (dB/year or %/year) or the increase in the time to event of VF deterioration. These VF outcomes have been reported in previous clinical trials such as the Early Manifest Glaucoma Trial (EMGT) (5), Low Tension Glaucoma Treatment Study (LoGTS) (6), Collaborative Normal Tension Glaucoma Study (CNTGS) (7), or the United Kingdom Glaucoma Treatment Study (UKGTS) (8). Although the general positive effect of IOP-lowering interventions on VF-related outcomes is accepted, there are conflicting results in the subgroup of patients with DHs. In the CNTGS, 11 treated and 12 untreated patients had DHs at baseline (9). There was no statistically significant difference in the time to VF deterioration between treated and untreated participants with DHs. However, there was a tendency toward a longer time to VF progression in the treated group (1533 days untreated compared to 1829 in treated eyes with p=0.305). In the EMGT, 55% of the participants had a DH during the full length of the study (10). The positive effect of treatment was not affected by an interaction between the treatment arm and DH status. However, the authors did not report a subgroup analysis of only DH+ participants and concluded that IOP-reduction in DH+ participants could not totally stop glaucoma progression.

Another variable that would be expected to change after IOP-lowering interventions is the number of DH+ visits compared to before the IOP was

lowered; this would be ideally measured as the percentage of DH+ visits before and after lowering IOP, but it would require patients to have multiple examinations before and after the IOP is lowered. Alternatively, in the setting of randomized clinical trials with untreated arms, despite not having multiple visits before the IOP was lowered, a comparison can be made between the untreated and treated arms. It would be expected that the number of DH+ visits would be lower in the treated arm. If DHs are the consequence of active glaucomatous damage to the optic nerve (irrespective of the mechanism), IOP reduction (that decreases VF deterioration as a functional surrogate of the active glaucomatous damage) would be expected to reduce the number of future DH+ visits. If the number of DH+ visits is not reduced, but VF deterioration is reduced, then it could be possible that the number of DH+ visits is independent of the reduction in IOP; this would also support the idea that DHs are not part of the glaucomatous neuropathy pathogenesis but only a collateral phenomenon present in some patients with glaucoma. In the EMGT, there was no difference in the percentage of DH+ visits between treated and untreated participants. The mean follow-up percentage of DH+ visits, detected clinically and photographically, was 8.4% and 12.4% in the treated arm compared to 8.5% and 11.2% in the untreated arm (p value of clinical and photographic detection was 0.943 and 0.356) (10). Furthermore, the authors did not find an association between the treatment group and participants categorized as DH+ (at least one visit with one eye with a DH). Other research groups have identified the opposite, with a reduction in the number of DH+ visits after IOP-lowering interventions. The Ocular Hypertension Treatment Study (OHTS) thirteen-year follow-up report identified a reduced risk of DH+ visits in the participants randomized to medication (HR=0.7; 95% CI 0.54-0.90; p=0.006) (11). However, a previous report did not find a difference in the cumulative proportion of eyes with a DH between treated and untreated eyes (data from the report with a median follow-up time of 96.3 months) (12).

The UKGTS is an ideal clinical trial to evaluate the effect of reducing IOP on VF deterioration and the number of DH+ visits. The trial used latanoprost (which is the most frequently prescribed glaucoma medication), there were frequent visits with imaging of the optic nerve to precisely categorize participants and visits as DH+ or DH-, the untreated group received a placebo, and there was a careful and

precise determination of VF deterioration with multiple and clustered examinations (13).

3.7.3 Methodology

The present analysis used data acquired during the scheduled visits of the 516 participants of the UKGTS. Previous publications have reported the methodology (13), baseline characteristics (14), primary outcome results (15), and risk factors for VF deterioration for the UKGTS (16). Participants were included in the present study if the VF outcome and imaging of the optic nerve were available. The methodology to define VF deterioration and DHs has been described in Chapter 2 and Chapter 6.

Eligible patients were recruited from consecutive new referrals. After a training visit, the patients signed informed consent and were enrolled in the study. At the baseline visit, participants were randomized using permuted blocks that were stratified by the study centre. Each participant received the same treatment that consisted of either latanoprost 0.005% or placebo at night. Pfizer, Inc. provided an unrestricted Investigator-Initiated Research Grant and the latanoprost and placebo eyedrops in identical bottles. The formulation of the latanoprost and placebo eyedrops included benzalkonium chloride (20mg/ml) and were almost identical apart from the active component of latanoprost 50µg/ml in the treatment arm and hydrochloric acid, sodium hydroxide and water for injection in the placebo arm. Pfizer and other funding organizations had no role in the design or conduct of this research (13).

All clinicians, patients, technicians, and the graders of images were masked to the treatment arms. The frequency of DHs was measured with the same methodology to that described in Chapter 4, the total number of DH+ visits per patient (adjusted for the number of visits) or as the frequency of DH+ visits (the number of DH+ visits divided by the number of visits with imaging of the optic nerve). Participants categorized as DH+ were divided into DH+ participants at the first visit or during follow-up.

The effect of treatment on VF deterioration was compared using hazard ratios from a Cox proportional hazard model. The interaction between DH+ status and the effect of treatment on VF deterioration was analysed in the Cox proportional hazard model with an interaction between DH+ status and treatment arm (one model with DH+ at baseline and another with DH+ at any time during follow-up). Additionally, a subgroup analysis was performed to compare the effect of treatment on the survival curves of participants with and without DHs. The comparison of the treatment effect of latanoprost for the subgroup analysis of DH+ and DH- participants, was performed with the method described by Altman and Bland (17); this method requires the calculation of the relative risk of VF deterioration with latanoprost in DH+ and DH- participants, then utilize the 95% CI to estimate the standard error and perform a test of interaction that produces a ratio of relative risks and a z-score that is finally transformed in two-tailed p-values. For the multivariate regression models, the study site was included in the model with a random effect because it was associated with VF deterioration in a previous publication from the UKGTS (15). To facilitate comparisons with the CNTGS, a Kaplan-Meier survival analysis of the effect of treatment on VF deterioration was performed with a comparison between DH- and DH+ at the first visit using the Log Rank, Breslow, and Tarone-Ware comparisons. The Log-Rank test is usually preferred for survival curves with greater differences toward the right side of the curves, the Breslow test is usually preferred for survival curves with greater differences toward the left side of the curves, and the Tarone-Ware test is usually preferred for survival curves with greater differences toward the middle portion of the curves (18). Additionally, the difference in the rate of VF deterioration was compared between treatment groups and in the subgroup analysis based on DH status. The rate of VF deterioration was measured in all participants included in the present analysis with more than five VF examinations, and it was reported as a change in mean deviation decibels per year (dB/year). The proportion of DH+ participants during all follow-up was compared between treatment arms using the Pearson Chi-Square test. The difference in the number of DH+ visits between treatment arms was compared with an independent sample T-test and with linear regression with the number of DH+ visits or frequency of DH+ visits as the dependent variable. The effect of treatment on the frequency of DH+ visits was adjusted for participants who had ever smoked, reported in Chapter 4 to be associated with the frequency or number of DH+ visits. All

statistical analysis was performed using SPSS (IBM Corp. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.).

3.7.4 Results

The UKGTS enrolled 516 participants and reported the primary VF outcome in 461 participants with sufficient VFs (15). Among those 461, 457 were included in the present analysis. The excluded cases did not have imaging of the optic nerve to categorize participants as DH+ or DH-.

Among the 457 participants included, there was no difference in the number of participants with DH+ status (at least one DH+ visit at any time during follow-up) between treatment arms (59 (25.4%) in the latanoprost arm vs 62 (28%) in the placebo arm; p=0.607). There was also no difference in the mean (SD) percentage of DH+ visits (8.7 (20.7) % in the latanoprost arm vs 8.5 (18.6) % in the placebo arm; p=0.915) or the mean (SD) total number of DH+ visits (0.85 (2.1) in the latanoprost arm vs 0.66 (1.4) in the placebo arm; p=0.254) (see Table 35).

Among the 121 participants with DH+ status, there was also no difference in the mean (SD) percentage of DH+ visits between treatment arms (34.3 (28.7) % in the latanoprost arm vs 32.0 (25.1) % in the placebo arm; p=0.643). There was also no difference in the mean (SD) number of DH+ visits between treatment arms (3.3 (3.1) in the latanoprost arm vs 2.5 (2.0) in the placebo arm; p= 0.088) (see Table 35).

Table 35 Effect of randomization on DHs

	Placebo arm	Treatment arm	p-value
Variables in all 457 participants			
Participants with DH+ status, n (%)	62 (28%)	59 (25.4%)	0.607
Percentage of DH+ visits, mean (SD)	8.5% (18.6)	8.7% (20.7)	0.915
Number of DH+ visits, mean (SD)	0.7 (1.4)	0.9 (2.1)	0.254
Variables in 121 DH+ participants			
Percentage of DH+ visits, mean (SD)	32.0 (25.1)	34.3 (28.7)	0.643
Number of DH+ visits, mean (SD)	2.5 (2.0)	3.3 (3.1)	0.088

Among the 457 participants included, the mean (SD) percentage of DH+ visits was higher in participants with VF deterioration (13.8 (23.9) % with vs 7.2 (18.1) % without VF deterioration; p= 0.003). However, the same analysis in the 121 participants with DH+ status did not identify a difference in the mean (SD) percentage of DH+ visits between participants with and without VF deterioration (37.5 (25.9) % with vs 31.3 (27.0) % without VF deterioration; p=0.240).

There was no association between the percentage of IOP reduction (mean percentage of IOP reduction in the treatment group of 23.3%, SD 18.5%) and the percentage of DH+ visits among the 121 DH+ participants (Unstandardized Coefficients β= 3.39, R^2= 6.8 X 10^{-4}; p=0.776, see Figure 38).

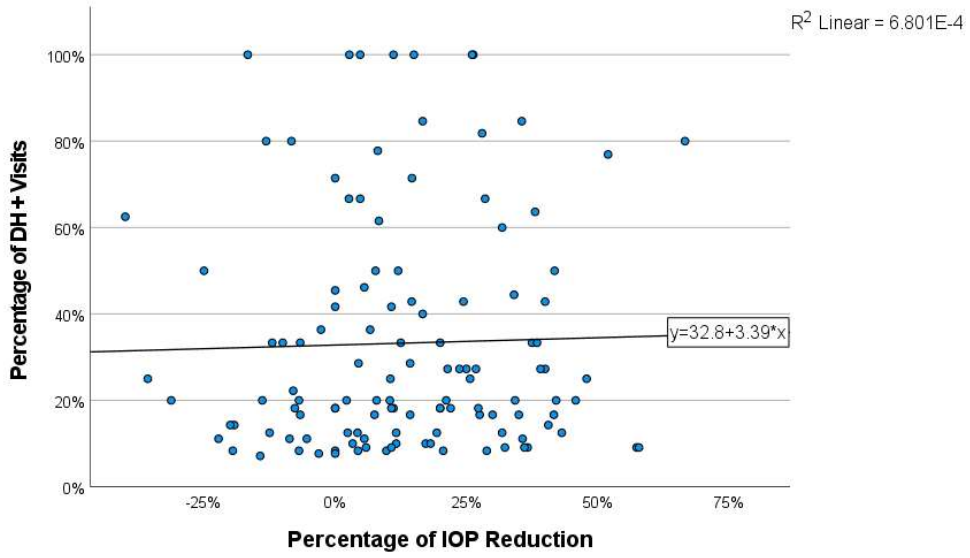

Figure 38 Scatterplot with the correlation between the percentage of IOP reduction and percentage of DH+ visits among the 121 DH+ participants.

More participants with DH+ status (at least one DH+ visit at any time during follow-up) had VF deterioration compared to the participant with DH- status (36 (30%) for DH+ status vs 62 (18%) for DH- status; p=0.009); this represents a 12% absolute risk reduction (40% relative risk reduction) for participants who never had a DH detected compared to DH+. Randomization to latanoprost reduced the number of participants with VF deterioration (61 (27%) in the placebo arm vs 39 (16%) in the latanoprost arm; p=0.004); this represents an 11% absolute risk

reduction (40% relative risk reduction) for participants randomized to the latanoprost arm.

In a subgroup analysis based on the DH status, the effect of latanoprost on reducing VF deterioration was analysed in participants with DH+ and DH- status (see the top part of Table 36) and in participants with first visit DH+ and DH- status (see the bottom part of Table 36).

- Overall DH- status: VF deterioration in 25 (14.5%) in the latanoprost arm vs 37 (22.7%) in the placebo arm (p=0.051). Absolute risk reduction of 8.2%
- Overall DH+ status: VF deterioration in (20%) in the latanoprost arm vs (39%) in the placebo arm (p=0.027). Absolute risk reduction of 19%
- Relative risk reduction of treatment with latanoprost was in DH- = 0.63 (95% CI 0.40-1.01) vs DH+ = 0.53 (95% CI 0.29-0.95)
- Ratio of relative risks = 1.21 (95% CI 0.57-2.57; p=0.616).

The effect of latanoprost on reducing VF deterioration had a tendency to also be present in participants with a DH during the first visit, but it was not statistically significant:

- First visit DH- status: VF deterioration in 32 (15%) in the latanoprost arm vs 52 (25%) in the placebo arm (p=0.014). Absolute risk reduction of 10%
- First visit DH+ status: VF deterioration in 5 (25%) in the latanoprost arm vs 9 (50%) in the placebo arm (p=0.179). Absolute risk reduction of 25%
- Relative risk reduction of treatment with latanoprost in DH- = 0.60 (95% CI 0.40-0.89) vs DH+ = 0.50 (95% CI 0.21-1.21)
- Ratio of relative risks = 1.20 (95% CI 0.45-3.18; p=0.711).

Overall, the number needed to treat (NNT) to reduce VF deterioration with latanoprost was 8.9. In the subgroup analysis based on the overall DH status, the NNT was 5.3 for participants with DH+ status and 12.2 for participants with DH- status. In the subgroup analysis based on the first visit DH+ status, the NNT was 4 for participants with first visit DH+ status and 10 for participants with first visit DH- status.

Table 36 Effect of randomization on the risk of VF deterioration depending on the DH status.

	Placebo arm	Treatment arm	p-value	RRR	NNT
Overall DH- status, n=336					
VF deterioration, n (%)	37 (22.7)	25 (14.5%)	0.051	36.1%	12.2
Overall DH+ status, n=121					
VF deterioration, n (%)	24 (39%)	12 (20%)	0.027	48.7%	5.3
First visit DH- status, n=419					
VF deterioration, n (%)	52 (25%)	32 (15%)	0.014	40%	10
First visit DH+ status, n=38					
VF deterioration, n (%)	9 (50%)	5 (25%)	0.179	50%	4

RRR= relative risk reduction
NNT= number needed to treat

Among the 457 participants included in the present study, 423 had more than five VFs and had the MD rate of VF deterioration calculated. The mean (SD) rate of VF deterioration was -0.007 dB/year (1.17) in the latanoprost group and -0.405 dB/year (1.69) in the placebo group. The mean reduction in the rate of VF deterioration in participants treated with latanoprost was 0.40 dB/year (95%CI 0.12 - 0.68; p=0.005).

The positive treatment effect of latanoprost was maintained irrespective of the DH status over the study visits. Participants randomized to the latanoprost arm had a mean MD rate of VF deterioration of -0.031 dB/year in participants with DH+ status and -0.002 dB/year in participants with DH- status. The mean reduction in the rate of VF deterioration in participants treated with latanoprost was 0.34 dB/year (95%CI 0.01 -0.67; p=0.046) in the participants with DH- status and 0.55 dB/year (95%CI 0.17 - 1.08; p=0.043) in the participants with DH+ status.

The same analysis on participants with first visit DH+ status (35 participants with sufficient VF to calculate the rate of VF deterioration) identified an even larger effect. The mean reduction in the rate of VF deterioration in participants treated with latanoprost was 0.31 dB/year (95%CI 0.03 - 0.59; p=0.030) in the participants with first visit DH- status and 1.38 dB/year (95%CI 0.07 - 2.68; p=0.040) in the participants with first visit DH+ status.

The risk of VF deterioration with the participants included in the present study was significantly reduced in the latanoprost arm with a univariable Cox regression HR of 0.49 (95% CI 0.33-0.75; p=0.001). Furthermore, this remained significant in the multivariable analysis after adjusting for the same variables included in a previous publication from the UKGTS study group (HR = 0.44, 95% CI 0.29-0.67; p=<0.001) (16). In addition, the positive effect of latanoprost was maintained in the 121 participants with DH+ status (Figure 39).

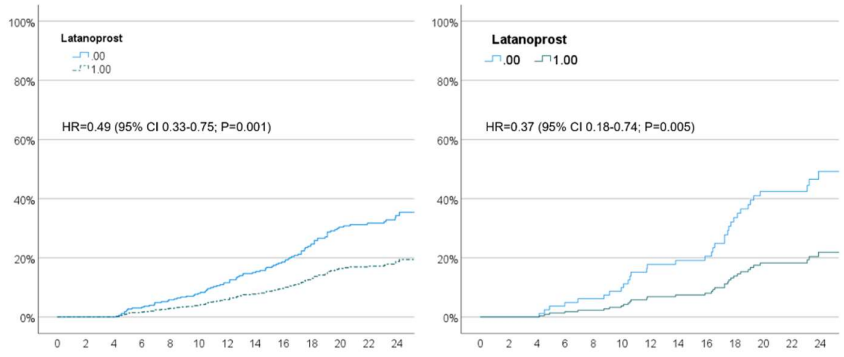

Figure 39 Cox survival analysis. On the right is the univariable effect of latanoprost on all participants. On the left is the univariable effect of latanoprost on participants with DH+ status at any visit. The Y-axis depicts the percentage of participants who progressed, and the X-axis depicts time in months from randomization.

The effect of DHs on VF deterioration was also confirmed in the participants included in the present study. A DH at the first visit had a univariable HR of 1.94 (95% CI 1.10-3.42; P=0.023). In the multivariable analysis, participants with first visit DH+ status had a higher risk of VF deterioration with a HR of 2.11 (95% CI 1.15-3.87; p=0.016) after accounting for the treatment arm, having both eyes eligible, IOP at baseline, sex, history of heart attack, previous smoking, and study site (Table 37 and top of Figure 40); these variables were taken from a similar analysis that the UKGTS investigators have recently published (16). The same multivariable analysis was constructed utilizing DH+ status at any visit during follow-up instead of first visit DH+ status. In this model (Table 38 and the bottom part of Figure 40), DH+ status was still significantly associated with VF deterioration but with a lower HR of 1.63 (95% CI 1.07-2.50; p=0.024).

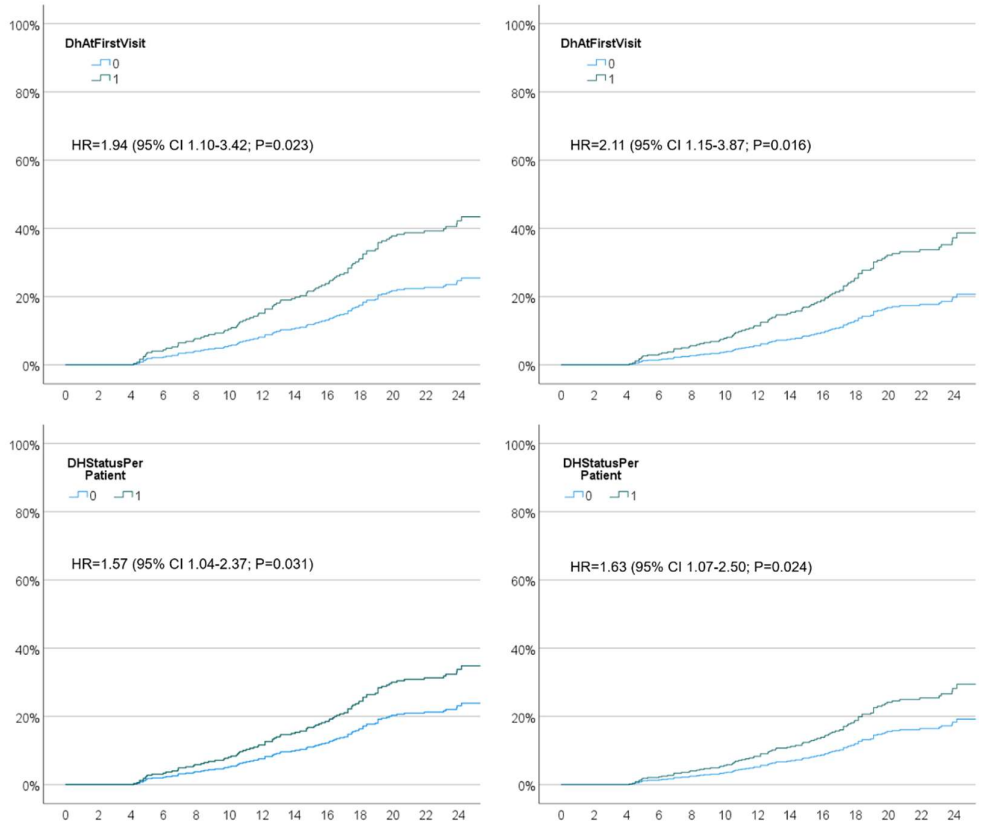

Figure 40 Cox survival analysis of the effect of DHs on VF deterioration. The top is for participants with a DH during the first visit, and the bottom is for a DH at any visit. Right is the univariable and left is the multivariable analysis. The Y-axis depicts the percentage of participants who progressed, and the X-axis depicts time in months from randomization.

Table 37 Multivariable Cox regression of factors associated with VF deterioration (with DH+ at first visit included).

Variables in 457 UKGTS participants	HR (95% CI)	p value
Latanoprost	**0.44 (0.29-0.67)**	**<0.001**
Both eyes eligible	**2.01 (1.29-3.11)**	**0.002**
IOP at baseline	**1.07 (1.03-1.13)**	**0.003**
Female	1.28 (0.84-1.95)	0.256
DH at first visit	**2.11 (1.15-3.87)**	**0.016**
History of hearth attack	0.25 (0.03-1.82)	0.171
Previous and current smoker	**0.54 (0.35-0.85)**	**0.008**

P values and HR are bold when the p-value is <0.05

Table 38 Multivariable Cox regression of factors associated with VF deterioration (with DH+ at any visit during follow-up included).

Variables in 457 UKGTS participants	HR (95% CI)	p value
Latanoprost	**0.45 (0.29-0.68)**	**<0.001**
Both eyes eligible	**1.93 (1.25-3.00)**	**0.003**
IOP at baseline	**1.07 (1.03-1.13)**	**0.003**
Female	1.38 (0.90-2.12)	0.140
DH at any visit	**1.63 (1.07-2.50)**	**0.024**
History of hearth attack	0.22 (0.03-1.62)	0.137
Previous and current smoker	**0.52 (0.33-0.81)**	**0.004**

P values and HR are bold when the p-value is <0.05

The positive effect of latanoprost on reducing VF deterioration was also confirmed in the subgroup analysis based on DH status at any visit during the study. The univariable HR for latanoprost in the DH- subgroup was 0.56 (95% CI 0.34-0.94; p=0.027) and for the DH+ subgroup was 0.37 (95% CI 0.18 -0.74; p=0.005) (see Figure 41). The positive results were also maintained after adjustment in the multivariable analysis with a reduced risk of VF deterioration in the latanoprost arm in the DH- subgroup with a HR of 0.47 (95% CI 0.27-0.80; p=0.005) and for the DH+ subgroup with a HR of 0.35 (95% CI 0.16 -0.76; p=0.008).

These results were further confirmed when the DH status was defined based only on the presence or absence of DHs during the first visit. The univariable HR for latanoprost in the first visit DH- subgroup was 0.52 (95% CI 0.34-0.81; p=0.004) and for the first visit DH+ subgroup was 0.30 (95% CI 0.10 -0.91; p=0.033) (see Figure 42). These positive results were also analysed after adjustment in the multivariable analysis with a reduced risk of VF deterioration in the latanoprost arm in the DH- subgroup with a HR of 0.47 (95% CI 0.29-0.74; p=0.001) and for the DH+ subgroup with a HR of 0.27 (95% CI 0.04 -1.80; p=0.175).

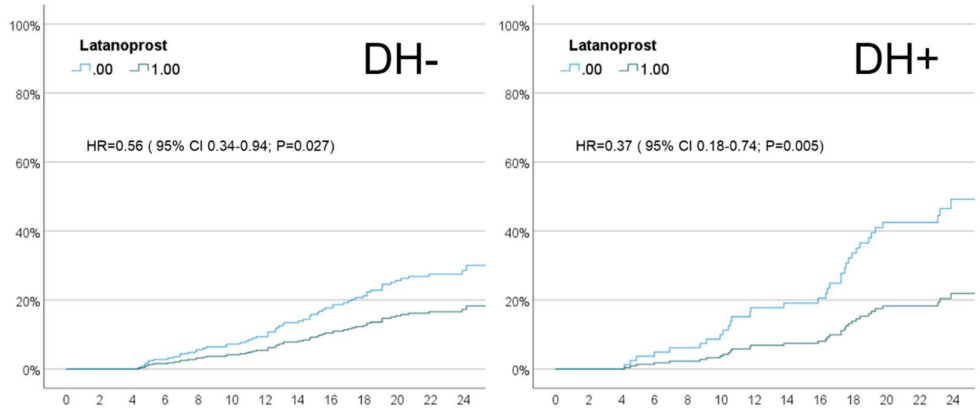

Figure 41 Univariable Cox survival analysis of the effect of latanoprost on VF deterioration depending on DH status at any visit. The Y-axis depicts the percentage of participants who progressed, and the X-axis depicts time in months from randomization.

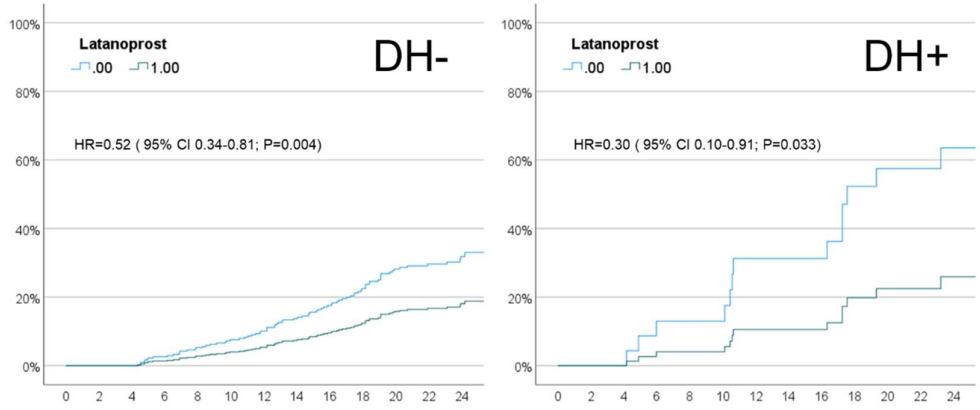

Figure 42 Univariable Cox survival analysis of the effect of latanoprost on VF deterioration depending on the DH status at the first visit. The Y-axis depicts the percentage of participants who progressed, and the X-axis depicts time in months from randomization.

Another multivariable analysis that included the same predictors was constructed to include the interaction between the treatment arm and DH status, but this interaction was not statistically significant when added to the model (HR= 0.59, 95% CI 0.17-2.04; p=0.405). The Kaplan-Meier survival analysis comparing treatment arms only in participants with a DH+ at the first visit (38 participants) identified a mean (95%CI) time to VF deterioration of 17.9 (14.2-21.6) months in

the placebo group and 25.4 (22.6-28.2) months in the latanoprost group; this was statistically significant with a Log Rank (p=0.041), Breslow (p=0.016), and Tarone-Ware (p=0.021) comparisons. The same analysis was performed in the 322 participants with an IOP at baseline below 21mmHg to simulate the inclusion criteria of the CNTGS. In these participants, the mean (SD) IOP was 16.3 (2.7) mmHg, and 27 participants had a DH during the first visit (14 treated and 13 untreated). Among the 27 participants with a DH at the first visit, the mean (95%CI) time to VF deterioration was 24.8 (21.3-28.3) months in treated and 19.0 (14.9-23.2) months in untreated participants; this was not statistically significant with a Log Rank (p=0.236), Breslow (p=0.122), and Tarone-Ware (p=0.151) comparisons. However, in this subgroup of 27 participants, 23 (12 treated and 11 untreated) had sufficient VFs to calculate the rate of VF deterioration. The mean (SD) MD rate of VF deterioration was +0.25dB/year (0.96) in treated participants and -1.65dB/year (2.9) in untreated participants. The mean reduction in the rate of MD VF deterioration in this subgroup of participants treated with latanoprost was 1.90 (95% CI 0.05 – 3.75; p=0.044).

3.7.5 Discussion

The treatment of new OAG patients with latanoprost reduced VF deterioration irrespective of the detection of a DH, either at the first or any visit. Furthermore, the positive effect of latanoprost on reducing VF deterioration had a tendency to be greater in the DH+ participants compared to DH-. The NNT to avoid a case of VF deterioration was 12.2 for DH- participants and 5.3 for DH+. Treatment with latanoprost had an absolute risk reduction on VF deterioration of 8.2% in DH- participants and 19% in DH+ participants. Despite the tendency toward a greater reduction of VF deterioration when IOP is lowered in DH+ participants, the differences in the ratio of relative risks between the DH+ and DH- subgroups were not statistically significant. Therefore, it cannot be confirmed that the VF preservation induced by treatment with latanoprost differs between patients based on the DHs status. The survival analysis also identified a positive effect of latanoprost on VF preservation, with a reduction of 50% in the risk of VF deterioration that was maintained irrespective of the presence of DHs (Overall HR=0.50, DH- HR=0.56, DH+ HR=0.37). The positive effect of lowering IOP in participants with a DH at any visit during follow-up was also confirmed to be

statistically significant when the VF endpoint was investigated with a trend-based analysis instead of an event-based analysis. The mean reduction in the rate of MD VF deterioration in participants treated with latanoprost was 0.34 dB/year without DHs and 0.55 dB/year with DHs. From the previous results of the effect of latanoprost on VF deterioration, it can be clearly concluded that the absolute risk of deterioration, the time to deterioration, and the speed of the deterioration are all reduced with latanoprost in patients with or without DHs. It is important to highlight the higher risk of VF deterioration in placebo-treated participants who had a DH+ status compared to the participants with a DH- status (see survival curves of Figure 41). Therefore, it appears that treatment with latanoprost removes the excess risk of VF deterioration conferred by the DH status.

In contrast, the EMGT concluded that IOP-reduction in DH+ participants could not totally stop glaucoma progression (19). In the EMGT, 55% of the participants had a DH in at least one visit, which increased the risk of VF progression with a HR of 1.02 per additional per cent higher in the percentage of DH+ visits (p=0.001) (20). However, there was no difference in the time to progression when the interaction between the treatment group and DH+ participants was analysed (HR=0.99 with p=0.53). The lack of interaction between the treatment group and DH status suggests that the latanoprost maintained a positive effect on preserving VF independently of the DH status. However, the authors did not report a subgroup analysis comparing the survival analysis between DH+ and DH- participants, but they concluded that "…glaucoma progression cannot be totally halted by IOP reduction in eyes with disc hemorrhages". In the UKGTS, the interaction between the treatment group and the DH+ status was analysed as in the EMGT, and the results were in the direction of a greater reduction in the risk of VF deterioration of latanoprost when participants had a DH, although the results were not statistically significant (HR= 0.59 with p=0.405). Despite the non-significance of this interaction term, visual inspection of the survival curves (see Figure 41) shows a greater reduction in the risk of VF deterioration in participants with a DH+ status that lowers the risk of VF deterioration to a level similar to participants with a DH- status.

A difference between the conclusions of the EMGT and UKGTS regarding the effect of lowering IOP on VF deterioration in DH+ participants was unexpected.

These trials have strong similarities in participants' baseline characteristics, methodology, and VF endpoint (13). However, some aspects of these trials could explain a difference in the effect of lowering IOP on VF deterioration in DH+ participants. The IOP-lowering intervention differed between the trials, latanoprost in the UKGTS and betaxolol plus argon laser trabeculoplasty (ALT) in the EMGT. Despite using different medications to lower the IOP, the percentage of IOP reduction was very similar in both trials (24.8% in the EMGT and 23.3% in the UKGTS). Although latanoprost reduces the IOP more than betaxolol, in the EMGT, it was accompanied by ALT, which resulted in a very similar IOP reduction in both trials. There are three possible scenarios to explain why in the UKGTS latanoprost seemed to eliminate the excess risk of progression conferred by a DH, whereas in the EMGT (19), the authors did not find evidence of an interaction between time to progression in treatment groups and DHs: 1) latanoprost has a non-IOP related effect on preventing VF deterioration in patients with DHs, 2) betaxolol has a deleterious effect on VF function (greater in DH+ patients) that is greater than the positive effect on reducing IOP, and 3) the previous options are partially true with a mild positive effect of latanoprost and a mild negative effect of betaxolol.

In the LoGTS, participants randomized to timolol had a 20% absolute increased risk of VF progression, relative to participants randomized to brimonidine, despite having a similar percentage IOP reduction (21). In addition, participants who were using systemic beta-blockers had a higher risk of having a DH (HR=5.59, p=0.036) (22), and participants randomized to topical timolol had a tendency towards a higher risk of developing DHs (brimonidine HR = 0.30; p=0.072). The LoGTS concluded that timolol was effective at reducing IOP but at the expense of possibly increasing the risk of developing DHs and having a lesser positive effect on VF preservation than brimonidine.

In comparison to the UKGTS, participants enrolled in the EMGT could have had a higher risk of developing DHs and VF deterioration caused by the use of betaxolol. Betaxolol is a β1 selective beta-blocker that was once considered ideal for patients with respiratory problems and patients with possible circulatory abnormalities at the optic nerve circulation due to its vasodilatatory effect (23), acting in particular against endothelin-1 (24). Although it has been repetitively

identified to be better than timolol to prevent VF deterioration (25), it seems possible that betaxolol still has a reduced beneficial effect on VF preservation when compared to latanoprost. The effect on modifying the blood flow to the optic nerve (26), the vasodilation of retinal and optic nerve circulation, and the balance between endothelin-1 and other vasodilating agents, or other factors could be involved as a mechanism to explain the differences found between the trials. Another alternative to explain the difference between these trials could be related to the method used to diagnose DHs. In the EMGT, fundus photographs were acquired every 3-6 months and in the UKGTS HRT scans were acquired on all visits (mean interval of two months). It is more likely that participants in the EMGT developed DHs between visits undetected with imaging which would have misclassified some of the participants as DH-. The difference in the imaging technique to identify DHs could have also influenced the different results between the trials. The HRT that was used in the UKGTS was more likely to miss DHs that were only localized in the cup, which could have caused a selection of DH+ participants who only had DHs that involved the rim and peripapillary tissue. A Korean publication reported the DH characteristics of 168,044 patients older than 20 years who participated in a comprehensive health screening programme and had fundus photographs acquired (27). The authors identified DHs located in the optic nerve head cup base to be more frequently associated with RNFL defects in comparison to DHs in the peripapillary region. If DHs' location affects the risk of VF progression, the under-detection of DHs located in the cup in the UKGTS could have affected the results. It is also important to consider the difference in the length of follow-up between trials (eight years EMGT and two years UKGTS). During the much longer follow-up of the EMGT, more participants could have had a DH secondary to a non-glaucomatous mechanism such as vitreous detachment or vascular retinopathies. Finally, it is also possible that the difference between the result of the trials is not caused by the different drugs or the methodological difference but due to the ALT. However, other than the transient and local inflammatory response, there are no biologically plausible mechanisms that could explain the difference in the VF preservation between DH+ participants treated in the EMGT and UKGTS. The recent Laser in Glaucoma and Ocular Hypertension (LiGHT) trial could help to further test this hypothesis (28).

The results of the Diagnostic Innovations in Glaucoma Study (DIGS) are in favour of a positive effect on VF preservation of lowering IOP in DH+ participants (29). In this longitudinal cohort study, 348 patients were followed up for an average of 8.2 years. DHs were detected based on yearly fundus photography, and VF progression was measured as the percentage of visual field index (VFI) loss per year. Ninety-seven (19%) eyes of 67patients had at least one DH; the rate of VFI change in this group was -0.88%/year compared to -0.31%/year in the patients without DHs. Among the 97 eyes with at least one visit with a DH, 49 eyes had more than four VF before and after the first detected DH. In this group of patients, the reduction in the rate of VFI loss after the first DH was detected was associated with the percentage of IOP reduction (r= -0.61; P= <0.001). The authors concluded that the rate of VFI loss could be reduced by 0.31%/year for every 1 mmHg of IOP reduction. Additionally, the authors identified a significant association between higher rates of VFI loss and the number of DH+ visits. The results of this study are consistent with the results of the UKGTS in which IOP reduction with latanoprost reduced the rate of MD progression in DH+ (0.55 dB/year) and DH- (0.34 dB/year) participants. However, in the DIGS, at the discretion of the attending ophthalmologist, the patients received very different interventions (31% underwent surgery and the remaining were on different medications) to reduce the IOP after the first DH was identified, and these could have had different effects on preventing VF loss.

In randomized clinical trials that evaluate the time to an event of interest, it is controversial to investigate the effect on the survival of a variable that was not present at randomization. Variables that appear during the follow-up and are affected by time are described as time-dependent covariates. For instance, in the UKGTS, DHs during the first visit were considered as baseline variables and were analysed as binary independent variables, although this visit was 4-6 weeks after participants initiated latanoprost or placebo. For this reason, in the CNTGS, the effect of DHs on survival was only investigated when they were detected at baseline (9). However, DHs are a transient phenomenon that cannot correctly categorize patients in a binary fashion based on a single baseline examination. Therefore, investigators of other previous RCTs, such as the EMGT, have decided to evaluate the effect on survival of the presence of a DH at any trial visit (19). Since the UKGTS was designed with a similar methodology as the EMGT,

DHs were also described similarly as the EMGT, based on the ever presence of a DH in at least one eye and one visit; this variable was considered as a time-dependent covariate with time defined as the first visit with a DH detected. At the first visit in the UKGTS, 38 (8.3%) participants had a DH compared to 121 (26.5%) during all study visits. Despite the differences in the statistical analysis required to include variables that were not present at baseline, it is valuable to analyse DHs during the first visit and follow-up because it is possible that with these different approaches, more clinical questions are resolved. There are advantages and disadvantages to each approach. The presence of a DH at any visit allows more patients to be categorized as DH+ and, as the number of examinations increases, it is more likely to be accurate in the categorization of participants as DH+ or DH-. If the risk of VF deterioration in DH+ patients is binary and stable over time, with no interaction with the time when a DH is detected, this approach would be ideal. However, if the risk of VF deterioration is higher after the DH is detected, then this approach would be suboptimal. For example, in the UKGTS, DH+ status based on the presence of a DH at any visit during follow-up includes patients who early in the trial had a DH with numerous subsequent visits in which VF deterioration could have been detected. However, it also included participants with a DH at the last visit in whom it could have been impossible to detect VF deterioration resulting after the DH was detected.

In the UKGTS, treatment with latanoprost reduced VF deterioration in participants with a DH at the first visit. However, the results were statistically significant in the survival analysis but not in the risk reduction analysis. The absolute risk reduction of latanoprost was 10% in DH- participants (p=0.014) and 25% in DH+ (p=0.179). The lack of statistical significance in the DH+ group was probably due to the smaller size of this subgroup analysis (38 participants with first visit DH+ status) and the lesser accuracy of the risk reduction analysis in comparison to the survival analysis. Survival analysis (with the Cox proportional hazards model in the present study) better analysed the effect of an intervention with the time to the event of interest (VF deterioration). Therefore it was more sensitive to detect a statistically significant effect of treatment with latanoprost on VF deterioration irrespective of the presence of DHs during the first visit (HR=0.52, p=0.004 for DH-, and HR=0.30, p=0.033 for DH+). Alternatively, as suggested by the EMGT investigators ‘glaucoma progression in eyes with disc hemorrhages cannot be

totally halted by IOP reduction' (19), the subgroup of participants with first visit DH+ status, in whom there was a longer follow-up to detect VF deterioration, might have had some reduction in the risk of VF deterioration but not complete elimination of the risk. A visual inspection of the survival curves in Figure 42 shows how the risk of VF deterioration cannot be totally halted in participants with either DH+ or DH- status. However, the survival curve of participants in the treated arm with first visit DH+ status was close to the survival curve of the treated arm in participants with first visit DH- status. In addition, the positive effect of lowering IOP in participants with a DH during the first visit was further confirmed when the VF endpoint was investigated with a trend-based analysis instead of an event-based analysis. The reduction in the rate of MD VF deterioration in participants treated with latanoprost was 0.31 dB/year in participants with first visit DH- status and went up to 1.38 dB/year in participants with first visit DH+ status. The present results contrast with those of the CNTGS, which did not find a benefit in the time to VF deterioration when IOP was lowered in participants with a DH at baseline (9). In the CNTGS, 23 participants had a DH at baseline (12 untreated and 11 treated), and in this subgroup, there was no statistically significant difference in the time to VF progression between treatment groups (1829 days in treated vs 1533 in untreated with a p=0.305) (9). The authors of the CNTGS concluded that the DH+ participants at baseline were high-risk patients who could have benefited from IOP reduction, but this was not enough to eliminate the risk of progression. In the UKGTS, a similar analysis but with DH at the first visit found the opposite results. Thirty-eight participants had a DH at the first visit (20 treated and 18 placebo). The mean time to VF deterioration was significantly longer in treated participants (25.4 months in treated vs 17.9 in untreated with a Log Rank p=0.041). Additionally, in the CNTGS, treatment of DH+ participants at baseline reduced the rate of VF deterioration at 0.08dB/year (p=0.741) compared to the UKGTS with 1.38 dB/year (p=0.040). The differences in the effect of IOP reduction between these trials could have been related to the inclusion of patients with an IOP over 21 mmHg in the UKGTS. However, an analysis of only the 322 UKGTS participants with an IOP below 21 mmHg and first visit DH+ status also identified a statistically significant effect of treatment on reducing the rate of VF deterioration and a tendency toward reducing the time to VF deterioration. Therefore, the difference in the results of these trials are more likely due to the type of IOP lowering interventions. Latanoprost alone was used in the UKGTS,

without a target IOP reduction, while in the CNTGS a minimum level of 30% of IOP reduction was defined and it was achieved in 57% of patients with pilocarpine with or without ALT and in 43% when fistulizing surgery was required (30). Although beta-blockers and adrenergic drugs were already available when the CNTGS included participants, these were not allowed due to their potential vasoactive actions (30). Pilocarpine was the only eyedrop allowed to reduce the IOP during the CNTGS due to the extensive experience of using this drug that was isolated in 1874 and used for glaucoma management for more than a century. Pilocarpine reduces IOP and pupil size by its agonist action on the muscarinic receptors found in the iris sphincter muscle, trabecular meshwork, and ciliary muscle. Although it was believed to have a lower vasoactive action compared to beta-blocker, the most current evidence is unclear. A radioactive microsphere technique, used in an animal model treated with topical pilocarpine, showed a trend toward a reduction in the ocular blood flow, although it was not statistically significant (31). Later, the pulsatile ocular blood flow derived from the IOP pulse measured with a pneumotonometer linked to the Langham ocular blood flow system showed no change in patients treated with topical pilocarpine (32). Another animal study identified that pilocarpine induces muscle relaxation of the ciliary arteries dependent on endothelium nitric oxide synthesis (33). The controversial evidence, suggesting a possible vasoactive effect of topical pilocarpine, opened the possibility that a non-IOP effect of this drug could have affected the optic nerve's microcirculation. In addition to the research on the vasoactive effect of pilocarpine, there are publications that have shown a lower capacity of pilocarpine to reduce VF progression in comparison to timolol (34, 35). It is possible that the differences in the UKGTS and the CNTGS are due to lower ability of pilocarpine to reduce VF deterioration due to a possible vasoactive effect on the optic nerve. An alternative explanation to the very different reduction in the rates of MD loss per year between the treatment arms of these trials is the effect of pilocarpine on reducing pupil size. A smaller pupil size reduces the mean deviation of the VF but in the CNTGS the baseline VF was measured under the effect of pilocarpine and all future examinations were required to have a pupil size of at least 2.5mm (36).

Treatment with latanoprost did not reduce the proportion of DH+ participants (25% on latanoprost and 28% on placebo) or the frequency/number of DH+ visits.

However, more DH+ participants had VF deterioration compared to DH- (30% vs 18%). These results are similar to those of the EMGT, which reported over a median follow-up of eight years at least one visit with a DH in 50.4% of treated and 44.4% of untreated participants (19). The CNTGS, unfortunately, did not report the number of DH+ participants over the complete follow-up in the treatment arms. The lack of effect of medical treatment to reduce DHs does not seem to be specific to a single intervention because the EMGT and the UKGTS had similar reductions of IOP (4.5 and 4.6 mmHg) but different IOP-lowering interventions. In the EMGT, participants were treated with ALT and betaxolol, while a participant in the UKGTS only received latanoprost irrespective of the IOP response. It is possible that categorising patients as DH+ based on at least one DH represents a susceptibility to developing DHs that is not reduced by lowering IOP. In the UKGTS, there was no relationship between the percentage of IOP reduction and the frequency/number of DH+ visits or the proportion of DH+ participants. However, it is possible that greater reductions in IOP are required to reduce the frequency/number of DH+ visits. A previous retrospective publication of patients with high and normal-tension glaucoma who underwent trabeculectomy identified a reduction in the frequency/number of DH+ visits after surgery (37). In a publication that pooled data from the DIGS and the African Descent and Glaucoma Evaluation Study (ADAGES), there were fewer DH+ visits after IOP was reduced (38). A total of 166 patients with POAG were analysed, and 37 had a DH during a mean follow-up of over four years. Among the 37 patients with a DH, 24 (64.9%) had a DH recurrence. However, only two eyes recurred after the last procedure to lower IOP, and all the other recurrences were before the last IOP-lowering procedure. Although this was not compared statistically, it seems that the number of DH+ visits was more frequent before IOP was definitively reduced. From these RCT and cohort studies, it seems unlikely that latanoprost or ALT reduces the subsequent number of DH+ visits, but it is possible that larger IOP-reduction may reduce but not eliminate the subsequent DH+ visits.

There are limitations in the present study. The identification of DHs was based only on HRT in 23% of the visits, which could have led to underestimating DHs that were only located in the cup or neuroretinal rim without extending to the peripapillary tissue. However, most of the DHs extended to the peripapillary area

and the HRT technique, described in Chapter 2, showed a good diagnostic ability to detect DHs when compared to fundus photography. Another limitation is concerning the DHs identified during the first visit; this visit was the earliest image-based identification of DHs, but it was 4-6 weeks after treatment was initiated in the baseline visit. Although the gap between these visits was small, it was different from other trials such as the CNTGS or the EMGT and the results should be compared accordingly. Regarding the analysis of the frequency/number of DH+ visits, the analysis of the present study was based only on the comparison between the treatment and placebo group and was unable to compare the frequency/number of DH+ visits before and after treatment was started. In addition, the comparison among the DH+ visits of the total number of DH+ visits between treatment arms should be analysed carefully because participants from the placebo group progressed earlier and had fewer visits in which to have a DH identified. Unfortunately, after the participants exited the study, the DH status information was not available to investigate an equal number of visits between treatment groups. However, the frequency of DH+ visits and the regression analysis (adjusted for the number of study visits) accounted for the total number of visits. Finally, the generalization of the present results should be made cautiously. The UKGTS patients had mild to moderate glaucoma and IOP below 30 mmHg. The benefit of lowering IOP in participants with a DH at the first visit should also be generalized only to a population similar to the UKGTS. The UKGTS population was very different from that clinicians face during routine clinical practice when patients who are already treated for glaucoma develop a new DH and the clinician has the dilemma of whether or not to intensify the treatment (similar to the cases presented in Chapter 1). The results from the UKGTS cannot be easily extrapolated to this clinical scenario because all patients were naïve to treatment and the magnitude of IOP reduction achieved medically is greater when the first eye drop is started in comparison to the addition of a second or third drug. However, evidence from the DIGS (with different medical and surgical interventions to reduce IOP) also suggested that lowering IOP when a new DH is detected in patients already treated was beneficial to reduce VF deterioration (29, 38). Important strengths of the results of the UKGTS were that only 59 of the 516 randomized participants were excluded (11.4%) from the present analysis, participants were randomized to treatment with a fixed regimen

of latanoprost or placebo, and a frequent and standardized method was used to identify DHs and the VF endpoint.

To conclude, the risk of VF deterioration associated with DH+ status can be significantly reduced with latanoprost. Patients with DHs who were once considered to have a reduced benefit of lowering IOP should be treated with latanoprost. Despite the strong benefit of reducing VF deterioration, the number of DH+ patients or DH+ visits was not reduced with latanoprost.

*Part of this work was accepted for a paper presentation in ARVO 2019 and is accessible as an abstract (39).

3.7.6 Bibliography

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Chapter 8 The effect of trabeculectomy on patients with DHs. Reduction in the frequency of disc haemorrhages and visual field deterioration.

3.8.1 Abstract

Purpose: to investigate the effect of trabeculectomy on reducing the frequency of disc haemorrhages (DH) and visual field (VF) deterioration.

Methods: The notes from all consecutive patients that underwent trabeculectomy between May 2007 and September 2013 in the normal tension glaucoma (NTG) clinic at Moorfields eye hospital were reviewed as part of a clinical audit (CA15/GL/12). During routine visits to the NTG clinic, patients underwent VF and Heidelberg retina tomograph (HRT) scans. The DH status was defined per patient, eye and visit based on the retrospective assessment by an ophthalmologist (masked to treatment allocation and VF outcomes) of all clinical notes and HRT scans with a flickering method between baseline and each follow-up scan. VF deterioration was defined with the aid of the guided progression analysis (GPA). The success of the trabeculectomy was defined as the absence of any further glaucoma surgery or deterioration of visual acuity and IOP reduction >20 or 30% of the preoperative value irrespective of the use of glaucoma drops. The success of the surgery was analyzed using Kaplan-Meier survival curves with a subgroup analysis comparing patients with and without DHs. The frequency of DH+ visits was compared before and after trabeculectomy.

Results: 149 eyes of 97 patients underwent trabeculectomy and were included in the present study with a mean (SD) follow-up of 16.5 (5.2) years. Patients had a median (IQR) of 18 (14) visits before trabeculectomy and 18 (7) visits after trabeculectomy and a mean (SD) of 5.4 (2.5) HRT scans before trabeculectomy and 5.7 (2.2) HRT scans after trabeculectomy. A DH was detected, in at least one eye and one visit, in 69 patients (71.1%) based on either clinical examination or HRT images. 77 eyes (56 patients) had a DH+ visit before trabeculectomy and only 16 eyes (16 patients) had a DH+ visit after trabeculectomy. The mean (SD) percentage of DH+ visits before trabeculectomy was 4.0 (4.5) % and 0.8 (1.9) %

after trabeculectomy. The median difference in the percentage of DH+ visits before and after trabeculectomy was 2.4% (IQR 1.9 – 2.9; p=<0.001). The mean trabeculectomy survival for the 20% IOP reduction criterion was 8.5 (95% CI 7.7 – 9.2) years for the DH- eyes and 9.2 (95% CI 8.6 – 9.7) years for the DH+ eyes (log-rank, p=0.204). The mean trabeculectomy survival for the criterion '20% IOP reduction and no GPA event-based progression' was 7.7 (95% CI 6.9 – 8.4) years for the DH- eyes and 8.3 (95% CI 7.6 – 9.0) years for the DH+ eyes (log-rank, p=0.451).

Conclusions: The cumulative incidence of DHs in NTG patients after a long follow-up was 71%; it seems that despite being very common, DHs were not a universal sign present in all NTG patients. Surgical reduction of IOP with trabeculectomy significantly reduced the number of postoperative DH+ visits. The preoperative DH+ status does not affect the positive effect of trabeculectomy in lowering IOP and reducing the risk of VF deterioration.

3.8.2 Introduction

Clinicians usually change the management plan of patients with glaucoma when a new DH is detected. The most frequent change is the reduction in the interval between office visits, and only occasionally some clinicians would recommend trabeculectomy (see Chapter 1). Trabeculectomy is still the most frequently performed glaucoma surgery (1). The recommendation to proceed with trabeculectomy in a patient with DHs assumes that this surgery will benefit patients with DHs in the same way as patients without DHs.

The effect of trabeculectomy on patients with glaucoma has been extensively described (2). Filtrating surgeries for glaucoma have been described since 1858, when De Wecker described the sclerotomy (3). In 1961, Sugar published the clinical results of trabeculectomy performed in nine eyes; in two eyes, the surgery involved the removal of a small piece of the trabecular meshwork, similarly to modern trabeculectomy (4). Since then, important aspects of this surgery have been improved, such as the suturing technique and the use of anti-scarring agents. The Moorfields safer surgery system (5) incorporates most of the modern

improvements in trabeculectomy and its safety and efficacy have been reported in different populations (6, 7).

Despite the numerous publications reporting on trabeculectomy or DHs, no publications have reported potential differences in outcomes of trabeculectomy between patients with or without previous DHs. Due to the frequent detection of DHs in more than 50% of all POAG patients (8), it would be important to know if trabeculectomy achieves the same IOP control and reduction in the risk of VF deterioration in patients with and without DHs. It would also be important to know if the frequency of postoperative DH+ visits, bleb morphology, and complications are affected by the DH status of patients. It would also be important to compare the risk of bleb-related infections and late bleb leaks (associated with thin and avascular blebs) between DH+ and DH- patients. It could be hypothesised that the known abnormalities in the vascular autoregulation in patients with DHs could affect the bleb vasculature, morphology and remodelling process.

A retrospective study of 99 Japanese patients with high-tension glaucoma (HTG) and 50 with normal-tension glaucoma (NTG) reported the number of DH+ visits before and after trabeculectomy (9). The authors identified a reduction in the mean percentage of IOP of 43.4% in HTG and 26.1% in NTG and a reduction in the number of DH+ visits/year from 0.08 (range 0 – 1.95) during the pre-operative years to 0.01 (range 0 – 0.47) during the postoperative years. The results of the Japanese study contrast with the EMGT in which IOP reduction (with laser and drops) did not reduce the number of DH+ visits in the treated compared to the untreated group (8, 10). A retrospective study by Hendrickx et al. observed a reduction in the number of DH+ visits/year after IOP-reduction therapy was initiated in patients with HTG but not in NTG (11).

Data from the NTG clinic at Moorfields Eye Hospital has been used previously to report the safety and efficacy of trabeculectomy in NTG patients (6). However, in the previous report, the effect of reducing the IOP with trabeculectomy on the number of postoperative DH+ visits was not reported; this consecutive cohort of patients with NTG that underwent trabeculectomy is ideal for investigating the effect of surgical IOP reduction on the subsequent frequency/number of DH+ visits and the risk of VF deterioration.

3.8.3 Methodology

Professor Roger Hitchings started the NTG clinic to investigate and care for this group of patients with a standardised system of diagnostic and monitoring methods and a well-defined pathway of treatment that escalated from observation to surgery. Multiple publications from this clinic have been reported previously on different topics such as genetic risk factors (12), asymmetric VF defects (13), the rationale for different therapeutic options (14), use of antimetabolites during glaucoma surgery (15), the relationship between retinal light sensitivity and optic nerve head structure (16), VF progression (17), among others. The NTG clinic is based in Moorfields Eye Hospital NHS Foundation Trust City Road, and it has been continuously supervised by two consultants, Prof Roger Hitchings and Miss Deborah Kamal. Patients were originally seen in the Glaucoma research unit and, more recently, in a regular outpatient clinic. The patients underwent a regular and standardised set of examinations which included diurnal phasing of IOP, VF testing, imaging (mostly with HRT and more recently with OCT), and vigilant examination of the presence of DHs.

A consecutive series of patients who underwent trabeculectomy from May 2007 to September 2013 under the supervision of the same surgeon (Miss Deborah Kamal) was previously audited to assess the safety and efficacy of the surgery. This audit was originally approved in 2014 by the Clinical Audit Assessment Committee from Moorfields Eye Hospital, London, UK, and the results have been published previously (6). The original audit did not explore the DH status of the patients or the characteristics of the fellow eye. In 2015, a re-audit (CA15/GL/12) was approved to investigate (in the same cohort of patients) the effect that the DH status before trabeculectomy could have on the outcomes of trabeculectomy, including the risk of VF deterioration. Another objective of the re-audit was to investigate the effect of trabeculectomy on the frequency/number of DH+ visits. The last clinical notes and HRT scans included in the re-audit were from May 2018. During the original audit, 131 eyes (98 patients) were included. For the re-audit, the DH status of both eyes of all patients was assessed. Additionally, fellow eyes of patients already included in the original audit that underwent trabeculectomy outside the original audit period were included in the comparison of the frequency of DH+ visits before and after trabeculectomy.

The trabeculectomy technique used in all the patients included in the present study was the Moorfields Safer Surgery System, which has been described before (5, 6). In brief, it aims to create a diffuse and posterior bleb that is less likely to develop future complications. A fornix-based conjunctival flap is constructed, followed by the application of subconjunctival mitomycin-C 0.2mg/ml to 0.4mg/ml (adjusted by the surgeon based on the risk of scarring). A scleral flap is secured with three releasable 10-0 nylon sutures aiming for no aqueous flow and finally, the conjunctiva is sutured with 10-0 nylon sutures.

The VF testing was performed with the Humphrey Visual Field Analyser (HFA 2, Carl Zeiss Meditec, Dublin, California, USA) using the 24-2 test grid and the Swedish Interactive Threshold Algorithm (SITA) standard strategy. During the re-audit, VF deterioration was assessed in all patients with more than five VFs after trabeculectomy. Visual field deterioration was defined with the Guided Progression Analysis (GPA) software as deterioration in three locations of the test grid with a P-value < 5% (in comparison with the two first VF tests which are defined as the baseline tests) on three consecutive visits (18). The first two VF tests after trabeculectomy were used to define the baseline tests. The date of the third VF examination in which progression was confirmed was defined as the date of VF deterioration. The assessment of the series of VF tests to define if the deterioration was present was done in the FORUM® Glaucoma Workplace (Carl Zeiss Meditec AG, Jena, Germany) by two glaucoma specialists (Brigid Ning and Jibran Mohamed-Noriega).

Imaging of the optic nerve with the HRT 2 and 3 (Heidelberg Engineering, Germany) was acquired by experienced technicians at the clinicians' request (based on their clinical judgment) on the same day as the clinical examination. During the re-audit, to investigate the DH status of each eye on all visits, the reflectivity images of the optic nerve acquired with the HRT were assessed with the method described in Chapter 2. All images were assessed retrospectively by one glaucoma specialist (Jibran Mohamed-Noriega) with experience in detecting DHs with the HRT-based technique, masked to the history of trabeculectomy in each eye, trabeculectomy outcomes and the DH status defined by the clinician during the routine clinical examination.

During the re-audit process, the clinical notes of all patients from the original audit were reviewed. The DH+ status of both eyes during the complete follow-up (since the first visit at the NTG clinic) of all patients was categorized as DH+ or DH- based on what was recorded by clinicians during routine clinical care. The postoperative visits after any laser procedure (first visit), cataract surgery (visits during the first month), or trabeculectomy (visits during the first three months) were not reviewed to assess the DH status because these types of visits were mainly focused on postoperative care and very rarely had the DH status described. A DH+ visit was defined as a visit with a DH described in the clinical notes, identified in the HRT images or both. The DH status based on each HRT scan or office visit was recorded in a database using a predefined form in Microsoft Access (Microsoft, Redmond, USA; version 16.0) that only allows the observer to register in a blank box the DH status of each eye for a specific date and type of visit (HRT or office visit).

The years of follow-up, the number of clinical examinations and HRT scans were compared before and after trabeculectomy in the following groups: all patients, DH+ and DH- eyes, DH+ and DH- patients, patients with and without post-trabeculectomy DH+ visits. The difference between these groups was analysed using the paired samples T-test or the independent samples T-test. The frequency of DH+ visits was calculated per eye by dividing the number of DH+ visits by the total number of visits; it is reported as mean (SD) and median (IQR). The comparison between the frequency of DH+ visits before and after trabeculectomy was calculated using the Wilcoxon Signed-Rank Test. Due to the asymmetry in the shape of the distribution of the differences between the frequency of DH+ visits before and after trabeculectomy, a sign test was also used.

The criteria to define the success of the trabeculectomy followed the recommendations of the World Glaucoma Association 11th consensus series (19). Success was defined as the absence of any further glaucoma surgery (excluding needling), the absence of deterioration of visual acuity to light perception or worse, and any of the following criteria irrespective of the use of glaucoma drops:

1. IOP reduction of >20% of the preoperative value on two consecutive visits
2. IOP reduction of >30% of the preoperative value on two consecutive visits
3. Absence of GPA event-based VF progression
4. Criteria 1 and 3
5. Criteria 2 and 3

All new patients to the NTG clinic underwent diurnal phasing to confirm the diagnosis of NTG (no IOP measurements exceeding 21 mmHg). The IOP of all preoperative and postoperative visits was recorded; the preoperative IOP was defined as the IOP on the day when the patient was listed for surgery. The IOP on the last visit was used to calculate the percentage of IOP reduction in patients that did not have other reasons for failure, such as further glaucoma surgery or IOP not reduced >20% or 30% of the preoperative value on two consecutive visits. The mean survival of trabeculectomy was calculated using Kaplan-Meier survival curves for DH- and DH+ eyes. The difference in the survival depending on the DH status was compared with the log-rank test. Intraoperative and postoperative complications were recorded and compared between patients with DH+ and DH- status. The statistical analyses were performed with SPSS (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.)

3.8.4 Results

From May 2007 to September 2012, 134 eyes of 101 patients from the NTG clinic underwent trabeculectomy. For the original audit, 131 eyes (98 patients) were included. One patient was excluded because the complete medical records were not available and the other two because they moved outside the UK during the first postoperative year. For the re-audit, one patient was excluded because secondary glaucoma was identified later in the follow-up. The re-audit included 20 fellow eyes of patients included in the original audit with only one eye. These patients had trabeculectomy in one eye during the time of the original audit (May 2007 to May 2012) and the fellow eye underwent trabeculectomy outside this period and was included in the present analysis (see Figure 43). The final number of eyes included was 149 of 97 patients who attended the clinic for a mean (SD) of 38.6 (12.2) visits over a mean (SD) follow-up of 16.5 (5.2) years. During the total follow-up, 3736 clinical examinations were reviewed in the clinical notes,

1917 (51.3%) before trabeculectomy and 1819 (48.7%) after trabeculectomy. Patients had a median (IQR) of 18 (14) visits before trabeculectomy and 18 (7) visits after trabeculectomy. There was no statistically significant difference between the number of clinical examinations before and after trabeculectomy (p=0.784). During 994 of the clinical examinations, an HRT scan was performed on the same day, 479 (48.2%) before trabeculectomy and 515 (51.8%) after trabeculectomy. Patients had a mean (SD) of 5.4 (2.5) HRT scans before trabeculectomy and 5.7 (2.2) HRT scans after trabeculectomy. There was no statistically significant difference between the number of HRT scans before and after trabeculectomy (p=0.437).

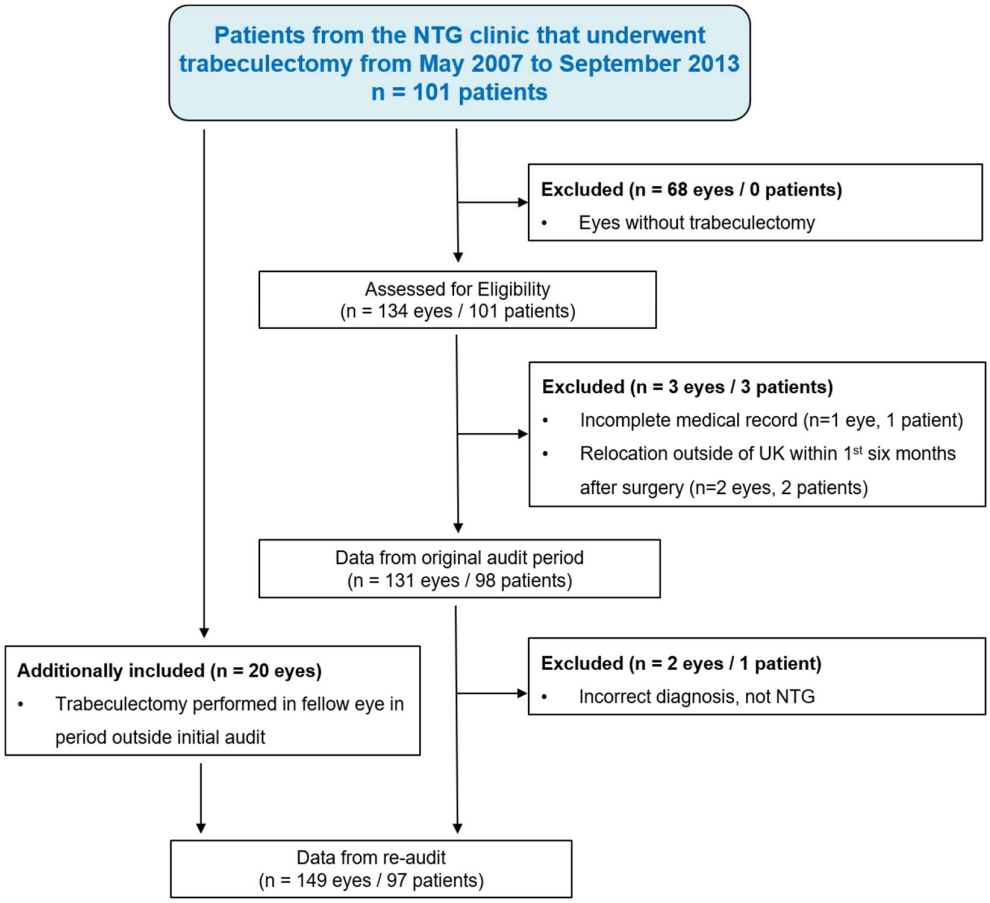

Figure 43 Flowchart of patients included in the comparison of DHs before and after trabeculectomy.

The demographic characteristics of the 97 patients included in the re-audit are described in Table 39.

Table 39 Characteristics of the 97 patients included in the re-audit.

	Participants
Female, n (%)	59 (60.8%)
Age at first trabeculectomy, mean (SD)	63.6 (10.7)
Both eyes had trabeculectomy, n (%)	52 (53.6%)
Only right eye had trabeculectomy, n (%)	15 (15.5%)
Only left eye had trabeculectomy, n (%)	30 (30.9%)
Left eye operated first when both eyes had surgery, n (%)	29 (55.8%)
Ethnicity:	
White, n (%)	67 (69.1%)
Black, n (%)	10 (10.3%)
Asian, n (%)	15 (15.5%)
Mixed, n (%)	1 (1.0%)
Not specified, n (%)	4 (4.1%)
Family History of glaucoma, n (%)	45 (46.4%)
Years of follow-up, mean (SD)	16.5 (5.2)
Number of visits, mean (SD)	38.6 (12.2)
Range of follow-up, years (visits)	4.6-33.6 (11-78)

A DH was detected, in at least one visit, in 101 eyes (52.1% of all eyes) of 69 patients (71.1%) based on either clinical examination or HRT images. Fifty-six patients (77 eyes) had a DH detected based on clinical examination, while 49 patients (66 eyes) had a DH detected based on HRT images. Among the 994 visits in which patients had a clinical examination and an HRT scan on the same day, the DH was detected by either method in 123 (12.4%) visits, by both methods in 32 (26.0% of DH+ visits), only by clinical examination in 7 (5.7% of DH+ visits) and only by HRT in 84 (68.3% of DH+ visits). The difference in the detection of DHs between clinical examination and the HRT-based method was described in Chapter 3. During the complete follow-up, among the 69 DH+ patients, 26 (37.7%) had a DH in BE at the same or different visit (some of the patients with a DH in BE had a DH in eyes that never underwent a trabeculectomy). Among the 101 DH+ eyes, 77 eyes underwent a trabeculectomy, while the rest did not have surgery during the complete follow-up.

There tended to be more women amongst the 69 DH+ patients compared to the 28 DH- patients, with more follow-up visits, both before and after trabeculectomy; Table 40 shows the characteristics of these subgroups of patients. At an eye level,

the DH+ and DH- eyes that underwent trabeculectomy were similar, as shown in Table 41.

Table 40 Characteristics of DH+ and DH- patients.

	DH+ (69 patients)	DH- (28 patients)	P value
Female, n (%)	46 (66.7%)	13 (46.4%)	0.064**
Age at first trabeculectomy, mean (SD)	64.1 (10.2)	62.2 (11.9)	0.428*
Ethnicity (not white), n (%)	19 (27.5%)	11 (39.3%)	0.257**
Family History of glaucoma, n (%)	34 (49.3%)	11 (39.3%)	0.371**
Years of follow-up, mean (SD)	17.1 (5.3)	15.2 (4.7)	0.112*
Number of visits, mean (SD)	40.0 (12.4)	35.0 (11.3)	0.066*
Number of visits before trabeculectomy, mean (SD)	21.0 (11.3)	16.8 (8.9)	0.086*
Number of visits after trabeculectomy, mean (SD)	19.0 (7.2)	18.1 (6.0)	0.579*

*Independent samples T-test
**Pearson Chi-Square

Table 41 Characteristics of DH+ and DH- eyes.

	DH+ (77 eyes)	DH- (72 eyes)	P value
Left eye, n (%)	46 (59.2%)	40 (54.9%)	0.630**
Phakic eyes, n (%)	65 (84.2%)	62 (86.3%)	0.749**
Central corneal thickness, mean (SD)	530 (32)	527 (34)	0.581*
Pre-op vision logMAR, mean (SD)	0.1 (0.1)	0.1 (0.3)	0.103*
Pre-op IOP, mean (SD)	14.8 (1.2)	14.8 (2.0)	0.870*
Pre-op IOP, maximum (SD)	16.8 (2.1)	16.8 (2.2)	0.970*
Pre-op IOP fluctuation, mean (SD)	2.1 (1.2)	2.3 (1.3)	0.399*
Pre-op number of drops, mean (SD)	2.0 (0.6)	2.0 (0.5)	0.743*
Pre-op Drops length, mean years (SD)	7.1 (3.9)	7.1 (3.8)	0.925*
Intraoperative complications, n (%)	4 (5.6%)	1 (1.4%)	0.182**
Post-op IOP reduction, mean percentage (SD)	44.3% (24%)	41.9% (23%)	0.578*

*Independent samples T-test
**Pearson Chi-Square

Among the 77 trabeculectomy eyes with a DH during the complete follow-up, all had a DH+ visit before trabeculectomy and only 16 eyes (16 patients) had a DH+ visit after trabeculectomy. The 16 eyes that developed a DH after trabeculectomy tended to have a higher maximum pre-operative IOP, more intraoperative

complications (conjunctival tear and buttonhole) and more of these patients had a non-white self-described ethnicity (see Table 42).

Table 42 Characteristics of eyes with and without DHs after trabeculectomy.

	DH+ post-op (16 eyes)	DH- post-op (61 eyes)	P value
Left eye, n (%)	12 (75%)	34 (55.7%)	0.162**
Female, n (%)	4 (25%)	20 (32.8%)	0.549**
Ethnicity (not white), n (%)	**8 (50%)**	**15 (24.6%)**	**0.048****
Number of visits, mean (SD)	41.3 (9.3)	40.4 (14.2)	0.810*
Number of pre-op visits, mean (SD)	20.6 (11.4)	20.7 (11.3)	0.973*
Number of post-op visits, mean (SD)	20.8 (5.3)	19.8 (8.7)	0.660*
Phakic eyes, n (%)	3 (18.8%)	9 (14.8%)	0.695**
Central corneal thickness, mean (SD)	535 (28)	528 (34)	0.437*
Pre-op IOP, mean (SD)	15.4 (1.9)	14.7 (1.9)	0.184*
Pre-op IOP, maximum (SD)	**17.8 (2.0)**	**16.5 (2.1)**	**0.023***
Pre-op IOP fluctuation, mean (SD)	2.4 (0.9)	2.0 (1.3)	0.198*
Pre-op number of drops, mean (SD)	2.2 (0.8)	2.0 (0.5)	0.173*
Pre-op Drops length, mean years (SD)	7.2 (4.0)	7.2 (3.9)	0.995*
Intraoperative complications, n (%)	**2 (12.5%)**	**1 (1.6%)**	**0.046****
Post-op IOP reduction, mean percentage (SD)	45.4% (27%)	43.9% (23%)	0.833*
Patients who required further glaucoma surgery, n (%)	12 (75.0%)	34 (55.7%)	0.162**

Values are in bold when the p-value is <0.05
*Independent samples T-test
**Pearson Chi-Square

The mean (SD, median, IQR) percentage of DH+ visits before trabeculectomy was 4.0% (4.5, 2.6, 0 – 5.5) and 0.8% (1.9, 0, 0 - 0) after trabeculectomy. The median difference in the percentage of DH+ visits before and after trabeculectomy was 2.4% (IQR 1.9 – 2.9; p=<0.001). Among the 16 eyes (16 patients) with a DH after trabeculectomy, the mean (SD, median, IQR) percentage of DH+ visits before trabeculectomy was 6.7% (4.7, 5.6, 2.7 – 13.0) and 4.6% (2.4, 4.1, 2.6 – 6.1) after trabeculectomy. In this subgroup of patients, the difference in the percentage of DH+ visits before and after trabeculectomy was not statistically significant (IQR of the difference 0 – 4.4; p=<0.180). Among the eyes with a DH before trabeculectomy, 79.2% never had a DH identified after trabeculectomy.

Among the 131 eyes (98 patients) included in the original audit, 120 eyes (90 patients) had sufficient data to re-analyse the survival outcomes of the trabeculectomy. The mean (SD) follow-up time after trabeculectomy for this group

of patients was 6.9 (1.8) years. The mean (SD) percentage of IOP reduction at the last follow-up visit was 45.6% (26.3) in the DH+ eyes and 41.6% (21.4) in the DH- eyes (p=0.343). The effect that the preoperative DH status had on the success of trabeculectomy is depicted in the Kaplan-Meier survival curves in Figure 44. The mean trabeculectomy survival for the 20% IOP reduction criterion was 8.5 (95% CI 7.7 – 9.2) years for the DH- eyes and 9.2 (95% CI 8.6 – 9.7) years for the DH+ eyes (log-rank, p=0.204). The mean survival for the 30% IOP reduction criterion was 8.0 (95% CI 7.2 – 8.7) years for the DH- eyes and 8.1 (95% CI 7.5 – 8.6) years for the DH+ eyes (log-rank, p=0.595). Among the 120 eyes (90 patients) in whom the long-term success of trabeculectomy was analysed, 96 eyes (74 patients) had more than five VFs, allowing event-based GPA assessment; these patients had a mean (SD) of 11.1 (2.9) VF tests during the complete follow-up after trabeculectomy. The mean trabeculectomy survival for the GPA criterion was 8.6 years (95% CI 7.8 – 9.3) years for the DH- eyes and 9.6 years (95% CI 8.0 – 10.1) years for the DH+ eyes (log-rank, p=0.103). The mean trabeculectomy survival for the criterion '20% IOP reduction and no GPA event-based progression' was 7.7 (95% CI 6.9 – 8.4) years for the DH- eyes and 8.3 (95% CI 7.6 – 9.0) years for the DH+ eyes (log-rank, p=0.451). The mean trabeculectomy survival for the '30% IOP reduction and no GPA event-based progression' was 7.4 (95% CI 6.7 – 8.2) years for the DH- eyes and 7.4 (95% CI 6.7 – 8.0) years for the DH+ eyes (log-rank, p=0.552).

The mean (SD) number of glaucoma drops was 2 (0.5) before trabeculectomy and 0.7 (0.9) after surgery. The mean reduction in glaucoma drops after surgery was -1.2 (95% CI 0.9 - 1.5; p=<0.001) for DH- eyes and -1.4 (95% CI 1.1 - 1.6; p=<0.001) for DH+ eyes. At the last follow-up visit, 71 eyes (59%) were not using glaucoma drops (53% of the DH- and 63% of DH+). None of the patients who underwent trabeculectomy had a blebitis or bleb-related endophthalmitis. Early and late complications occurred in less than 4% of the patients and there was no tendency toward more of these in patients with DH+ or DH- status. Three patients had intraoperative complications: two conjunctival buttonholes and one conjunctival tear. Eight patients had postoperative complications: two early bleb leaks, three early hypotony (with anatomical changes), one late hypotony and three patients who had rapidly progressing cataracts that required surgery.

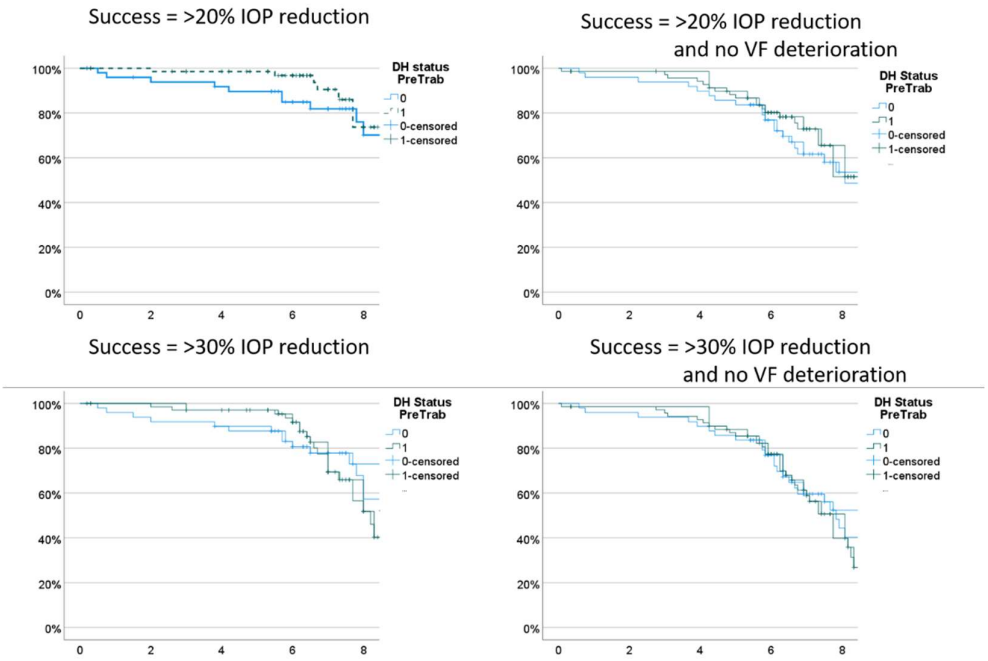

Figure 44 Kaplan-Meier survival curves of trabeculectomy comparing the preoperative DH status. 0 = no DHs before trabeculectomy and 1 = at least one DH before trabeculectomy. Censoring of patients is displayed as marks on the survival curves. The Y-axis depicts the percentage of participants who have a successful trabeculectomy, and the X-axis depicts time in years from surgery.

All of the previous results were based on all eyes. The comparison between the percentage of DH+ visits before and after trabeculectomy and the survival analysis of trabeculectomy were reanalysed using only one randomly selected eye per patient and the results remained with the same effect and statistical significance.

3.8.5 Discussion

Surgical reduction of IOP in patients with NTG reduced the mean frequency of DH+ visits from 4% before trabeculectomy to 0.8% after trabeculectomy. Among the patients with a DH before trabeculectomy, 79% did not have a DH detected during the mean postoperative follow-up time of 7 years. The present results are similar to Miyake et al., who reported no further DHs after trabeculectomy in 63% (10/16) and 89% (16/18) NTG and HTG patients (9).

The reduction in the frequency of DH+ visits identified in the present study with surgical reduction of IOP was in contrast to the results from the UKGTS reported in Chapter 7, the EMGT (8) and a retrospective publication by Hendrickx et al. (11). In the UKGTS, treatment with latanoprost induced a mean reduction in IOP of 4.6 mmHg, but the mean frequency of DH+ visits was similar between the treatment (8.7%) and placebo group (8.5%). In the EMGT, treatment with betaxolol and ALT induced a mean reduction of IOP of 4.5 mmHg, but the mean frequency of DH+ visits was similar between the treatment (8.4%) and placebo group (8.5%) (8). In the Hendrickx et al. retrospective study, a mix of therapies (timolol, pilocarpine, acetazolamide, guanethidine/epinephrine, ALT and trabeculectomy) induced a mean reduction in IOP of 5.1 mmHg in HTG eyes and 2.3 mmHg in NTG eyes. However, the number of DH+ visits/year was not reduced (0.41 before therapy vs 0.48 after therapy) in NTG patients and it was only lowered (0.51 before therapy vs 0.14 after therapy) in HTG patients (11). The reason why the previous studies could not identify a reduction in the frequency of DH+ visits with IOP-lowering interventions may be explained by the difference in the magnitude of the IOP reduction between the previous and the present study. The present study and the Miyake et al. study had IOP reductions of more than 40% and achieved a significant reduction in the number of DH+ visits after trabeculectomy. However, there was no difference in the mean percentage of IOP reduction between the 16 patients who had DH+ visits after trabeculectomy and the rest of the patients who did not have subsequent DHs after surgery. As discussed in Chapter 5, it seems possible that DH+ status reflects an endotype of glaucoma patients with a spectrum of manifestations. In most of the patients, the DH+ visits can be reduced to zero (79% of patients in the present study) after a profound reduction in IOP (such as trabeculectomy) in comparison to a smaller group of DH+ patients in whom DH+ visits might become less frequent but continue appearing irrespective of the magnitude of IOP reduction. The group in whom DH+ visits were no longer detected after lowering IOP seemed to require a minimum threshold of IOP reduction (higher than 40% in the present study and Miyake et al.) to reduce the number of DH+ visits, because in the UKGTS and EMGT, there was no association between the DH+ visits and the percentage of IOP reduction (Figure 39). Alternative explanations for why trabeculectomy reduces the frequency of DHs and the other therapies do not are the effect of surgery on IOP fluctuation, the compliance to medication, and the possible risk

that some glaucoma drops might increase DH occurrences. IOP fluctuations have been associated with a higher risk (OR = 1.05 per mmHg) of DHs in a Korean retrospective study that investigated risk factors for DH+ status in a group of 281 NTG patients (113 eyes with DHs) (20). A German retrospective study of 92 patients who underwent trabeculectomy identified a mean reduction of IOP fluctuation from 8.6 mmHg before surgery to 3.2 mmHg after surgery (21). The IOP fluctuation was carefully monitored before and six months postoperatively while patients were admitted to the hospital for 48 hours phasing of the IOP. Although IOP fluctuation has been associated with DHs, and trabeculectomy reduces the IOP fluctuation, in the UKGTS (see Chapter 4), IOP fluctuation was not associated with the DH+ status or the frequency of DH+ visits. The lack of compliance with glaucoma drops or the improper instillation technique has been reported to be present in more than 50% of patients (22), and this could partially explain why medical treatment does not reduce the frequency of DHs. However, in the UKGTS (see Chapter 7) and the EMGT (8), medical treatment reduced VF deterioration but did not reduce the frequency of DH+ visits between treated and untreated groups, despite being subject to poor compliance. Patients treated with systemic β-blockers had a higher risk of DHs in the LoGTS and patients on topical timolol had a tendency toward a higher risk of DHs (23). It is possible that treatment with betaxolol in the EMGT increased the risk of DHs and counteracted the positive effect of reducing IOP on the risk of DHs. However, UKGTS participants were treated with latanoprost and the frequency of DH+ visits was the same in the placebo and treated groups. The possibility that benzalkonium chloride (BAK) could increase the risk of DHs is very unlikely considering the findings of the EMGT and UKGTS. In the EMGT, participants in the untreated arm did not receive eyedrops, however, the number of DH+ participants was the same in both treatment arms. In the UKGTS, the untreated arm received a placebo eyedrop that contained BAK, however, the number of DH+ participants was the same in both treatment arms. If BAK could increase the risk of DHs, the untreated arm of the EMGT would have had a lower frequency of DH+ patients, or in the UKGTS, the placebo group could have had a higher frequency of DH+ patients compared to the treated group, which could have potentially benefited from the reduction in the risk of having DHs by reducing the IOP with latanoprost.

During the complete follow-up (mean 16.5 years) of this cohort of patients with progressive NTG who required trabeculectomy, 71.1% had at least one eye with a DH. The cumulative incidence of DHs in the present NTG cohort is higher than the 26.3% identified in the UKGTS during the 24 months of the trial (see Chapter 6), the 55% identified in the EMGT during a median follow-up of 8 years (8), the 10.4% identified in the OHTS during a median follow-up of 13 years, or the 43.1% identified in Kitazawa's retrospective publication during a mean follow-up of 15.7 months. However, it is similar to the Dalby survey (24) which identified a DH in 10 (66.7%) of the 15 patients with glaucoma and it is lower than the 93% mentioned by Krakau (25) referring to an unpublished result in which 37 out of 40 POAG patients had a DH over a long follow-up. The previous publications from the Dalby survey had a small number of glaucoma patients and, despite having been published almost 20 years ago, have not been confirmed by other independent groups. The present results over a mean of 16 years support the findings from the investigators of the Dalby survey that a large proportion of glaucoma patients develop at least one DH during their lifetime. However, it seems that DHs are not a universal sign of glaucoma. In addition, the present study only represents the NTG patients who progress and require trabeculectomy.

The efficacy of trabeculectomy is frequently analysed with different criteria that include different percentages of IOP reduction (26). In the present study, the DH status before trabeculectomy did not affect the success of trabeculectomy based on any of the success criteria. For the criterion of > 20% of IOP reduction, the mean survival was 8.5 years for DH- eyes and 9.2 years for DH+ eyes. The frequent use of VFs in the NTG clinic at Moorfields Eye Hospital facilitated the implementation of a VF event-based criterion of success for trabeculectomy. The VF outcome was not affected by the DH status. In addition, the combination of a stringent criterion for IOP reduction (>30% of reduction) and the VF criterion was also not affected by the DH status and the mean survival was 7.4 years for DH- and DH+ eyes. The risk of intraoperative or postoperative complications was not higher in patients with DH+ status. To the best of our knowledge, this is the first time that the efficacy of trabeculectomy has been compared between DH+ and DH- patients. In addition, the present NTG cohort has as a strength the careful categorization of the DH status based on a long follow-up of clinical examination and HRT imaging which reduces the risk of misclassification of the DH status.

Due to the high risk of DHs in NTG patients and the better chances of achieving a target IOP in these patients with trabeculectomy, it is important to advise patients that although a DH+ status increases the risk of VF deterioration, this risk can be reduced with trabeculectomy in the same way as in patients with a DH- status.

The results of the present study cannot be generalized to OAG patients with HTG. However, a previous retrospective study by Hendrickx (11) reported that patients with NTG had a worse outcome compared to HTG concerning the frequency of post-therapy DH+ visits. As the present study identified a reduction in the number of postoperative DH+ visits in the group that Hendrickx previously published to have no reduction, then it would be expected that a similar or greater reduction in the number of postoperative DH+ visits would be present in HTG patients. A limitation of the present study was the use of imaging to define the DH status in only 26.6% of the visits. In the remaining visits, the DH status was defined based only on clinical examinations, which have been shown to miss up to 84% of DHs that are detected photographically (27). In the same cohort of patients as the present study, in the visits in which an HRT scan was acquired on the same day as clinical examination, 68% of the clinical examinations missed a DH (see Chapter 3). Although it is possible that some patients were incorrectly misclassified as DH-, the risk is low because patients had a median of 18 clinical examinations before and after trabeculectomy. In addition, there was a mean of 5.4 and 5.7 HRT scans before and after trabeculectomy. As reported in Chapter 5, 80% of the DH+ participants over the complete follow-up of the UKGTS were categorized as DH+ during the first five examinations using the HRT-based method to detect DHs. A limitation to the analysis of the frequency of DHs is that at a visit level, the frequency of DH+ visits seems to be lower than in previous publications. The present study identified a frequency of DH+ visits of 4% compared to ~8% identified in the EMGT (8) and the UKGTS. The under-detection of DHs at a visit level is probably due to using the HRT-based method to detect DHs only in 26.6% of the visits of the present study, compared to ~100% of the UKGTS visits. However, the under detection is very likely equally distributed before and after trabeculectomy; therefore, the effect of surgery on reducing the frequency of DH+ would very likely remain the same if all DHs would have been identified. Another limitation to analysing the frequency of DH+ visits before and

after trabeculectomy is the risk of having regression-to-the-mean. In some cases, an observed DH was the trigger to proceed with trabeculectomy. In these cases, it would be expected to observe a reduced number of postoperative DHs compared to the preoperative visits. However, in almost half of all included patients, the DH+ status before trabeculectomy was unknown by the clinician and it was detected retrospectively during the re-audit of the HRT images. Therefore, the risk of regression-to-the-mean should not be considered as a relevant factor. A limitation of the eye-level analysis was the inclusion of both eyes of patients who underwent trabeculectomy in the analysis of the frequency of DH+ visits (8 patients with BE included, 10% of DH+ eyes before trabeculectomy) and the survival analysis (30 patients with BE included, 25% of eyes with the success of trabeculectomy analysed). However, the effect of trabeculectomy on reducing the frequency of DH+ visits and the survival of trabeculectomy were analysed with only one eye selected randomly per patient, and the result remained with the same effect and statistical significance.

To conclude, in this cohort of NTG patients with a long follow-up, the incidence of at least one DH in one eye was very high (71% of the patients), but DHs were not a universal sign present in all NTG patients. Surgical reduction of IOP with trabeculectomy significantly reduced the number of postoperative DH+ visits. The positive effect of trabeculectomy in lowering IOP and reducing the risk of VF deterioration was not affected by the preoperative DH status.

*The long-term success of trabeculectomy to reduce the IOP and reduce the risk of VF deterioration, not including the analysis of the DH status, was previously presented in the American Academy of Ophthalmology 2019 Congress by Brigid Ning (28). The effect of trabeculectomy on the frequency/number of DH+ visits was accepted for a paper presentation in ARVO 2019 and is accessible as an abstract (29).

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Section 4 Conclusions

4.1.1 Conclusion

The timely identification of patients with DHs could accelerate appropriate risk stratification and improve the quality of the care that some patients with glaucoma receive. Clinicians tend to change their management plans based on the detection of DHs. However, there is practice variation among glaucoma specialists on how to manage patients with new DHs. Despite the high frequency of DHs, international guidelines do not have specific consensus-based recommendations on how to manage patients with new DHs.

Consecutive flickering SLO images acquired with the HRT can be used to detect DHs, but the accuracy is slightly lower compared to fundus photography. The retrospective implementation of an SLO-based method to detect DHs in a real-life clinical setting identified that clinical examination alone missed 68% of the detected DHs. Although fundus photography is the gold standard to detect DHs, the SLO technology used in the HRT and some OCT instruments could be used to detect DHs without requiring additional equipment.

Risk factors for DHs such as age, sex and refraction have been confirmed. Novel risk factors widely common in society, such as smoking, tea or coffee consumption, use of SSRIs and CCBs, are associated with DHs. These systemic risk factors and the occurrence of more simultaneous and bilateral DHs than expected just by chance suggest a systemic mechanism involved in the pathogenesis of DHs. However, patients with DHs are a very heterogeneous group of patients with glaucoma. A large group with frequent but mostly unilateral DHs, and two small groups, one with only one DH, and another with very frequent and more bilateral DHs.

In patients who will develop DHs, the detection of DHs seems to change during a patient's lifetime. From occasional DHs before unequivocal glaucoma is diagnosed to more frequent DHs in more advanced stages of the disease. Patients with a DH at any stage of the natural history of glaucoma have a higher

risk of VF deterioration. The increased risk of glaucoma progression conferred by DHs was lowered with latanoprost, but a reduction in the frequency of subsequent DHs was only seen after surgical reduction of IOP with trabeculectomy.

The role of DHs in the pathogenesis of glaucoma is still unknown, and it is clear that not all patients with glaucoma require to have observable DHs to develop glaucomatous neuropathy. However, it seems possible that patients with glaucoma who have detectable DHs represent a different endotype of glaucoma. In these patients, systemic mechanisms that affect both eyes may play a more important role in the pathogenesis of glaucomatous neuropathy.

The role that DHs may play in the clinical care of patients with glaucoma cannot be overstated. Most clinicians look carefully for DHs and change their management plan accordingly. Patients with glaucoma who have a DH have a higher risk of deterioration. However, medical or surgical reduction of IOP seems to reduce and eliminate, in most cases, the additional risk of progression induced by DHs.

4.1.2 Future work

The questions about DHs that have been answered with this thesis opened even more questions about the role of DHs in glaucoma. Of special interest, the following areas of future research could help us better understand the role of DHs in glaucoma:

1. A genome-wide association study of data from RCTs or longitudinal studies of patients with glaucoma with and without DHs who had the DH status defined by an imaging modality
2. Animal models of DHs could be developed using a technique similar to the animal models of stroke with microhaemorrhages. This model could help in the evaluation of the effect of blood in RNFL and glial cells
3. In vivo detection of nitric oxide activity in patients with and without DHs using agents that sensitize MRI to the biological nitric oxide
4. To automatize the SLO-based method to aid in the detection of DHs and evaluate the diagnostic performance of this method in a real-life clinical practice
5. Investigate the role of DHs in healthy individuals using data from large-scale biomedical databases
6. To identify the proportion of patients with glaucoma that will develop VF deterioration and the risk factors. This may help in the stratification of the risk that patients with new DHs have to develop VF deterioration
7. To evaluate in more detail the relationship between peripapillary atrophy and DHs
8. Head to head clinical trials of new IOP-lowering medications. To compare the effect of these new drugs on preserving visual function in DH+ patients and their effect on the number of subsequent DHs
9. Analyse DHs in trials of neuroprotection. Evaluate the inclusion of DHs as a baseline variable to stratify patients enrolled in these trials and consider an appropriate sample size to power the study to perform a subgroup analysis based on the DH status
10. Evaluate in animals models the possibility of using drugs that enhance erythrolysis or erythrophagocytosis to reduce the harm that DHs may produce in the neighbouring tissues.

Section 5 Appendices

5.1.1 Pilot versions of the disc haemorrhage survey.

5.1.1.1 First option (Case based)

Case 1

You receive a new referral from an optician who discovered a new disc haemorrhage (DH) in a healthy 50 year old patient with no past ocular history and no family history of glaucoma or blindness. IOP 13 mmHg on both eyes. Disc haemorrhage in his right eye and otherwise normal eye examination including a healthy optic disc with no RNFL defect or rim thinning. No posterior vitreous detachment (PVD).

1. How would you manage this patient?
2. Discharge without baseline test.
3. Ask for imaging scans of the optic nerve (OCT or other), then discharge.
4. Follow-up yearly for 3-5 years and then discharge
5. Start treatment to reduce the IOP

Case 2

50 year old ocular hypertensive patient that has been under observation yearly for 5 years. Maximum IOP 23 BE with CCT 560 BE. Healthy optic disc VCDR 0.3, no RNFL defects. Normal visual fields (VF) and normal OCT. On the last visit you observed a new DH not associated with PVD on the left eye, what would you do next?

1. Discharge
2. Continue monitoring every 12-24 months
3. Monitor every 3-6 months
4. Start treatment to reduce the IOP

Case 3

65 year old patient with early/moderate glaucoma on both eyes. Glaucoma was diagnosed 3 years ago after an optician found and IOP of 28 RE and 26 LE. During the last 3 years the IOP has been controlled under Latanoprost treatment with 40% reduction, no side effects and stable VF. On the last visit you observe a new DH not associated with PVD on the left eye, what would you do next if IOP and VF are stable?

1. Continue with same treatment and monitor in 6-12 months.
2. Continue with same treatment and monitor in 3-6 months.
3. Increase treatment to reduce the IOP (add a second drop)
4. Offer surgery to increase the reduction in IOP

Case 4

75 year old patient with early/moderate glaucoma on both eyes diagnosed 6 years ago after an optician found and IOP of 30 RE and 28 LE. During the first 3 years of treatment the patient had a labile IOP and tried many different treatments. Finally during the last 3 years the IOP has been between 12-14 on BE with no progression on visual fields. The patient is using 3 drugs to reduce the IOP with no side effects. On the last visit you observed a new DH not associated with PVD on the right eye, what would you do next?

1. Continue with same treatment and monitor in 6-12 months.
2. Continue with same treatment and monitor in 3-6 months.
3. Increase treatment to reduce the IOP (add a fourth drop or systemic treatment)
4. Offer surgery to increase the reduction in IOP

Finally, as a general rule, in your clinic when a glaucoma patient with a new DH is present what do you do. (Question for three options of surverys).

1. Continue same treatment and monitoring interval
2. Reduce the monitoring interval
3. Increase treatment

4. Offer surgery

5.1.1.2 Second option (Based on change in management)

Please answer what would you do in your current practice in the following clinical scenarios if you discover a new DH?

Healthy patient with DH as an incidental finding with an optometrist. In your examination everything looks normal except for a DH.

1. Discharge
2. Monitor every 12-24 months
3. Monitor every 6-12 months
4. Order an extra test (OCT) and monitor in 2-6 months
5. Start medical treatment

OHT patient with IOP 23, CCT 560, and normal VF for 5 year with no treatment.

1. Discharge
2. Monitor every 12-24 months
3. Monitor every 6-12 months
4. Order an extra test (OCT) and monitor in 2-6 months
5. Start medical treatment

OHT patient with good IOP and normal VF under three drops to reduce the IOP.

1. Monitor every 12-24 months
2. Monitor every 6-12 months
3. Order an extra test (OCT) and monitor in 2-6 months
4. Increase medical treatment
5. Offer Surgery

Early/moderate POAG patient with good IOP and no progression in VF treated with one drop.

1. Monitor every 6-12 months
2. Monitor every 2-6 months
3. Order an extra test (OCT)
4. Increase medical treatment
5. Offer Surgery

Early/moderate POAG with good IOP and no progression in VF treated with three drops.

1. Monitor every 6-12 months
2. Monitor every 2-6 months
3. Order an extra test (OCT)
4. Increase medical treatment
5. Offer Surgery

5.1.1.3 Third option (based on how the clinician agrees to a statement)

To what extent do you agree or disagree with the next statements?

Disc haemorrhages are an important sign of active glaucoma.

1. Strongly agree
2. Partially agree
3. Neutral
4. Partially disagree
5. Strongly disagree

I consider healthy patients with a new DH as glaucoma suspects.

1. Strongly agree
2. Partially agree
3. Neutral
4. Partially disagree
5. Strongly disagree

I start or increase treatment in my OHT patients with a new DH even if IOP and VF are stable.

1. Strongly agree
2. Partially agree
3. Neutral
4. Partially disagree
5. Strongly disagree

I reduce the monitoring interval to my OHT patients with a new DH even if IOP and VF are stable.

1. Strongly agree
2. Partially agree
3. Neutral
4. Partially disagree
5. Strongly disagree

I increase treatment in my POAG patients with new DH even if IOP and VF are stable.

1. Strongly agree
2. Partially agree
3. Neutral
4. Partially disagree
5. Strongly disagree

I reduce the monitoring interval to my POAG patients with a new DH even if IOP and VF are stable.

1. Strongly agree
2. Partially agree
3. Neutral
4. Partially disagree
5. Strongly disagree

I offer surgery to my POAG patients on maximal therapy and new DH even if IOP and VF are stable.

1. Strongly agree
2. Partially agree
3. Neutral
4. Partially disagree
5. Strongly disagree

5.1.2 Results from the pilot survey.

Question 1. "Healthy individual with DH"

1. Discharge 17%
2. Imaging then discharge 50%
3. FU yearly for 3-5 years then discharge 33%
4. Start treatment 0%

Question 2. “OHT patient stable after 5 years”

1. Discharge 17%
2. Continue monitoring every 12-24 months 50%
3. Monitor every 4-8 months 33%
4. Start treatment 0%

Question 3. "Stable glaucoma with 40% IOP reduction but new DH"

1. Continue with same treatment and monitor in 6-12 months 50%
2. Continue with same treatment and monitor in 3-6 months 50%
3. Increase treatment to reduce the IOP 0%
4. Offer surgery to increase the reduction of IOP 0%

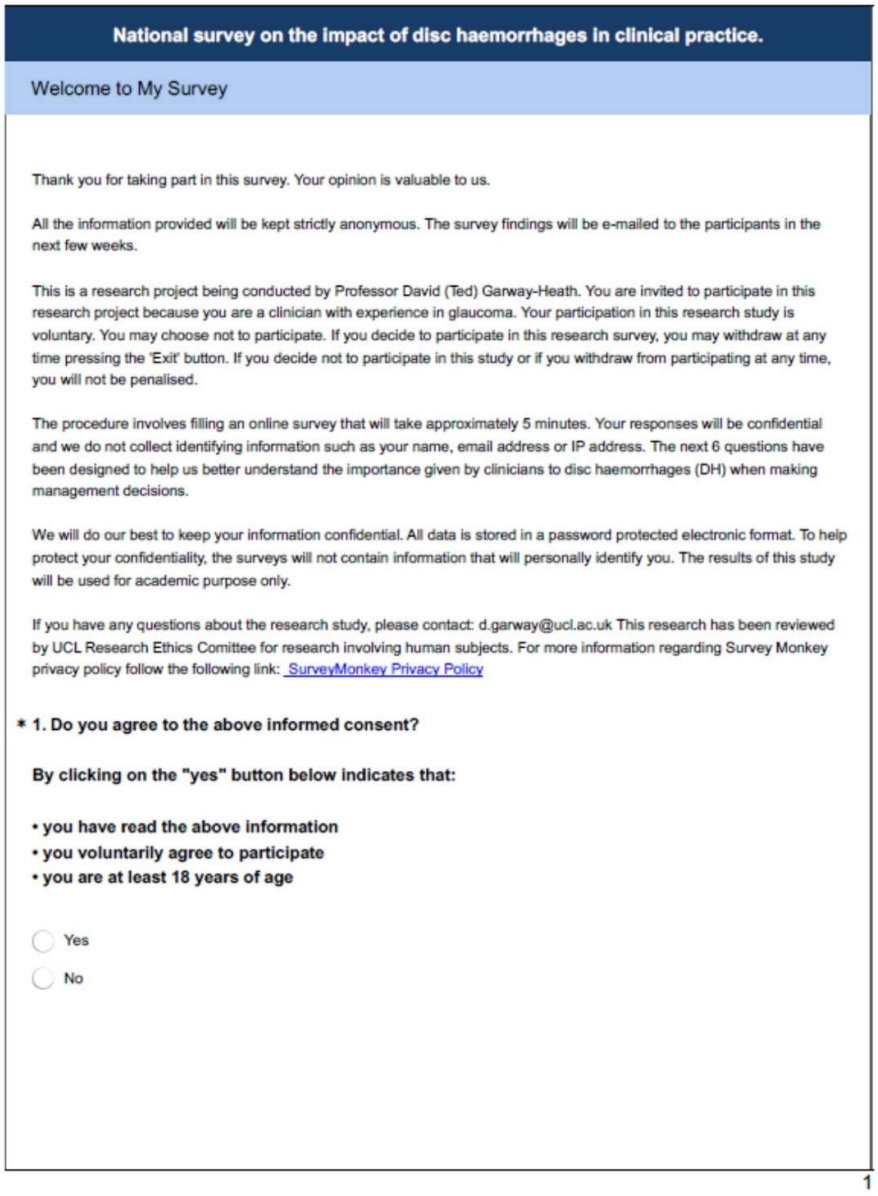
Question 4. "Stable glaucoma on maximum medical therapy with good IOP but new DH"

1. Continue with same treatment and monitor in 6-12 months 33%
2. Continue with same treatment and monitor in 3-6 months 50%
3. Increase treatment to reduce the IOP (systemic treatment) 0%
4. Offer surgery to increase the reduction of IOP 17%

Question 5. As a general rule in your clinic, what do you do with stable patients with new DH

1. Continue same treatment and monitoring interval 17%
2. Reduce the monitoring interval and ask for more tests 83%
3. Increase treatment only medically but never surgically 0%
4. Increase treatment medically or surgically 0%

5.1.3 SurveyMonkey version of the final survey.



National survey on the impact of disc haemorrhages in clinical practice.

Welcome to My Survey

Thank you for taking part in this survey. Your opinion is valuable to us.

All the information provided will be kept strictly anonymous. The survey findings will be e-mailed to the participants in the next few weeks.

This is a research project being conducted by Professor David (Ted) Garway-Heath. You are invited to participate in this research project because you are a clinician with experience in glaucoma. Your participation in this research study is voluntary. You may choose not to participate. If you decide to participate in this research survey, you may withdraw at any time pressing the 'Exit' button. If you decide not to participate in this study or if you withdraw from participating at any time, you will not be penalised.

The procedure involves filling an online survey that will take approximately 5 minutes. Your responses will be confidential and we do not collect identifying information such as your name, email address or IP address. The next 6 questions have been designed to help us better understand the importance given by clinicians to disc haemorrhages (DH) when making management decisions.

We will do our best to keep your information confidential. All data is stored in a password protected electronic format. To help protect your confidentiality, the surveys will not contain information that will personally identify you. The results of this study will be used for academic purpose only.

If you have any questions about the research study, please contact: d.garway@ucl.ac.uk This research has been reviewed by UCL Research Ethics Comittee for research involving human subjects. For more information regarding Survey Monkey privacy policy follow the following link: SurveyMonkey Privacy Policy

*** 1. Do you agree to the above informed consent?**

By clicking on the "yes" button below indicates that:

- **you have read the above information**
- **you voluntarily agree to participate**
- **you are at least 18 years of age**

○ Yes

○ No

1

*** 2. I grant permission for the data generated from this survey to be used for academic purpose (scientific meetings and/or publications)?**

- ◯ Yes
- ◯ No

2

National survey on the impact of disc haemorrhages in clinical practice.

Welcome to our survey.

3. Clinical case

A new patient has been referred to you by an optometrist. The reason for referral is a disc haemorrhage (DH) in the right eye. It is a healthy 50-year-old man with no past ocular history and no family history of glaucoma or blindness. IOP is 13mmHg in both eyes and, apart from the DH, eye examination is normal including a healthy optic disc without retinal nerve fibre layer (RNFL) defect or rim narrowing. There is no posterior vitreous detachment (PVD). What would you do?

- ○ Discharge without baseline test.
- ○ Ask for imaging scans of the optic nerve, then discharge.
- ○ Follow up yearly for 3-5 years and then discharge.
- ○ Start treatment to reduce IOP.

4. Clinical case

A 50-year-old ocular hypertensive patient has been under observation for 5 years with a yearly examination. There has been no change over the last 5 years until a DH not associated with PVD was noted in the left eye at the last visit. Maximum IOP is 23mmHg in both eyes with a central corneal thickness of 560µm. The optic discs are healthy with a vertical cup:disc ratio of 0.3 and there is no RNFL defect. Both visual fields (VFs) and OCT scans are normal. What would you do?

- ○ Discharge.
- ○ Continue monitoring every 12 months.
- ○ Monitor every 4–8 months.
- ○ Start treatment to reduce IOP.

5. Clinical case

A 65-year-old patient was diagnosed with early/moderate glaucoma in both eyes 3 years ago, subsequent to IOP measurements of 28mmHg in the right eye and 26mmHg in the left eye by an optometrist. IOP has been controlled since with latanoprost resulting in a 40% reduction, no side effects and stable VFs. On the patient's last visit, a DH not associated with PVD was observed in the left eye. What would you do if IOP and VFs are stable?

- ○ Continue with same treatment and follow up in 6–12 months.
- ○ Continue with same treatment and follow up in 3–6 months.
- ○ Increase treatment to reduce IOP.
- ○ Offer surgery to increase reduction in IOP.

6. Clinical case

A 75-year-old patient was diagnosed with early/moderate glaucoma in both eyes 6 years ago, subsequent to IOP measurements of 30 in the right eye and 28 in the left eye by an optometrist. During the first 3 years, the patient was put on different glaucoma eye drops because of labile IOP, which eventually stabilized to between 12-14mmHg in both eyes with three drugs in the last 3 years with no side effects and no VF progression. On the last visit, a DH not associated with PVD was observed in the right eye. What would you do?

- ○ Continue with same treatment and follow up in 6–12 months.
- ○ Continue with same treatment and follow up in 3–6 months.
- ○ Increase treatment to reduce IOP (add a fourth drug or systemic treatment).
- ○ Offer surgery to increase IOP reduction.

7. As a general rule in your clinic: what do you usually do in a glaucoma patient with a newly detected DH but stable disc and visual fields?

- ○ Continue same treatment and monitoring interval.
- ○ Reduce monitoring interval and perform more tests.
- ○ Increase treatment, only medically but not surgically.
- ○ Increase treatment, either medically or surgically.
- ○ Other (please specify)

4

8. Which of the following best describes where you trained?

- East Midlands - North
- East Midlands - South
- East of England
- Kent, Surrey, Sussex
- London - North
- London - South
- Mersey
- North East
- North West
- Northern Ireland
- Peninsula
- Scotland - East
- Scotland - North
- Scotland - South East
- Scotland - West
- Severn
- Thames Valley
- Wales
- Wessex
- West Midlands
- Yorkshire - North
- Yorkshire – South

9. Which of the following best describes your practice?

- I only have glaucoma patients
- Most of my patients have glaucoma
- About half of my patients have glaucoma
- Few of my patients have glaucoma
- I don't have patients with glaucoma

5

10. Finally, how old are you?

- ◯ <40 years
- ◯ 40-50 years
- ◯ 50-60 years
- ◯ >60 years

Thank you very much for your time.

6

5.1.4 Research ethic committee approval.

UCL RESEARCH ETHICS COMMITTEE
ACADEMIC SERVICES

UCL

5th June 2017

Professor David Garway-Heath
Institute of Ophthalmology
UCL

Dear Professor Garway-Heath

Notification of Ethical Approval
Re: Ethics Application 8335/001: National survey on the impact of haemorrhages (DH) in clinical practice

I am pleased to confirm in my capacity as Chair of the UCL Research Ethics Committee that I have ethically approved your study until **5th June 2018.**

Approval is subject to the following conditions:

Notification of Amendments to the Research
You must seek Chair's approval for proposed amendments (to include extensions to the duration of the project) to the research for which this approval has been given. Ethical approval is specific to this project and must not be treated as applicable to research of a similar nature. Each research project is reviewed separately and if there are significant changes to the research protocol you should seek confirmation of continued ethical approval by completing the 'Amendment Approval Request Form':
http://ethics.grad.ucl.ac.uk/responsibilities.php

Adverse Event Reporting – Serious and Non-Serious
It is your responsibility to report to the Committee any unanticipated problems or adverse events involving risks to participants or others. The Ethics Committee should be notified of all serious adverse events via the Ethics Committee Administrator (ethics@ucl.ac.uk) immediately the incident occurs. Where the adverse incident is unexpected and serious, the Chair or Vice-Chair will decide whether the study should be terminated pending the opinion of an independent expert. For non-serious adverse events the Chair or Vice-Chair of the Ethics Committee should again be notified via the Ethics Committee Administrator within ten days of the incident occurring and provide a full written report that should include any amendments to the participant information sheet and study protocol. The Chair or Vice-Chair will confirm that the incident is non-serious and report to the Committee at the next meeting. The final view of the Committee will be communicated to you.

Final Report
At the end of the data collection element of your research we ask that you submit a very brief report (1-2 paragraphs will suffice) which includes in particular issues relating to the ethical implications of the research i.e. issues obtaining consent, participants withdrawing from the research, confidentiality, protection of participants from physical and mental harm etc.

Academic Services, 1-19 Torrington Place (9th Floor),
University College London
Tel: +44 (0)20 3108 8216
Email: ethics@ucl.ac.uk
http://ethics.grad.ucl.ac.uk/

With best wishes for the research.

Yours sincerely

[redacted]

Dr Lynn Ang
Interim Chair, UCL Research Ethics Committee

Cc: Jibran Mohamed Noriega

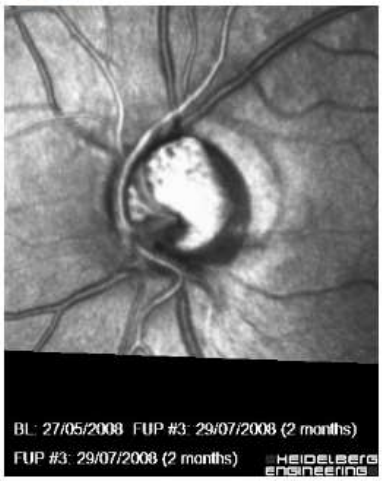
5.1.5 Standard operating procedure for the grading of DHs based on HRT scans.

1. Record the patient UKGTS ID, date of visit, and eye being graded
2. The images of the right and left eye of the same participant will be assessed separately
3. Check the image quality
4. Analyse the raw image video for abnormal data
5. Convert the raw data to a topographical image
6. Use the flicker feature to compare between the baseline image and all the following scans. (or use different dates to compare presence or absence of DH)
7. Look for changes in reflectivity
8. Observe the shape of the changes in reflectivity with special attention to flamed shape
9. A DH is defined as the presence of a change in reflection in the disc, rim or in peripapillary retina with flamed or dot shape
10. When more than one scan is available for the same day double-check that the probable DH is in the same place in all the scans. This will help to avoid confusion between shadows/artefacts and DH.
11. Confirm that the probable DH changes over time in scans of different days. To avoid confusion between artefacts of areas of variation in reflectivity
12. A definite DH is a change in reflectivity with flame or dot shape that maintains the same size during different scans of the same day and changes in scans of different days
13. When a DH is identified, a grid is located over the disc with clock hours mark. The clock hour that is located more closely to the centre of the disc haemorrhage at the point where the DH cross the scleral ring will be marked as the meridian in which the DH was found.
14. Register the results of the grading in the access database that only allows the visualisation of the visit and eye that is being graded

5.1.6 Survey Monkey view of the agreement study questionnaire.

Pilot DH agreement study.

Patient 1



1. Please describe this image as DH+ or DH-. If positive please mention in which clock hour.

- ◯ DH-
- ◯ DH+

2. If positive, please mention in which clock hour.

- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ 6
- ◯ 7
- ◯ 8
- ◯ 9
- ◯ 10
- ◯ 11
- ◯ 12