Screening for Diabetic Retinopathy
Aspects of Photographic Methods

Gunvor von Wendt

Stockholm 2005
To my patients
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ABSTRACT

Diabetic retinopathy (DRP) is a major cause of acquired blindness and visual impairment among people of working age as well as those aged 65 years or more. About 3-4% of the population has diabetes mellitus, 35-65% of the diabetic patients have some type of DRP and 10-35% have sight-threatening retinopathy. Regular eye examinations with a sensitive method are important in order to detect the treatment needing, usually asymptomatic, lesions in time. Only then laser treatment reduces the risk of visual impairment by 50-90%. Forty-five degree photography from one or two fields using colour transparencies or Polaroid pictures has so far been the predominant photographic screening method. Field definitions, especially for the macular field, have varied according to different recommendations. Sixty degree wide-field photography offers large field coverage and might, despite less magnification, improve detection of diabetic retinopathy abnormalities. Furthermore, the detectability of vascular structures and red lesions might improve when using red-free light at photography. The technique of using a monochromatic green filter enhances the contrast of retinal blood vessels and haemoglobin containing structures.

In this methodological study varying photographic screening methods for the detection of DRP were evaluated. Using wide-field 60° cameras it was studied whether DRP was more easily detected from red-free film based or digital black-and-white pictures as compared to corresponding colour transparencies. Furthermore, it was evaluated whether two 60° photographic fields were needed or whether one 60° field was enough for screening purposes. The field coverage of one and two 60° fields was compared with that of the Gold Standard (30° seven-field photography). We also studied how retinal neovascularizations (NVEs) were detected from one and two 45° fields and compared the results with that of one- and two-field 60° photography. Furthermore, in order to find out whether any of three varying 45° macular fields was superior in detecting NVEs, the number of NVEs detected in each of them was compared with the number detected from the 60° fovea-centred field.

Our results show that especially early DRP lesions (red dots) but also intraretinal microvascular abnormalities and venous beading, both indicating severe DRP, are more easily detected from monochrome red-free digital images and photographs, compared to colour transparencies. This is important as the detection of the first abnormalities as well as of the severe DRP lesions influences both future screening intervals and decisions for referral or treatment. “White lesions”, e.g. cotton wool spots, were the only abnormalities which were less easily detected with the red-free technique. For further prognosis these lesions are, however, not considered as important as the former ones. Single-field 60° photography can be advocated only when the finding in this field is normal, otherwise severe lesions can be missed. One and two-field 60° photography covers 60% and 80%, respectively, of the areal coverage of that of 30° seven-field photography (Gold Standard). One- and two-field 45° photography disclosed 53% and 77%, respectively, of the NVEs which were detected from two-field 60° photography. Of the 45° macular fields investigated, the field centred most temporally turned out to disclose NVEs most appropriately.

Keywords: Diabetic retinopathy, diabetic maculopathy, screening, retinal photography, photographic fields, grading scale, inter-observer agreement, inter-method agreement, monochromatic photography, retinal neovascularizations, sight-threatening diabetic retinopathy

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LIST OF PUBLICATIONS

This thesis is based on the following papers, which are subsequently referred to by their Roman numerals, as well as previously unpublished data on photographic screening for diabetic retinopathy:


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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>Ang-2</td>
<td>Angiopoietin2</td>
</tr>
<tr>
<td>ARVO</td>
<td>The Association for Research in Vision and Ophthalmology</td>
</tr>
<tr>
<td>bFGF</td>
<td>Basic fibroblast growth factor</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CSMO</td>
<td>Clinically significant macular oedema</td>
</tr>
<tr>
<td>CWS</td>
<td>Cotton wool spot</td>
</tr>
<tr>
<td>ERG</td>
<td>Electroretinography</td>
</tr>
<tr>
<td>DCCT</td>
<td>Diabetes Control and Complications Trial</td>
</tr>
<tr>
<td>DD</td>
<td>Disc diameter</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes mellitus</td>
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<tr>
<td>DMO</td>
<td>Diabetic macular oedema</td>
</tr>
<tr>
<td>DRP</td>
<td>Diabetic retinopathy</td>
</tr>
<tr>
<td>DRS</td>
<td>Diabetic Retinopathy Study</td>
</tr>
<tr>
<td>ETDRS</td>
<td>Early Treatment Diabetic Retinopathy Study</td>
</tr>
<tr>
<td>EURODIAB</td>
<td>The European Diabetes Study</td>
</tr>
<tr>
<td>FA</td>
<td>Fluorescein angiography</td>
</tr>
<tr>
<td>GP</td>
<td>General practitioner</td>
</tr>
<tr>
<td>H</td>
<td>Haemorrhage</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycolysated haemoglobin</td>
</tr>
<tr>
<td>He</td>
<td>Hard exudate</td>
</tr>
<tr>
<td>HGF</td>
<td>Hepatocyte growth factor</td>
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<tr>
<td>IDDM</td>
<td>Insulin dependent diabetes mellitus</td>
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<tr>
<td>IDF</td>
<td>International Diabetes Federation</td>
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<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor 1</td>
</tr>
<tr>
<td>IRMA</td>
<td>Intra retinal microvascular abnormality</td>
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<tr>
<td>k</td>
<td>Kappa</td>
</tr>
<tr>
<td>Ma</td>
<td>Microaneurysm</td>
</tr>
<tr>
<td>Mi</td>
<td>Microinfarct</td>
</tr>
<tr>
<td>NIDDM</td>
<td>Non-insulin dependent diabetes mellitus</td>
</tr>
<tr>
<td>NPDR</td>
<td>Non-proliferative diabetic retinopathy</td>
</tr>
<tr>
<td>NV</td>
<td>Neovascularization</td>
</tr>
<tr>
<td>NVD</td>
<td>Neovascularization on the disc</td>
</tr>
<tr>
<td>NVE</td>
<td>Neovascularization elsewhere</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet-derived growth factor</td>
</tr>
<tr>
<td>PDR</td>
<td>Proliferative retinopathy</td>
</tr>
<tr>
<td>PEDF</td>
<td>Pigment epithelium-derived factor</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein kinase C</td>
</tr>
<tr>
<td>PIGF</td>
<td>Placenta growth factor</td>
</tr>
<tr>
<td>PPDR</td>
<td>Preproliferative retinopathy</td>
</tr>
<tr>
<td>PRH</td>
<td>Preretinal haemorrhage</td>
</tr>
<tr>
<td>Sp</td>
<td>Standard photograph</td>
</tr>
<tr>
<td>STDR</td>
<td>Sight-threatening diabetic retinopathy</td>
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<tr>
<td>TGF</td>
<td>Transforming growth factor</td>
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<tr>
<td>UKPDS</td>
<td>United Kingdom Prospective Diabetes Study</td>
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<tr>
<td>VA</td>
<td>Visual aquity</td>
</tr>
<tr>
<td>VB</td>
<td>Venous beading</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>VH</td>
<td>Vitreous haemorrhage</td>
</tr>
<tr>
<td>WESDR</td>
<td>Wisconsin Epidemiologic Study of Diabetic Retinopathy</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>wk</td>
<td>Weighted kappa</td>
</tr>
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</table>
1. INTRODUCTION

1.1. Prevalence and incidence of diabetes mellitus

Diabetes mellitus (DM) is considered one of the main worldwide health threats in the 21st century (Zimmet 2000). The global prevalence of DM is estimated to rise from 150 million people in 2000 to 220 million in 2010 and 300 million in 2025 (Amos et al. 1997, King et al. 1998). In Sweden about 3-4% of the population, that is 300,000 people, have diabetes (The Swedish National Board of Health and Welfare 1999).

There are two main types of DM, Type 1 and 2 (WHO 1999). Type 1 DM is due to an autoimmune process leading to the destruction of pancreatic insulin-producing β-cells resulting in an absolute insulin deficiency affecting mostly children and adolescents. Type 1 DM is the most common chronic disease of children. This type of DM comprises about 10-15% of the diabetic population. The world’s highest incidence of childhood Type 1 DM, 36.5 cases per 100,000, is reported from Finland (Karvonen et al. 2000).

Most patients with diabetes, 85-90%, have type 2 DM (The Swedish National Board of Health and Welfare 1999). Insulin resistance and/or abnormal insulin secretion, either of which may predominate, characterize this type of DM. Changes in our lifestyle induced by a higher standard of living have resulted in a dramatic increase in the incidence of specially Type 2 DM. Overly rich nutrition and reduced physical activity leading to obesity, are considered to be the main reasons leading to the metabolic syndrome and Type 2 DM (Zimmet 1999). Although the onset of type 2 DM in Europeans is usually after age 50, onset in younger age groups and even in children, has been reported from Japan, the United States, Pacific Islands, Australia and the United Kingdom (Rosenbloom et al. 1999, The American Diabetes Association 2000, Ehtisham et al. 2000, Fagot-Campagna et al. 2000).

Apart from the microvascular late complications, DRP, nephropathy and neuropathy, there is a significantly higher morbidity and mortality in macrovascular diseases such as heart disease and stroke, in subjects with type 2 DM. In the Botnia study, a multicentre study from Finland and Sweden, there was a threefold risk for coronary heart disease. Stroke and cardiovascular mortality was markedly increased (from 2% to 12%) in subjects with the metabolic syndrome (Isomaa et al. 2001). Furthermore, DRP is one of the most important causes of visual impairment and blindness in the industrialised world. Therefore, prevention of the metabolic syndrome and diabetes should be one of the main tasks for health professionals in the decades to come.

1.2. The diabetic eye

DM affects the eye in varying ways. Apart from hyperglycaemia-induced variations of the refractive power of the lens, cataract occurs earlier in patients with DM compared to persons without DM. Patients with DM have approximately a two-fold risk of cataract (Leske et al. 1999, Rowe et al. 2000). A special type of fast-maturing diabetic cataract involving both eyes that especially affects young people may occur (Falck and Laatikainen 1998). Diabetic neuropathy can manifest itself as as extraocular muscle palsies and also as autonomous neuropathy causing miosis. The most important diabetes induced pathologic finding is, however, DRP. Advanced DRP with retinal non-perfusion may also lead to neovascularizations in the anterior segment of the eye causing therapy-resistant neovascular glaucoma.
1.3 Diabetic retinopathy

1.3.1. History
After the invention and development of the direct ophthalmoscope in 1851 by Helmholtz, diabetic macular changes were for the first time described by Jaeger (1856). Jaeger’s findings were controversial and it was not until Nettleship (1872) verified histopathologically macular cystoid oedema that the causal relationship between diabetes and maculopathy was proven. Despite this the debate whether maculopathy was caused by diabetes, hypertension or arteriosclerosis, continued until the beginning of the next century. Manz (1876) reported proliferative retinopathy in diabetes for the first time. Increasing longevity of diabetic patients due to the discovery of insulin by Best and Banting in 1921 allowed late complications to occur in much greater numbers. Around the middle of the 20th century Ballantyne and Loewenstein (1943) showed more evidence of a retinal vasculopathy characteristic for DM. Many publications on DRP appeared in the 1950s and 1960s. After the Second World War the German ophthalmologist Meyer-Schwickerath reported treatment of retinal diseases with light coagulation. Today laser photocoagulation for PDR and DMO are well-documented treatment modalities. In 1968 an international symposium, the Airlie House Symposium, was held in the USA to evaluate the current knowledge of the natural history of DRP. It brought together leading American, British and Scandinavian ophthalmologists interested in retinal disease and DM. This symposium emphasized the need for standardized classifications of DRP, which was an important step towards quantifying the natural history of DRP and documenting the value of varying therapeutic interventions. Since then several multicenter studies, such as the Diabetic Retinopathy Study (DRS), Early Treatment Diabetic Retinopathy Study (ETDRS), Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), Diabetes Control and Complications Trial (DCCT), United Kingdom Prospective Diabetes Study (UKPDS), have provided evidence that now are considered the foundations of not only laser and surgical therapy but also of the general management, screening for and follow-up of DRP.

1.3.2 Aspects of pathogenesis
To date, several possible mechanisms including the polyol pathway, non-enzymatic glycation, oxidative stress and activation of PKC have been implicated in the development of DRP (Giugliano et al. 1996, Chibber at al. 1999, Gürler et al. 2000, Ways et al 2001, Yoshii et al. 2001). Functional as well as structural changes take place.

The functional changes comprise of an increase in and heterogeneity of the distribution of retinal blood flow. The retinal circulation is controlled locally in response to local and systemic influences and this auto-regulation is impaired in DM (Sinclair et al.1982, Harris et al. 1998, Kohner et al. 1995). So far at least ten humoral regulating substances have been proposed for the control of retinal blood flow (Chen et al. 2000). They fall into two main categories: firstly endothelium-derived relaxing factors such as nitric oxide, prostaglandin and endothelium-derived hyperpolarizing factor and secondly endothelium-derived contracting factors such as endothelin and cyclooxygenase products.

In retinal blood vessels both endothelial cells as well as pericytes are affected in DRP. Furthermore the basement membrane is thickened which in turn may contribute to capillary closure (Stitt et al.1995). The endothelial cells constitute the blood-retinal barrier and the pericytes regulate the vascular blood flow. The tight junctions of the endothelial cells, essential for the inner blood-retinal barrier, are dependent on an assembly of different proteins such as occludins, claudins and zonula occludens-1, -2 and -3 (Antonetti et al.1999, Gardner et al.1999). The quantity of occludin is reduced and the junction proteins are disorganized in experimental diabetes (Antonetti et al.1998, Barber et al. 2000). Morover, cytokines, such as VEGF altering the tight junction proteins,
induces increased vascular permeability (Aiello 1997). Other cytokines such as IGF-1 and bFGF may also be involved.

Apart from the well-known microvascular functional and structural lesions, hyperglycemia also induces retinal neurodegeneration which affects the macroglial, neuronal and microglial cells (Gardner et al. 2002). The macroglial cells, Müller cells and astrocytes are support cells that regulate retinal metabolism and modulate the function of neurons and blood vessels (Abbott et al. 1992). Recent studies have shown that astrocytes undergo prominent changes in DM (Barber et al. 2000, Runger-Brändle et al. 2000). Astrocytes extend end-feet that wrap around blood vessels of the inner retina. This interaction is important to induce the expression of tight junction proteins and to maintain the blood-retinal barrier (Abbott et al. 1992, Gardner et al.1997). In addition, Müller cells proliferate in the formation of epiretinal membranes in PDR (Hiscott et al. 1984). Furthermore, many studies have shown that glial cells as well as neurons produce cytokines as VEGF, which increase blood vessel permeability (Gilbert et al. 1998, Cooper et al. 1999). Microglial cells also become activated by DM and become phagocytic (Broderick et al. 2000). Furthermore, cytokines and chemokines, such as VEGF and tumor necrosis factor, are released from the affected microglial cells and increase retinal vascular permeability.

Neuronal cells, of which there are four primary types, photoreceptors, bipolar cells, amacrine cells and ganglion cells, are influenced by DM as well. Pathologic ERG, impaired colour vision and contrast sensitivity, in fact, may precede the clinically detectable DRP (Daley et al 1987, Di Leo M 1992, Parisi et al. 2001). In fact retinal ganglion cells and inner nuclear layer cells die by apoptosis early in DM, explaining these findings (Barber et al.1998). This may explain why some patients lose vision even if DMO is successfully treated or in patients with clinical manifestations of DRP but without any signs of macular thickening in ophthalmoscopy, fluorescein angiography or optical coherent tomography.

However, it is still unclear which cells, vascular or glial and neuronal cells, are primarily affected in DM. Moreover, other systemic conditions such as hypertension and renal and cardiac failure affecting the hydrostatic pressure and colloid osmotic pressure are of importance as etiologic factors for DMO (Aiello et al. 2001, Gardner et al. 2002).

Besides increased vascular leakage, microvascular occlusion is the other important component of DRP. The mechanisms behind vascular closure are many: microthrombosis, leucostasis and the invasion of Müller cells into the vascular lumen all contribute to the occlusion (Bek 1997, Barouch et al 2000, Boeri et al. 2001,). Occluded vessels cause retinal hypoxemia, which in turn increases the excretion of angiogenic factors as VEGF, bFGF, IGF-1, HGF, PlGF, Ang-2, TGF-α and TGF-β which stimulate the formation of new vessels (Boulton et.al.1997, 1998, Cai and Boulton 2002). PEDF, on the other hand, seems to inhibit the formation of new vessels (Chader 2001).

The mechanisms behind DRP, as described previously, include many signs of classic inflammation at a microscopic level (Adamis 2002): vessel dilatation, altered flow, exudation of fluids including plasma proteins and leukocyte accumulation and migration. This is of interest as intravitreous steroids may reduce DMO (Jonas 2003). Antonetti and co-workers (2002) also showed that hydrocortisone increases the tight junction proteins, essential for the blood-retinal barrier, in retinal endothelial cells.
1.3.3. Individual DRP lesions
The previously described functional and structural changes of the retina and retinal vessels in DM lead to vascular abnormalities, which in turn cause retinal hypoxia and oedema.

The first signs of DRP are usually small, 10 µm-100 µm sized, saccular capillary extensions called microaneurysms (Mas), which may disappear in early DRP (Kohner 1999). Only a small part of the Mas detected on FA are seen in a clinical examination or on fundus photography (Friberg et al 1986, Hellstedt et al. 1996).

Intraretinal haemorrhages (Hs) may appear on various levels in the retina: superficial flame shaped Hs are oriented in the direction of the nervfiber layer, small dot Hs usually lie in the superficial capillary plexus of the retina and can be indistinguishable from Mas, while larger blotch and cluster Hs lie deeper in the retina and are a hallmark of capillary damage.

DMO, the most common cause of decreased vision in NPDR, arise when plasma constituents accumulate in the extra cellular space. It can be seen as a local or a diffuse thickening of the retina or as a cystoid swelling in the centre of the fovea.

Leaking lipoproteins are seen as white streaks, clusters or circinate rings called hard exudates (Hes). They usually appear in the posterior pole with a preference for the macula.

Capillary closure leads to microinfarcts (Mis), also called cotton wool spots (CWS), local swellings of the nerve fiber layer due to disturbed axoplasmic flow, which appear as white fluffy spots. They may appear early as isolated lesions but in combination with other abnormalities are a sign of a more advanced DRP.

Venous dilation occurs early in DM, often before the clinical DRP, whereas venous beading (VB), which appears as focal areas of segmental venous dilation and narrowing, is a sign of advanced DRP. Other venous abnormalities are omega loops and segmental reduplication.

Intraretinal microvascular abnormalities (IRMAs), indicating STDR, are seen as pathologic networks of dilated and teleangiectatic capillaries. The morphology of IRMAs is consistent with the features of blood vessels in general in DRP: pericyte degeneration, thickening of basement membranes and endotehelial cell loss, but there are also histopathologic features indicating intraretinal neovascularization (Imesch et al. 1997).

When the capillary nonperfusion proceeds, it results in increased excretion of varying angiogenic factors and new blood vessels develop, on the retina (NVE) and/or on the optic disc (NVD). Neovascular vessels are fragile and may bleed either in the retrohyaloid space or in the vitreous and can be seen as either a preretinal haemorrhage (PRH) or vitreous haemorrhage (VH). In advanced DRP fibrous tissue develops in connection with neovascularizations. Fibrovascular proliferations may adhere to the undetached posterior surface of the vitreous body, the contraction of which can cause traction to the retina and finally retinal detachment.

1.3.4 Classification of DRP and maculopathy
DRP is classified in two main categories: non-proliferative (NPDR) and proliferative DRP (PDR) with neovascular vessels. DMO is the other type of STDR involving the macular region. One of the main tasks for the international Airlie House Symposium held in 1968 was to create an uniform, internationally adopted, detailed grading-scale for DRP (Davis et al.1969). This grading-scale
became the foundation for later modified classifications used in epidemiological and therapeutic intervention studies such as ETDRS (1980), DRS (1981), WESDR (Klein et al. 1984a,b, 1986), DCCT (1986), and the UKPDS (1991). The grading was based on seven-field stereoscopic 30° photographs using colour transparencies. The severity level of individual lesions was assessed field-by-field comparing them with 30° standard photographs. The overall DRP for each eye was obtained by summarizing the findings. This detailed grading-scale for DRP is still considered the Gold Standard with which other methods should be compared.

The DRS identified the special characteristics of PDR with a high risk for severe visual impairment (VA< 0.025) in the following years. High-risk PDR as defined below, if untreated, leads to severe visual impairment in about one half of the affected eyes within the next four years (DRS 1981).

**High-risk PDR**
- NVD ≥ Sp 10A or
- NVD < Sp 10A plus VH or PRH
- NVE ≥ 0.5 disc area plus VH or PRH
- VH or PRH obscuring ≥ 1 disc area

(For standard photographs, Sps, see ETDRS report nr 10, 1991)

In the ETDR study Nr 12 (1991c), gradings of baseline 30° seven-field stereoscopic fundusphotographs of eyes with NPDR assigned to deferral of photocoagulation, were used to examine the power of various lesions and the combination of lesions to predict the progression to PDR. The severity and extent of IRMAs, Hs and/or Mas and VB were the most important factors in predicting progression. A detailed 12-level DRP severity scale was developed based on these findings.

**The gradingscale consists of 12 levels (ETDRS 1991c)**

<table>
<thead>
<tr>
<th>Level</th>
<th>Description</th>
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<tbody>
<tr>
<td>Level 10</td>
<td>DRP absent</td>
</tr>
<tr>
<td>Level 14-15</td>
<td>Questionable DRP</td>
</tr>
<tr>
<td>Level 20</td>
<td>Minimal DRP</td>
</tr>
<tr>
<td>Level 35</td>
<td>Mild DRP</td>
</tr>
<tr>
<td>Level 43</td>
<td>Moderate DRP</td>
</tr>
<tr>
<td>Level 47</td>
<td>Moderately severe DRP</td>
</tr>
<tr>
<td>Level 53</td>
<td>Severe DRP</td>
</tr>
<tr>
<td>Level 61</td>
<td>Mild PDR</td>
</tr>
<tr>
<td>Level 65</td>
<td>Moderate PDR</td>
</tr>
<tr>
<td>Levels 71-75</td>
<td>High-risk PDR</td>
</tr>
<tr>
<td>Levels 81-85</td>
<td>Advanced PDR</td>
</tr>
<tr>
<td>Level 90</td>
<td>Cannot grade</td>
</tr>
</tbody>
</table>
Of special interest is the concept of moderately severe and severe DRP levels which are crucial for further prognosis. The definitions in general are complex, as demonstrated below, and they are not easily applied to wide-field photography as they are based on 30° fields.

<table>
<thead>
<tr>
<th>Definitions for minimal to severe (levels 20-53) DRP (ETDRS 1991c)</th>
</tr>
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<tr>
<td><strong>Level 20</strong></td>
</tr>
</tbody>
</table>
| **Level 30** | One or more of the following:  
Venous loops ≥ D/1*;  
CWS, IRMA or VB = Q;  
Retinal Hs present;  
He ≥ D/1;  
CWS ≥ D/1 |
| **Level 43** | H/Ma = M/4-5 – S/1 or Irma=D/1-3 (not both) |
| **Level 47** | Both characterics for moderate DRP and/or one only of the following:  
IRMA = D4-5  
H/Ma = S/2-3  
VB = D/1 |
| **Level 53** | One or more of the following:  
≥ 2 of the 3 of level 47 characteristics  
H/Ma ≥ S/4-5  
IRMA ≥ M/1  
VB ≥ D/2-3 |

*) D=definite, M=moderate, S=severe. The number after the letter indicates number of 30° standard fields (3-7) affected

For the classification of maculopathy the concept of DMO and CSMO has been presented. The latter is relevant for the ophthalmic clinician, as it defines treatment-needling, sight-threatening DMO according to ETDRS studies (ETDRS 1985, ETDRS 1987a,b).

**CSMO** as defined by the ETDRS is one or more of the following:
- Retinal thickening at or within 500µm of the foveal avascular zone.
- He at or within 500µm of the center of the foveal avascular zone, if associated with thickening of the adjacent retina.
- Retinal thickening > 1,500 µm within 1,500µm of the center of the foveal avascular zone.
1.3.5. Prevalence and incidence of DRP
Type 1 DM
Generally the prevalence of DRP at diagnosis of Type 1 DM has been reported to be low, between 0 to 3% (Dorf et al, 1976, Frank et al, 1980, Klein et al, 1997, Wan Nazaimoon et al, 1999). In the DCCT study from Canada and the USA involving 29 centres, however, photographic evidence of DRP was found in 54% at baseline (DM < 5 years duration) and 67% within five years of diabetes duration (Malone et al, 2001). In the same study 20% of patients with a one year-duration of DM had some DRP. In the EURODIAB, a multicentre study from 31 centres in 16 countries, the mean overall prevalence of DRP was 36% (range 19%-69%) (Toeller et al,1999). In the WESDR, as well as in a Swedish study, the prevalence of DRP was strongly correlated with the duration of DM. In the WESDR, prevalence of DRP rose sharply from 2% in those with DM less than two years to 98% after 15 years (Klein et al,1984a). In a Swedish study, DRP was found in 4% in patients with disease duration less than five years and in 92% after 15 years (Henricsson et al, 1996a).

Children with the onset of DM before the age of ten do not usually have DRP and definitely not treatment needing lesions. In a population-based survey in the county of Oulu, Finland, 194 of 216 subjects (90%) aged 4.6-16.6 years, 11% had minimal or mild DRP but no PPDR, PDR or maculopathy was found (Falck et al, 1993). The youngest patient with DRP was a 12-year old boy with DM from the age of 1.3 years. The children in the DRP group had an average DM-duration of 8.5 years. DRP was not found in any of the children who were prepubertal. Prevalence of DRP increased in puberty so that 17% (13/79) of the children examined in puberty and 32% (8/25) examined in the postpubertal stage had DRP.

In a Swedish population-based study, 780 IDDM patients aged > 9 years with DM diagnosis before the age of 15 years, disease duration under 12 years, were invited, of whom 71% participated (Kernell et al, 1997). The prevalence for any DRP and for PPDR or PDR was 15% and 2.3 %, respectively. In this series one patient (1/19) in the age group under ten years had minimal DRP (Mas only). Six percent of patients (4/66) in pubertal stage 1 (Tanner) and 18% (41/224) in pubertal stage 5 had DRP (p< 0.03). The duration of DM was not significantly different in the patients with signs of DRP in pubertal stage 1 compared to the postpubertal patients with DRP. In other Swedish studies the prevalence of any DRP in patients with Type 1 DM varied from 61% to 68% (Agardh et al, 1989, Reuterving et al, 1999, Lövestam-Adrian et al, 2001). This is somewhat lower compared with the result (71%) presented in the WESDR (Klein et al, 1984a).

Kristinsson and co-workers (1997) reported a four-year incidence of any retinopathy of 38% in Type 1 diabetic patients in Iceland which is significantly lower than that (59%) reported by Klein et al. (1989c) in the Wisconsin study.

These results confirm the necessity of regular retinal screening examinations among IDDM children after ten years of age. Patients with Type 1 DM diagnosed later should be examined regularly from the onset of the disease.

Type 2 DM
From 13% to 39% of patients with Type 2 DM have some DRP at baseline ophthalmologic examination (two to five years from diagnosis of DM) and these patients are at risk to develop STDR in the follow-up period of ten years (Klein et al,1984b, Henricsson et al,1996a, UKPDS 1998b). Twenty years from the onset of DM more than 60% of people with Type 2 DM will have
some kind of DRP (Klein et al.1984b, Henricsson et al.1996a). The prevalence of DRP is higher for those patients needing insulin treatment compared to those maintaining diet or oral agents only, 62% and 36%, respectively (Klein et al 1985a). Comparable figures were presented by Henricsson and co-workers (1996a). In a recent study from Sweden 66% of the insulin treated, 30% of the oral agent-treated and 7% of the diet-treated older onset diabetic patients (age at diagnosis ≥ 30 years) presented some DRP (Reuteriving et al.1999). Patients with Type 2 DM and with a normal baseline status or with minimal DRP (Ma in one eye only) are not likely to develop treatment-needing lesions in the next three years (Kohner et al.1999, UKPDS 2001a). Notably, patients who are maintaining on diet-treatment only and who at baseline examination have a normal retinal status, are at low risk to develop treatment-needing retinopathy in the next four to five years (Henricsson et al.1996b, Hansson-Lundblad et al.1997).

In the UKPDS study, the number of Mas and level of DRP at baseline examination strongly correlated with the risk for STDR in the next nine years (Kohner et al.1999). Interestingly, about 50% of single Mas disappeared over the course of three years (Kohner et al. 1999).

These findings suggest that the screening interval for DRP, for patients with Type 2 DM with a good glycaemic control without or with minimal DRP (1-2 Mas) at baseline examination, could be two or even three years instead of one year, if screened with a reliable screening method. Likewise patients with a normal baseline retinal status, maintaining on diet-treatment only, could be screened every fourth to fifth year.

1.3.6. Prevalence and incidence of STDR and blindness
In developed countries DRP is one of the leading acquired and treatable causes of visual impairment in people of working-age as well as in people older than 65 years. (Ojamo 2001, Kocur et al. 2002). STDR comprises DMO, which is the more frequent cause of visual impairment in patients with Type 2 DM, and PDR which is the more common cause of visual impairment among patients with Type 1 DM (Klein et al.1984d, Fong et al. 1999). The prevalence of PDR and DMO are both related to the duration of the disease.

None of the patients with Type 1 DM in the WESDR had DMO with disease duration of less than five years, compared with 29% after 20 years. Corresponding figures for Type 2 diabetic patients were 3% and 28% (Klein et al.1984c). Prevalence of DMO varies also, depending on whether the disease is insulin treatment-needing. In a recent Swedish cross-sectional study the prevalence for CSMO for patients with Type 1 DM was 16% and for Type 2 DM 26%, 9% and 0.6% depending on whether the patient was treated with insulin, oral agents or diet only (Reuterventing 1999).

The prevalence of PDR in the WESDR for patients with Type 1 DM was 4% when the disease duration was nine to ten years and 51% after 20 years disease duration. In patients with Type 2 DM with disease duration less than four years the prevalence was 4% versus 3% depending on whether the patients were insulin dependent or not. With disease duration of more than 15 years corresponding figures were 20% versus 4% (Klein et al. 1987). The mean prevalence for PDR for Type 1 diabetic patients in Europe in the EURODIAB study was 10.8% (range 3-19.5%) (Toeller et al.1999). Again the patients who were insulin dependent had a higher prevalence of PDR compared with the non-insulin dependent ones, 25% versus 5%, respectively (Klein et al. 1985a).

The four-year incidence of PDR in IDDM patients in the studies from Iceland and Wisconsin were 6.6% and 11%, respectively (Klein et al. 1989c, Kristinsson et al. 1997). The four-year risk of developing PDR in the Icelandic study was 44% in those with PPDR, 5.3% in those with background DRP and in 2.4% in those without any DRP at the baseline examination.
Klein and co-workers (1989c) found a four-year risk of developing PDR in 42% of those patients with level 41 baseline DRP characteristics, which corresponds with the definition of PPDR in the Icelandic study.

Sjølie and Green (1987) showed that the prevalence of blindness in a Danish Type 1 diabetes population was 5.9% in patients with disease duration of more than eight years and estimated the risk of blindness for patients with DM to be 50-80 times higher than in the non-diabetic population. In the WESDR the four-year incidence of blindness was 1.5% for patients Type 1 DM and 3.2% versus 2.7% for insulin-treated and oral medication or diet-treated patients with Type 2 DM, respectively (Moss et al. 1988). In a recent Swedish study, the prevalence of social blindness (VA ≤ 0.1 in the better eye) was 2.5% versus 1.5% in the younger-onset (DM-diagnosis < 30 years of age) versus the older-onset patients (Reuterving et al. 1999).

1.3.7. Risk factors for DRP

Glycaemic control

Apart from the duration of DM, the most important risk factor for DRP is unsatisfactory glycaemic control. Normoglycaemia, or blood glucose values as close to normoglycaemia as possible, protect the eye from DRP as such or the progression of pre-existing DRP in patients with Type 1 as well as Type 2 DM.

The DCCT multicentre study (1993) from the USA involved altogether 1441 patients with Type 1 DM, 726 patients without DRP (primary prevention cohort) and 715 patients with mild DRP (secondary prevention cohort) at baseline. The patients in both groups were randomly assigned to intensive therapy or to conventional therapy. The mean follow-up time was 6.5 years. The average HbA1c value in the intensive treatment group was 2% lower than that in the conventional treatment group (7% versus 9%). In the primary prevention cohort intensive therapy reduced the adjusted mean risk for the development of DRP by 76% and in the secondary intervention cohort intensive therapy slowed the progression of DRP by 54% as compared with conventional therapy. In another large prospective complication study including 3250 Type 1 diabetic patients, the EURODIAB study, HbA1c value was the strongest predictor of progression to PDR (Porta et al. 2001).

The UKPDS study, a multicentre randomised intervention study, included 3867 newly diagnosed patients with Type 2 DM who were randomly assigned to two groups, one on intensive treatment and one on diet-therapy only (UKPDS 1998a). The target fasting blood glucose in the intensive therapy group was 6 mmol/l. In ten years the average HbA1c value was 11% lower in the intensive therapy group as compared to the conservative treatment group (7% versus 7.9%). The risk for DRP and the need for photocoagulation treatment were reduced by 25% in the former group.

In a smaller (n= 110) randomised study from Japan patients with Type 2 DM were assigned to two groups, a multiple insulin injection therapy group (MIT group) and a conventional insulin injection treatment group (CIT group) (Ohkubo et al. 1995). In accordance with the DCCT study a primary intervention cohort and a secondary intervention cohort were included in each therapy group. The follow-up time was six years during which the cumulative percentages of development of DRP in the MIT and CIT group were 7.7% and 32%, respectively. In the secondary prevention group the cumulative percentages of progression of DRP (two steps or more on a modified ETDRS scale) were 19% versus 44%, respectively.

Rapid improvement of glycaemic control might cause an early worsening of DRP (DCCT 1993, Henricsson et al. 1999). However, long-term outcomes in the intensively treated group who had an
early worsening, were similar or more favourable than outcomes in conventionally treated patients who had not (DCCT 1993).

**Blood pressure**

Strictly controlled blood pressure appears to reduce the risk of clinical complications from diabetic eye disease.

The UKPDS (2004) included 1148 patients with Type 2 DM and arterial hypertension, of whom 758 patients were allocated to a tight BP control either with angiotensin-converting enzyme inhibitor or β-blocker as the main therapy and 390 patients were allocated to a less tight BP control. The mean BP during the study over nine-years follow-up was 144/82 mm Hg in the tight BP therapy group and 154/87 mm Hg in the conventional therapy group. It became evident that there was a statistically significant reduction in the progression of DRP and a reduced need of laser treatment due to maculopathy in the tight BP treatment group. After 7.5 years the relative risk for at least a two-step progression of DRP in the tight PB therapy group was 0.66 (p<0.001).

For patients with Type 1 DM similar results have been obtained (Janka et al. 1989, Klein et al. 1989a, Sjølie et al. 1997). Janka and co-workers (1989) showed in a prospective study comprising 153 IDDM patients that the severity of the progression of eye lesions was associated, among others, with elevated diastolic BP. The mean follow-up time was 46 months. The risk of severe progression of DRP was significantly higher in those individuals with a diastolic BP over 70 mm Hg. This finding persisted in multiple logistic regression analysis. In WESDR systolic BP was found to be a significant and independent predictor of the four-year incidence of DRP (Klein et al 1989a). Diastolic BP was associated with DRP progression but the relationship did not reach statistical significance. In the EURODIAB study diastolic blood pressure was independently related to moderate and severe NPDR (Sjølie et al. 1997).

**Blood lipids**

Contradictory results regarding dyslipidaemia as a risk factor for DRP have been presented. In the observational data from the ETDRS (1996) patients with an elevated baseline serum total cholesterol or LDL-cholesterol were twice as likely to have hard exudates as patients with normal levels. The risk of decreased visual acuity (loss of three lines) was associated with the extent of hard exudates. In the EURODIAB study, elevated triglycerides were associated with moderate-severe NPDR and PDR (Sjølie et al. 1997). Later, the same study group concluded using a nested case-control study approach and multivariate analysis adjusted for age, gender and glycaemic control, that there was a positive correlation between total cholesterol, LDL-cholesterol as well as triglycerides and the severity of DRP (Chaturvedi et al. 2001). However, part of the association could be accounted for by the association of an increased albumin excretion rate from nephropathy. In the DCCT study, the association between the severity of DRP and conventional lipid measures was shown in univariate analyses but they were lost in multivariate analyses (Lyons et al. 2004). However, detailed analyses of lipoprotein subclasses revealed associations which remained significant in multivariate analyses. In the UKPDS (1998b) there was no correlation between triglyceride or LDL-cholesterol levels and the presence and severity of DRP in the baseline univariate analysis. However, the same study group investigated risk factors related to the incidence and progression of DRP over six years and found a correlation between LDL-cholesterol and two-step progression of DRP but only in those individuals with baseline DRP (UKPDS 2001b).
Other risk factors

Pre-existing DRP is always a risk for the progression of DRP (Klein et al. 1989b, UKPDS 1998b, UKPDS 2001a). Klein et al. (1995) showed that an increase of 16 or more Mas over a four-year period increased the risk of PDR by 4.6 and the risk of CSMO by 9.1 times at the ten-year follow-up. Likewise, insulin-needing patients with Type 2 DM are more prone to develop DRP compared with patients on diet or oral medication only (Klein et al.1984b, Henricsson et al.1996a).

Hormonal alterations in puberty and pregnancy are risk factors for the development of or the progression of pre-existing DRP. Murphy and co-workers (1990) showed that puberty is a risk factor for the development of DRP. The relative odds of having DRP, after adjusting for DM duration and gender, was 4.8 in the post-pubescent group as compared to the prepubescent or pubescent groups of patients with Type 1 DM. The mechanisms behind the acceleration of microvascular disease is anticipated to be due in part to the combination of the deterioration of glycaemic control and an increase in growth and sexual hormones. In the DCCT study, pregnant women with Type 1 DM and conventional treatment had a 2.48-fold greater risk of any worsening of DRP during pregnancy compared with nonpregnant women. The risk for the pregnant women with better glycaemic control in the intensive treatment group was 1.63-fold (DCCT 2000).

1.3.9. Prevention of visual impairment

Apart from minimizing risk factors for DRP, the most important tool to reduce visual impairment caused by DM is regular screening examinations of the ocular fundus in order to detect treatment-needing, usually asymptomatic, lesions early enough to achieve optimal treatment results. Laser photocoagulation for high-risk PDR and CSMO is of proven benefit (DRS 1981, ETDRS 1985). Older patients with DM, in particular those with Type 2 DM and PPDR, also benefit, from photocoagulation before high-risk features develop (Ferris 1996). Since the current international therapy recommendations for PDR have been adopted, the risk for severe visual loss from PDR has diminished from about 50% to less than 2% (Ferris 1993). Treatment for DMO has not been as successful. Focal photocoagulation and grid photocoagulation may reduce the risk of moderate vision loss by 50 % (ETDRS 1985).

Several studies, showing the positive effects of early detection and treatment of STDR have been published. In Iceland the prevalence of legal blindness (VA in the better eye < 0.1) was 2.4% in 1980 when a national screening programme started. In 1994 the corresponding figure was 0.5%, demonstrating the substantial benefit of a regular screening programme (Kristinsson et al 1997). In a Swedish study, new DM related blindness was reduced by more than one third from 1981 to 1995 in Stockholm where systematic screening by a mobile team was initiated in 1990 (Bäcklund et al. 1997). In another Swedish clinic-based study, the five-year incidence of blindness (VA ≤ 0.1) and moderate visual impairment (VA > 0.1 ≤ 0.5) after introducing a screening programme for early detection of treatable DRP, was 0.5% and 1.2% for patients with Type 1 DM and 0.6% and 1.7% for patients with Type 2 DM (Agardh et al. 1993). The figures for blindness are concordant with those presented by Kristinsson.

However, a small number of patients end up visually impaired despite regular photographic screening mainly due to an unsuccessful outcome after laser treatment of DMO (Hansson-Lundblad et al. 2002).
1.4 Screening for diabetic retinopathy

1.4.1 History
Jackman and Webster (1886) described in vivo human retinal photography for the first time. Since then a tremendous development in ophthalmic imaging equipment, including those needed for FA and indocyanide angiography as well as digital imaging, has taken place. The development of FA in the early to mid-1960s made it possible to visualize, describe and also to quantify different pathologic processes in DRP. For large-scale DRP screening purposes fundus photography has been available since the 1980s. Until then ophthalmoscopy was the most commonly used examination method. A national diabetic eye screening programme was founded in Iceland in 1980 (Kristinsson et al.1994a,b). In 1989 WHO and IDF in Europe arranged a meeting in Saint Vincent in Italy. This meeting established general goals with relation to people with DM and targets for the health professionals (Diabetes care and research in Europe: The Saint Vincent Declaration 1990). One of the targets was to reduce new blindness due to DM by one third or more in the next five years. This declaration initiated the establishment of national DRP screening programmes in Europe. According to an inquiry sent to hospitals and non-institutional care offices in Sweden in 1990, 94 % of the hospitals had an established screening programme for DRP. Of the out-patient offices only 66% answered and of these 76% did regular screening. In 1991, a concensus meeting was held in Stockholm and recommendations concerning the management of DRP were published (Medicinska forskningsrådet & Spri 1992). In Finland consensus recommendations were published in 1992 and regular photographic screening was recommended (Karma et al 1992).

1.4.2 Criteria of a disease suitable for screening
DRP fulfils the criteria for a screening programme according to WHO (1971): The disorder which should be screened is well defined, there is available knowledge about the prevalence and the progression of the disorder, there are effective treatment modalities, there are simple and safe screening methods and the screening programme is cost effective.

1.4.3 Methods
Screening for DRP is generally performed by a variety of health professionals using ophthalmoscopy or non-stereoscopic retinal photography. There is a wide variability in screening services both in coverage and methods used.

Seven-field 30º stereoscopic photography, considered as the Gold Standard, is not usually a feasible method when performing large scale screening and is therefore not always used as the reference standard. An ophthalmologist’s examination using slit lamp biomicroscopy compares favourably with seven-field stereophotography and can therefore also be used as a reference standard (Scanlon et al. 2005).

FA is the most sensitive method for the detection of DRP lesions. Only a small part of the Mas as detected with FA are perceived when using colour or red-free photography, 7% and 13%, respectively, according to Hellstedt and co-workers (1996). However, as an invasive examination method, FA is time-consuming and expensive, and also prone to fatal risks (Yannuzzi et al. 1996). It is therefore not suitable as a screening or as a reference method for screening purposes.

Ophthalmoscopy, specially in the hands of a non-ophthalmologist, but even when used by experts, has proven to be insensitive (Sussman et al. 1982, Buxton et al 1991, Hutchinson et al. 2000) When screening for any STDR using direct or indirect ophthalmoscopy, the achieved specificity was over 91% but the sensitivity was poor in the hands of ophthalmologists (43-79%) as well as diabetologists (27-73%) and GPs (25-66%) (Hutchinson et al. 2000). Fundus photography has

1.4.4. Classification of DRP
For large-scale screening purposes the detailed ETDRS classification protocol is too time-consuming and expensive. Furthermore, 30° fields are not used for photographic screening purposes. Therefore the ETDRS classification scheme is not suitable for DRP-screening from wide-field photographs.

1.4.5 Cost-effectiveness of screening for DRP
In a recent Finnish study a yearly photographic screening programme using a non-mydriatic 45° one-field approach could lead to savings of FIM 63.9 million (€ 10.7 million) per year (Pajunpää 1999). This calculation was based on the fact that the screening and treatment costs in finding one patient with preventable visual impairment using this method were FIM 185,000 (€ 30,800) while the costs caused by visual impairment were FIM 594,000 (€ 99,000) per patient. Several other studies have proved the cost-effectiveness of varying screening strategies for DRP, especially of younger onset (< 30 years) and older onset (≥ 30 years) patients with IDDM (Javitt et al 1990, Dasbach et al 1991). These results are, however, not directly applicable to the Nordic countries with differing health care policies.

1.4.6 Screening intervals
Most DRP screening recommendations advocate screening intervals of one to two years depending on the baseline status and risk factors (Table 5). Generally the first eye examination for patients with childhood-onset Type 1 DM should take place at age ten years or at the onset of puberty and for patients with type 2 DM at diagnosis of DM. Recently Younis et al. (2003a) published the results of the Liverpool cohort study concerning the incidence of STDR in patients with Type 2 DM out of 20570 screening events. The yearly incidence of STDR in patients without DRP at baseline was 0.3% (95% CI 0.1-0.5) in the first year, rising to 1.8% (95% CI 1.2-2.5) in the fifth year. The cumulative incidence at five years was 3.9 (95% CI 2.8-5.0). For a 95% probability of being free from a STDR, mean screening intervals by baseline status were: no DRP 5.4 years (95% CI 4.7-6.3), background 1.0 years (95% CI 0.7-1.3) and mild PPDR 0.3 years (95% CI 0.2-0.5). The authors concluded that a three-year screening interval could be safely adopted for patients with no DRP at baseline. This is in concordance with the results from the UKPDS (2001a).

Likewise, the Liverpool group studied the incidence of STDR in patients with Type 1 DM based on 2742 screening events. They found that the cumulative incidence of STDR in patients without DRP at baseline was 0.3% (95% CI 0.0-0.9) at one year rising to 3.9% (95% CI 1.4-5.4) at five years. A screening interval of two to three years was suggested, rather than annual controls (Yonis et al. 2003b). This is in line with the results presented by Hansson-Lundblad and co-workers (1997) that patients with Type 2 DM with no DRP at baseline, maintaining only on diet-treatment are not likely to develop STDR in the next four to five years.

These results indicate that the screening interval in the category of patients mentioned above and in the absence of special risk factors, could be longer than those recommended in the guidelines of Table 1.

1.4.7 International and national recommendations
International and national guidelines for the screening of DRP in Europe and in the USA are presented in Table 1.
Table 1.

### International and national guidelines for screening of DRP

<table>
<thead>
<tr>
<th>Recommendation by Type of DM</th>
<th>First examination</th>
<th>Screening interval</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinopathy Working Party. A protocol for screening for DRP in Europe (1991)</td>
<td>Type 1 From onset of puberty</td>
<td>At least two-yearly. At least annually if retinopathy appears, and more frequently if deemed appropriate.</td>
<td>Ophthalmoscopy or photography with mydriasis. Two 45° or 50° fields (centred on macula and nasal to optic disc) or four 30° fields (temporal to macula, macula, optic disc, nasal to optic disc) using colour or red-free transparencies. Measurement of visual acuity.</td>
</tr>
<tr>
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<td>Type 2 At diagnosis of DM</td>
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<tr>
<td>The Finnish Diabetes Association (Karma et al. 1992)</td>
<td>Type 1 Patients entering puberty</td>
<td>Annually</td>
<td>Ophthalmoscopy or photography from two fields (macula- and optic disc-centred) with mydriasis. Red-free, black-and-white or colour photographs or colour transparencies</td>
</tr>
<tr>
<td></td>
<td>Type 2 At diagnosis of DM</td>
<td>Annually, in case of no DRP or mild DRP without decreased visual acuity</td>
<td></td>
</tr>
<tr>
<td>The Swedish Council on Technology Assessment in Health Care (1993) and The Swedish National Board of Health and Welfare (1999)</td>
<td>Type 1 From the age of ten years</td>
<td>At least every 2 years</td>
<td>Photography with mydriasis recommended</td>
</tr>
<tr>
<td></td>
<td>Type 2 At diagnosis of DM</td>
<td>At least every 2 years</td>
<td></td>
</tr>
<tr>
<td>The Royal College of Ophthalmologists (UK). Guidelines for diabetic retinopathy (1997).</td>
<td>Type 1 All patients aged over 12 years and/or entering puberty should be screened</td>
<td>Annually</td>
<td>Ophthalmoscopy or photography with mydriasis. Measurement of visual acuity.</td>
</tr>
<tr>
<td></td>
<td>Type 2 Within three to five years after diagnosis of diabetes once patient is age ten years or older, paying particular attention to the prepubertal duration of diabetes.</td>
<td>Annually, but less frequent examinations (every 2-3 years) may be considered with the advice of eye care professionals in the setting of a normal eye examination. Abnormal findings necessitate more frequent follow-up.</td>
<td>Dilated eye examination by an ophthalmologist or optometrist or 30° seven-field stereoscopic photography. The use of a non-mydriatic camera may be considered.</td>
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<tr>
<td></td>
<td>Type 2 At diagnosis of DM</td>
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1.5 Photographic screening of DRP

1.5.1 Photographic methods

General aspects
The main advantage of retinal photography, besides reliability and validity aspects, is that every screening event produces an objective document of the retinal status for future comparison. Furthermore, the photographic or digital material enables consultation with other health professionals.

In the WESDR three methods, direct ophthalmoscopy through an undilated pupil, nonstereoscopic one field 45° retinal photography through a pharmacologically undilated pupil and nonstereoscopic 45° retinal photography through a dilated pupil, were validated in a masked randomised fashion against three-field 30° stereoscopic colour transparencies as a reference standard in 99 patients (Klein et al. 1985b). When using three levels of severity (no DRP, NPDR, PDR) agreement between the results obtained from ophthalmoscopy and the standard method was only 54 %. When comparing the results of photography through undilated and dilated pupils for four severity levels of DRP the agreement was 83% and 87% (Klein et al. 1985b).

Pugh et al. (1993) evaluated four screening methods: an examination by an ophthalmologist through dilated pupils using direct and indirect ophthalmoscopy, an examination by a trained assistant through dilated pupils using direct ophthalmoscopy, a single 45° retinal photograph without pharmacological dilation, and a set of three dilated 45° retinal photographs. The reference standard was stereoscopic 30° seven-field photographs assessed by a central reading centre. The sensitivity and specificity for the detection of at least moderate DRP were as follows: ophthalmologist 0.33, 0.99; single-field photograph without pharmacological dilation assessed by a central reading center 0.61, 0.85; dilated three-field photography assessed by a central reading center 0.81, 0.97, physicians assistant 0.14, 0.99, respectively.

Recently several studies have been published regarding how DRP is detected from digital images versus colour photography (George et al. 1998, Liesenfeld et al. 2000, Tennant et al.2001, Fransen et al. 2002, Lin et al. 2002, Olson et al. 2003). Excellent agreement (93%) was obtained using colour digital images and colour transparencies (George et al.1998). Lin et al. (2002) investigated the sensitivity and specificity of single-field 45° nonmydriatic monochromatic digital photography with standardized mydriatic seven-field colour photography and found an exact agreement for five ETDRS retinopathy levels in 65%. Agreement using kappa statistics concerning referral-requiring DRP (≥ level 35 = mild DRP) was 0.97.

Retinal photography with mydriasis appears to be more sensitive than direct ophthalmoscopy. However, both methods may achieve high sensitivity or specificity under optimal conditions (Bachmann et al 1996, Hutchinson et al.2000). Although there is no international formal standard for an acceptable level of sensitivity and specificity for screening tests for DRP, the British Diabetic Association (1997) has proposed levels of at least 80% sensitivity and 95% specificity and a maximum failure rate of 5%.

A systematic review of literature published in English from 1983 to 1999 was recently presented in order to evaluate which screening method and screening strategy for DRP was the most appropriate one (Hutchinson et al. 2000). The main databases were searched and the studies with a prospective study design which compared at least one screening method in a blinded fashion with that of a
reference standard were included. None of the studies were randomised controlled studies. In this review it became evident how difficult it was to compare the efficacy of varying photographic screening methods. There was a considerable variation in the outcomes not only depending on the method but also depending on which DRP grading scale was used and which level of DRP was used for the calculation of sensitivity and specificity. Other confounding factors were varying reference standards and the experience of health professionals who assessed DRP. Thus, in some studies the grading was performed by diabetes physicians and in other studies by trained graders or optometrists or ophthalmologists working at retinal centres. Retinal photography with mydriasis appeared to be the most effective test, with reported sensitivity often in excess of 80%. Conversely, the specificity obtained by retinal photography was lower than when obtained with ophthalmoscopy. Specificity using ophthalmoscopy exceeded 91%.

The sensitivity and specificity for the detection of any DRP as well as STDR, using direct ophthalmoscopy and 45°-50° photography (film and digital) for GPs, diabetologists and ophthalmologists is presented in Table 2. The figures in Table 2 are data from the systematic review (1983-1999) by Hutchinson et al. (2000) and data from after 1999 concerning photographic and digital DRP screening (Newsom et al. 2000, Stellingwerf et al. 2001, Saari et al. 2004, Scanlon et al. 2005).
Table 2.

Sensitivity (Sens) and specificity (Spec) for detection of any and sight-threatening DRP (STDR) or referable DRP using ophthalmoscopy and retinal photography (film and digital)

<table>
<thead>
<tr>
<th>Examiner</th>
<th>Any DRP</th>
<th></th>
<th>STDR or referable DRP</th>
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</thead>
<tbody>
<tr>
<td></td>
<td><strong>0phthalmoscopy</strong></td>
<td><strong>Retinal photography</strong></td>
<td><strong>0phthalmoscopy</strong></td>
<td><strong>Retinal photography</strong></td>
</tr>
<tr>
<td></td>
<td>Sens %</td>
<td>Spec %</td>
<td>Sens %</td>
<td>Spec %</td>
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<tr>
<td>GP</td>
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<td>Buxton et al.</td>
<td>3</td>
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<td>8</td>
<td>94 *)</td>
<td>98 **)</td>
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<td>Scanlon et al.</td>
<td>9</td>
<td>80</td>
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</table>

1) Moss et al. (1985): Direct and/or indirect ophthalmoscopy with mydriasis. Reference standard: 30º 7-field photography. STDR: PR
4) Harding et al.(1995): Direct ophthalmoscopy with mydriasis. Reference standard: Stereoscopic slit lamp biomicroscopy by retinal specialist. Retinal photography: 45º 3-field evaluated by ophthalmologist clinical assistant. STDR: PPDR or more advanced DRP, maculopathy, VA≤ 0.5, other ophthalmic disease or ungradable photographs.
8) Saari et al. (2004): Reference standard: Stereoscopic slit lamp biomicroscopy by experienced ophthalmologist and colour and red-free images. Digital images: *) two 50º colour images (768 x 576 pixels), **) one black-and-white red-free 50º image (1320x1032 pixels), ***) two 45º colour images 2160 x 1440 pixels)
9) Scanlon et al. (2005): Reference standard: 30º 7-field photography. Referable DRP: Moderate or more severe and maculopathy. Photography: 45º 2-field digital colour images.
Use of mydriatic (pupil dilating) drops
Fundus photography using a non-mydriatic camera allows photography without mydriatic drops. However, it has been proven that pharmacological mydriasis improves significantly the quality of photographs or digital images and therefore the use of pupil dilating drops is recommended. In a British study it was shown that the use of mydriatic drops increased the proportion of gradable images from 5% to 26% when using one-field 45° digital images (Murgatroyd et al. 2004). The benefit of using pharmacologic mydriasis was most obvious in the elderly patients (> 55 years). In another study by Pugh and co-workers (1993) the proportion of non-gradable images, when using colour transparencies, was reduced from 14% to 3.7% with mydriatic drops. Furthermore, photography using pharmacological mydriasis gave statistically higher sensitivity and specificity values for referral-needing DRP compared with photography without it. However, photographs of eyes which, despite the use of mydriatic drops are ungradable should be considered as screening positives and the patients should be referred for a clinical examination.

Use of monochromatic light
Ocular fundus can be investigated or photographed with different wavelengths of light. First, when used with black-and-white film monochromatic light lightens structures of their own colour and darkens structures of the complementary colour. Second, the wavelength of monochromatic light determines its capacity for retinal penetration. Light of short wavelength (e.g. blue light) is scattered from the surface of the retina allowing us to visualize the surface structures (e.g. nerve fiber layer) particularly well. Red light, on the other hand, penetrates deep into the retina and makes it possible to analyse choroidal structures (Fig.1).

![Fig. 1. Schematic drawing of the retina. Different colours indicate the presumed depth of penetration of monochromatic light of varying wavelengths. Red light (640 nm) penetrates to the choroid, green light (540 nm) is reflected mainly from the retina, whereas blue light (490 nm) is scattered from the surface of the retina. Blood vessels in all layers are marked with dark red.](image-url)
Vogt’s (1925) studies with red-free light first demonstrated the clinical usefulness of spectral illumination. Later many researchers have investigated and reported results of examination and photography of the ocular fundus with different wavelengths of light (Kugelberg 1940, Behrendt et al. 1965). The light absorption of blood, or more specifically that of haemoglobin, was found to be high in the blue-green region of the light spectrum (Horecker 1943). Delory et al. (1977) and Ducrey et al. (1979) investigated how normal as well as pathological fundus structures were detected using monochromatic opthalmoscopy and fundus photography. They found that the wavelength for observation of large retinal vessels (first and second branches of retinal central artery and vein) was 570 nm. The contrast of vessels decreased as the wavelength decreased below 540 nm or increased above 580 nm. By introducing a green filter in the optical system of a fundus camera red-free light can be employed. The monochromatic green filter (often termed a red-free filter) thus enhances the contrast of retinal blood vessels and haemoglobin containing normal and abnormal structures.

Digital colour images can be converted red-free with the provided software. The red-free photographic technique has not earlier been an established screening approach although described by Falck et al. (1993) and Hirvelä and Laatikainen (1997). Digital red-free technique has also been used for automatic detection of microaneurysms as well as of other DRP abnormalities (Gardner et al. 1996, Hipwell et al. 2000).

**Films and digital imaging**

35 mm colour film for transparencies has been widely in use for screening for DRP (Kalm et al. 1989, Bäcklund et al. 1993). The advantage of 35 mm film is a superb resolution. The disadvantage is the need of magnifying equipment and that the assessment is time consuming.

Use of high-resolution black-and-white film (Kodak Panatomic-x) and green filter has been reported in a research setting, but this method has not generally been used in every-day screening work earlier (Falck et al. 1993).

Polaroid prints have also been widely used for screening of DRP. However, Jones et al. (1988) found that about 20% of the Polaroid prints were not assessable owing to poor quality. Despite of those discouraging experiences ten of the twelve participating centres in a British multicentre study in the 1990s still used Polaroid film (Taylor et al. 1996).

The development of digital imaging technology during the course of the last ten years has made it possible to switch to digital images when screening for DRP. Instead of light sensitive film these cameras are equipped with computer chips that transforms the image into electrical signals, which can be read by a computer. The advantages are many: immediate assessment, telemedical transmission possibilities, no expenses for film developing and convenient storage of the images, which will not degrade over time. In addition, technical failure rates are lower with digital imaging than conventional photography (Sharp et al. 2003). Furthermore, the digital images are annotated with information concerning technical data of images, name of patients and date of photography. The resolution of the digital image depends not only on the amount of pixels (picture elements) but also on the quality of the optics of the fundus camera. The amount of resolution needed for a digital image for DRP screening purposes has been discussed but there are no official international recommendations concerning the amount of pixels. However, most modern digital cameras produce high resolution and high quality images (Scanlon 2000). Digital images are in colour but can be manipulated by software to provide red-free images. Monochrome digital images can also be captured with a green filter in the camera.
Computerized lesion analysis and counting of Mas may be useful for screening of DRP (Gardner et al. 1996, Hipwell et al. 2000, Larsen et al. 2003, Hansen et al. 2004b). However, automatic detection of DRP is so far not used in everyday screening work.

**Fundus cameras**

Principally, there are two main types of fundus cameras in use: the nonmydriatic camera (45°) and the mydriatic camera (50-60°). The nonmydriatic camera, easy to use and transport, is suitable for mobile screening whereas the use of the mydriatic camera is more challenging and demanding and suitable for hospital and stationary use. As the name nonmydriatic indicates, photography is possible without pharmacological mydriasis but the use of mydriatic drops is recommended as it improves the quality and assessability of the photographs (Pugh et al. 1993, Murgatroyd et al. 2004). Fundus cameras are also described by the angle of view. An angle of 30° creates a film image 2.5 times larger than life. This angle is used for the stereoscopic photography from seven partially overlapping fields (Gold Standard). An angle of 45° is the maximum angle, which can be used in the non-mydriatic camera without pharmacological pupil dilation. The magnification of 45° pictures is x 1.4. Photographic angles of 50° to 60° always require pharmacological mydriasis. These cameras provide larger photographic areas but proportionally less magnification. The use of the 45° camera, with or without mydriasis, has been an established photographic screening method for DRP (Kalm et al. 1989, Taylor et al. 1996)

**1.5.2 Photographic fields**

Most guidelines advocate the use of two 45° fields, a macula-centred and an optic disc-centred field (Table 5). The definitions of the macular field, however, have varied and a macular field centred between the fovea and the optic disc, centred on the fovea and centred temporal to the fovea has been used (Klein 1985b, Retinopathy Working Party 1991, Aldington et al. 1995). Furthermore stereopairs of the macular field can be used in order to detect DMO more accurately (Hansson-Lundblad et al. 1997).

One-field 45° photography has been widely used for screening purposes (Klein et al. 1985b, Buxton et al. 1991). In a British multicentre study only two of the twelve participating centres used more than one 45° field (Taylor et al. 1996).

Retinal photography using one 60° field has been described for detection of DRP (Falck et al. 1993). However, the use of 60° photography from one or two fields has not been a generally established approach for screening of DRP.

For detection of STDR, Moss and co-workers (1989) showed the sensitivity for detecting PDR from two 30° standard fields to be 74% and to rise to 90% from four 30° standard fields. Joannou et al. (1996) studied how DRP were diagnosed from one and two 45° fields in comparison with one 60° field. According to their results some 30% and 11%, respectively, of serious retinal pathology may be missed in comparison with the single 60° field.
2. AIMS OF THE STUDY

- **PAPER I**
  To study how DRP lesions were detected from 60° two-field red-free paper prints compared with corresponding colour transparencies. To create an ETDRS-based grading scale for 60° photography emphasizing those lesions known to predict a poor prognosis and to select 60° novel red-free Sps for individual lesions corresponding with ETDRS 30° Sps.

- **PAPER II**
  To study how DRP lesions were detected from one macula-centred 60° photographic field compared to two-field photography when an optic disc-centred field was used in addition. The photographic coverage of one- and two-field 60° photography was compared with that of standard 30° seven-field photography (Gold Standard).

- **PAPER III**
  To study how retinal new vessels could be detected from three varying recommended macular 45° fields compared to a 60° fovea-centred field. To compare how retinal new vessels could be detected from one and two 45° fields (fovea- and optic disc-centred) compared with corresponding one and two 60° fields.

- **PAPER IV**
  To evaluate how DRP lesions were detected from 60° two-field digital red-free images compared with corresponding colour transparencies.
3. PATIENTS AND METHODS

3.1 Patients and data collection

The patients for the first two studies (I-II) consisted of 93 persons with Type 1 and Type 2 DM arbitrarily chosen to be photographed using two differing photographic methods; colour transparencies and red-free paper prints. The patients were referred by GPs or diabetologists to the retinal photographic screening unit in Helsinki, Finland, in 1993-1997. Nineteen patients were excluded due to the unsatisfactory quality of photographs or field alignment or previous laser treatment. Thus, 74 patients were included in the study. When transparencies and black-and-white photographs of sufficient quality and with correct field alignment were available, the grading results for the right eye (n = 56) were used to represent the patient in the statistical analysis. If not, the results for the left eye (n = 18) were used.

The patients for the third study (III) were 72 consecutive persons with DM who, during a three-year period from 1997 to 1999 were referred from the retinal photographic screening unit in Helsinki, Finland for laser treatment due to PDR. Of them 58 patients with NVEs were accepted for the study. Fourteen patients were excluded due to: NVEs outside the two 60º standard photographic fields, NVDs only, unacceptable field alignment, inactive treated NVs, PRHs obscuring NVs and NVEs due to branch retinal vein occlusion. Results for the right eye (n = 43) were used when NVEs were present in the right eye or in both eyes. Otherwise data for the left eye were used (n = 15).

The patient material for the fourth study (IV) comprised 110 persons with mainly Type 2 DM who were referred to the Department of Vitreoretinal Diseases at St Eriks Eye Hospital in Stockholm, Sweden for retinal screening photography. The patients were consecutively photographed using two differing methods colour transparencies and red-free digital images. Three patients were excluded; two due to inadequate quality of photographs and one patient who refused to participate. The results for the right eye (n =105) were used for the analysis when good-quality pictures with acceptable field projections were available, otherwise the results for the left eye (n = 2) were used.

3.2 Photographic methods

3.2.1 Mydriasis
The pupils were dilated for photography in all studies (I-IV) with 0.5% tropicamide and, in addition, with 2.5-10% neosynephrine and 1% cyclopentolate, if needed in studies I-III.

3.2.2 Cameras and filter
In all studies (I-IV) a wide-angle 60º fundus camera was used: in studies I-II either a Canon CF-60 U or a Canon CF-60 UV camera and in studies III-IV a Canon CF-60 UV camera. The red-free photographs in studies I-III were captures using a green absorption filter (Kodak Wratten 58). A Kodak Megaplus 1.4i digital camera giving a resolution of 1320 x 1032 pixels was used for the digital images.

3.2.3 Photographic fields, required quality of photographs and digital images
Two 60º photographic fields, one centred on the macula and one on the optic disc of each eye with each method were taken in all studies (I-IV). The field alignment was considered to be correct if the fovea (1 DD in diameter) and optic disc, respectively, were within the central third of the photographic field. Colour transparencies, red-free black-and-white prints and digital images were...
of adequate quality if they were of at least fair quality according to both graders; retinal vessels sharply defined or slightly fuzzy, but small lesions such as Mas and drusen clearly visible.

3.2.4 Film and its development
Kodachrome 64 (Kodak) film was used in studies I-III and Ectachrome 64 professional (Kodak) film in the fourth study (IV) for colour transparencies. For red-free prints T-Max 100 (Kodak) black-and-white film was used in studies I-III. All the films were commercially developed. The red-free black-and-white enlargements were done on Agfa Multicontrast Premium measuring 13 x 18 cm.

3.3 Grading of diabetic lesions

3.2.1 Grading scale for DRP
When using wide-field (45°- 60°) retinal photography, assessment of individual lesions from four retinal quadrants is more convenient. Likewise, wide-field standard photographs are more convenient than 30° photographs when grading individual lesions per retinal quadrants. A detailed 14-level grading scale was designed for 60° fundus photography based on the ETDRS classification. This grading scale emphasized DRP lesions known to predict a poor prognosis, e.g. Ma/H, IRMA and VB, according to the ETDRS (1991b,c). Furthermore, a simplified pooled five level grading scale was used. The severity level of each DRP abnormality was assessed per retinal quadrants. This grading scale designed in 1994 was used in studies I, II and IV.

3.2.2 Grading scale for diabetic maculopathy
In the first study (I) maculopathy was graded according to a detailed nine level and a pooled six level grading scale.

3.2.3 Standard photographs
Seven, 60° red-free black-and-white Sps were chosen in order to assess the severity level of individual DRP lesions in each retinal quadrant; three (Sp 1, 2A, 2B) to estimate the density of Mas/Hs, two to assess the severity level of IRMA (Sp 3, 4), the severity level of VB (Sp 5, 6) and in addition the severity level of Hes for the grading of maculopathy (Sp 7, 8) (I, II, IV). These standard photographs applicable to wide-field photography were chosen to correspond to the 30° Sps which were used in the ETDRS (1991b).

3.4 Equipment for assessment of colour transparencies and digital images
Grading from red-free black-and-white prints did not require any special viewing equipment. Colour transparencies were assessed in studies I-II at 7.5x magnification (Agfa Reflecta Diamator AFM) using a halogen light bulb (Osram Xenophot HLX 64640) and in the fourth study (IV) at 4x magnification using an anastigmatic loupe (Peak) on a lightboard providing light temperature around 5,000° Kelvin. The digital images in study IV were assessed using an Ophthalmic Imaging System (OIS) software version 4.0 and a Sony Trinitron Multiscan 20se 19-in. screen with a resolution of 1280 x 1024 pixels.

3.5 Grading procedure
In studies I, II, IV the photographic material/digital images were graded in a masked fashion by two independent retinal specialists. Colour transparencies and red-free black-and-white prints or digital images were graded on separate occasions.
In the second study (II) the DRP severity level was first assessed with each photographic method from the 60º macula-centred field alone and then from the two over-lapping 60º fields together. In the fourth study (IV) the two graders also scored together all colour transparencies and the digital images of patients with a discordant grading in order to get a consensus grading for each method. Finally, the two graders graded together both the colour transparencies and the digital images of each patient with a discordant consensus DRP level to achieve a final consensus of DRP using all available information.

In the third study (III) the two graders analysed the photographic material together, counted the NVEs in the fields which were studied and noted the location and size of the NVEs. First the NVEs detected in the standard 60º macula-centred field were counted. In order to assess the total NVEs that could be detected by two-field 60º photography, NVEs detected only by the 60º optic disc-centred field were counted. In order to study how the NVEs were detected from three varying 45º macular fields a transparent grid indicating 45º field borders was placed over the 60º fovea-centred macular field in three positions: centred on the fovea (Field A) (Retinopathy Working Party, 1991), centred between the disc and the fovea (Field B) (Klein et al.1985b) and with nasal field border along the disc margin (Field C) (Aldington et al. 1995) and the NVEs were counted.

3.6 Evaluation of the site of NVEs

A transparent grid designed for the third study (III) for 45º and 60º fields was used in order to define the fields and mark the location of NVEs in all the fields studied.

3.7 Planimetry

The photographic areal coverage of one- and two-field 60º photography compared with 30º seven-field photography in the second study (II) was calculated approximately from a schematic drawing using an Ushikata x-plan360i planimeter.

3.8 Statistical methods

The collected data were entered into a computer programme (Statistica®, Statsoft). Quantitative patient characteristics (e.g. age, age at the time of diagnosis of DM, duration of DM) were described by median and range, given by StatXact and SPSS software. Wilcoxon-Mann-Whitney and $\chi^2$ tests were used to determine whether patients included in the study differed from the patients excluded. All tests were two-sided and their results were regarded as statistically significant when $p<0.05$. In the first two studies (I-II) two-way and multiway frequency tables were used (BMDP, 4F) to find out which of the photographic methods was the most sensitive one to disclose DRP abnormalities. Inter-method and inter-observer variations were presented using kappa and weighted kappa statistics including 95% CIs using StatXact software. The applied weights were calculated as suggested by Liebetrau (1983) and Agresti (1990). Inter-method and inter-observer variations were also presented as percentages, including 95% CI, and using scatter diagrams using the STATA software programme. To test the error tendencies for under- and over-diagnosis in the fourth study (IV) a two-sided Mc Nemar’s test was applied using StatXact software.

Kappa statistics corrects for agreement by chance. Interpretation of kappa values according to Landis and Koch (1977) is presented in Table 3.
Table 3.

<table>
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4. SUMMARY OF RESULTS

PAPER I

Grading for DRP from red-free black-and-white photographs versus colour transparencies

Interrater agreement for DRP

Interrater agreement applying the unpooled grading scale for DRP using 60° red-free black-and-white prints was 70% (95% CI 59-80%). The corresponding figure for 60° colour transparencies was 68% (95% CI 56-78%).

When using the five pooled main levels, the two graders agreed in 88% (95% CI 78-94%) and 81% (95% CI 70-89%) for red-free black-and-white prints and colour transparencies, respectively. Kappa and weighted kappa values for agreement was 0.83 (95% CI 0.72-0.93) and 0.96 (95% CI 0.93-0.99) for red-free black-and-white prints. Corresponding values for colour transparencies were 0.73 (95% CI 0.60-0.85) and 0.89 (95% CI 0.81-0.98).

The extent of disagreement, as presented using scatter diagrams, between the observers in terms of the unpooled retinopathy levels was greater when using colour transparencies compared with red-free, black-and-white prints.

Interrater agreement for maculopathy

Interrater agreement applying the unpooled grading scale for maculopathy using red-free black-and-white prints was 88% (95% CI 78-94%). The corresponding figure for colour transparencies was 82% (95% CI 72-90%).

When using the six pooled main levels the interrater agreement was 96% (95% CI 87-99%) and 89% (95% CI 80-95%) for red-free black-and-white prints and colour transparencies, respectively. Kappa and weighted kappa values for agreement was 0.84 (95% CI 0.66-1.0) and 0.97 (95% CI 0.93-1.0) for red-free black-and-white prints. Corresponding values for colour transparencies were 0.67(95% CI 0.47-0.86) and 0.86 (95% CI 0.71-1.0).

Inter-method agreement for DRP

When comparing the unpooled retinopathy level Grader 1 agreed when grading 45/74 (61%; 95% CI 49-70%) of the eyes. For Grader 2 the corresponding figures were 49/74 (66%; 95% CI 54-77%). When pooling retinopathy in the five main levels Grader 1 agreed in 53/74 (72%; 95% CI 60-82) of the eyes. Corresponding figures for Grader 2 were 56/74 (76%; 95% CI 64-85%). The results revealed that in eyes with discordant grading results, the severity level of DRP in general was higher for both graders when the grading was performed using red-free black-and-white prints.
compared with colour transparencies. Thus, 24/29 (83%; 95% CI 64-94) of these discordantly judged eyes represented a higher severity level for Grader 1. For Grader 2 the corresponding figures were 19/25 (76%; 95% CI 55-91%). The most obvious difference was the detection of early DRP (eg. Mas), which was easier from the red-free black-and-white prints compared with the colour transparencies as presented in the scatter diagrams (I). Inter-method agreement in the assessment of five pooled DRP levels yielded kappa and weighted kappa values of 0.61 (95% CI 0.47-0.75) and 0.91 (95% CI 0.86-0.95) for Grader 1. Corresponding figures for Grader 2 were
0.65 (95% CI 0.52-0.79) and 0.88 (95% CI 0.82-0.95), respectively.

**Inter-method agreement for maculopathy**

When comparing the unpooled maculopathy level Grader 1 agreed when grading 65/74 (88%; 95% CI 78-94%) of the eyes. For Grader 2 corresponding figures were 54/74 (73%; 95% CI 61-83%). For the pooled six maculopathy levels corresponding figures for both graders were 66/74 (89%; 95% CI 80-95%). Inter-method agreement in the assessment of six pooled maculopathy levels yielded kappa and weighted kappa values of 0.62 (95% CI 0.43-0.80) and 0.84 (95% CI 0.61-1.0) for Grader 1. Corresponding values for Grader 2 were 0.59 (95% CI 0.38-0.80) and 0.81 (95% CI 0.64-0.98), respectively.

**PAPER II**

**Grading for DRP from one and two 60º fields**

When the assessment of retinopathy severity level, using the unpooled grading scale, was based on two-field 60º colour transparencies, it was more severe in 10/74 (13.5%; 95% CI 6.7-23.5%) of the eyes for Grader 1 and in 12/74 (16.2%; 95% CI 8.8-26.6%) of the eyes for Grader 2 as compared to assessment from only one macula-centred 60º field. When applying the pooled five-level grading scale the corresponding figures were 7/74 (9.5%; 95% CI 3.9-18.5%) and 8/74 (10.8%; 95% CI 4.8-20.2%). Kappa and weighted kappa values for intermethod agreement between one- and two-field photography, applying the unpooled DRP severity scale, for the two graders were 0.79 and 0.82 and 0.96 and 0.98, respectively, indicating substantial to almost perfect agreement (Landis and Koch 1977). When analysing divergences in the grading results of the two graders one (1/74) and four (4/74) eyes, respectively, requiring clinical assessment or treatment (Levels 47-71) were “underdiagnosed” when using data from only one macula-centred field.

When the assessment of retinopathy severity level, using the unpooled grading scale, was based on two-field 60º red-free black-and-white prints, it was more severe in 10/74 (13.5%; 95% CI 6.7-23.5%) of the eyes for Grader 1 and in 11/74 (14.9%; 95% CI 7.7-25.0%) of the eyes for Grader 2 as compared to assessment from only one macula-centred 60º field. When applying the pooled five-level grading scale the corresponding figures were 8/74 (10.8%; 95% CI 4.8-20.2%) and 7/74 (9.5%; 95% CI 3.9-18.5%). Kappa and weighted kappa values for intermethod agreement between one- and two-field photography, applying the unpooled DRP severity scale, for the two graders were 0.81 and 0.84 and 0.97 and 0.98, respectively, revealing almost perfect agreement (Landis and Koch 1977). Two eyes (2/74) with a retinopathy level requiring clinical assessment or treatment were “underdiagnosed” by Grader 1 and one eye (1/74) by Grader 2, when using data from only one macula-centred field.

**Field coverage**

The planimetric calculations revealed that one 60º macula-centred field covers 60% and two 60º fields, macula-and optic disc-centred field, 80% of the area covered by seven-field 30º photography (Gold Standard). However, there are areas around the posterior pole which are
covered by the two 60º fields but are left outside the area covered by standard seven-field 30º photography.

PAPER III

Detection of NVEs from varying photographic fields

In all 111 NVEs were detected by two-field 60º photography in one eye of 58 patients. Of these 81 (73%; 95% CI 64-81%) were detected in the 60º macula-centred photographic field alone. In 11/58 (19%; 95% CI 10-31 %) eyes in all 30 NVEs were detected in the 60º optic disc-centred field only. Of these 27 (90%; 95% CI 73-98%) were also detected in the corresponding 45º field. Two-field 45º photography allowed for detection of 86/111 (77%; 95% CI 69-85%) NVEs.

When comparing how NVEs were detected from the three 45º macular fields A, B and C which were evaluated, compared with the 60º fovea-centred field 59/81 (73%; 95% CI 62-82%), 50/81 (62%; 95% CI 50-72%) and 64/81 (79%; 95% CI 69-87%), respectively, of the NVEs were detected. Kappa coefficients for agreement, when the data were collapsed into two groups based on whether or not at least one NVE was visible, were 0.62 (95% CI 0.40-0.83), 0.38 (95% CI 0.19-0.57) and 0.80 (95% CI 0.62-0.99), respectively. When the 60º fovea-centred field revealed only one NVE (n=30), it could not be detected in 9/30 (30%; 95% CI 15-49%), 16/30 (53%; 95% CI 34-72%) and 4/30 (13%; 95% CI 4-31%) in fields A, B and C, respectively. Fields A, B and C disclosed 59/111 (53%; 95% CI 43-63%), 50/111 (45%; 95% CI 36-55%) and 64/111(58%; 95% CI 48-67%), respectively, of the NVEs which were detected from two-field 60º photography.

The location and size of NVEs were presented in schematic drawings (III).

PAPER IV

Detection of DRP from digital red-free images versus colour transparencies

Interrater agreement, when using colour transparencies applying the unpooled DRP severity scale was obtained in 81/107 eyes (76%; 95 % CI 66-83 %). Kappa- and weighted kappa values were 0.69 (95% CI 0.59-0.80) and 0.85 (95% CI 0.77-0.93), respectively. For four pooled main levels kappa and weighted kappa values were 0.85 (95% CI 0.75-0.95) and 0.88 (95% CI 0.79-0.97). The grading result was discordant in 26/107 (24 %; 95% CI 17-34 %) eyes.

Interrater agreement when using red-free digital images applying the unpooled DRP level was obtained in 91/107 eyes (85%; 95% CI 77-91 %). Kappa- and weighted kappa values were 0.65 (95% CI 0.54-0.76) and 0.88 (95% CI 0.82-0.95), respectively. For four pooled main levels kappa and weighted kappa values were 0.81 (95% CI 0.70-0.91) and 0.86 (95% CI 0.78-0.95). The grading result was discordant in 16/107 (15%; 95% CI 9-23 %) (unpublished data).

Agreement between the individual grading results of the two graders and the consenus results for the two methods is presented in Table 4.
Table 4.

Agreement between the individual grading results of the two graders and the consensus results for the two methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Comparison of grading result</th>
<th>Number of eyes</th>
<th>Percent (95% CI)</th>
<th>Kappa (95% CI)</th>
<th>Weighted kappa (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour transparencies</td>
<td>Grader 1 versus consensus result</td>
<td>100/107</td>
<td>93 (87-97)</td>
<td>0.90 (0.83-0.97)</td>
<td>0.97 (0.95-1.0)</td>
</tr>
<tr>
<td></td>
<td>Grader 2 versus consensus result</td>
<td>92/107</td>
<td>86 (78-92)</td>
<td>0.79 (0.69-0.88)</td>
<td>0.88 (0.80-0.95)</td>
</tr>
<tr>
<td>Red-free digital</td>
<td>Grader 1 versus consensus result</td>
<td>94/107</td>
<td>88 (80-93)</td>
<td>0.83 (0.74-0.91)</td>
<td>0.96 (0.92-1.0)</td>
</tr>
<tr>
<td></td>
<td>Grader 2 versus consensus result</td>
<td>90/107</td>
<td>84 (76-90)</td>
<td>0.78 (0.68-0.87)</td>
<td>0.91 (0.85-0.97)</td>
</tr>
</tbody>
</table>

Inter-method agreement was obtained in 76/107 eyes (71%; 95% CI 61-79%), when comparing the consensus level of DRP obtained from colour transparencies and red-free images digital images. Kappa and weighted kappa for agreement were 0.58 (95% CI 0.47-0.70) and 0.79 (95% CI 0.68-0.89), respectively. When the levels of DRP were pooled into four main categories corresponding kappa and weighted kappa values were 0.68 (95% CI 0.56-0.81) and 0.74 (95% CI 0.63-0.86), respectively.

A total of 62/107 eyes (58%) showed some degree of DRP with either or both methods. Of the eyes featuring DRP 31/62 (50%) were graded concordantly with both methods. Of the eyes with discordant gradings, the level of DRP obtained from red-free digital images was higher in 24 (77%) and lower in 7 (23%) than from colour slides. Discordant gradings occurred mainly in eyes with early DRP. For the total of 107 eyes, false “over-diagnosis” occurred in 3/107 (3%) and 1/107 (1%) and false “under-diagnosis” in 7/107 (7%) and 20/107 (19%) eyes from red-free digital images and colour slides, respectively. False “over-diagnosis”, depending on false interpretation of pigmentary lesions as Hs or Mas, occurred more often when the grading was done from red-free digital images.

The principal DRP lesions, which led to “under-diagnosis” from colour transparencies were haemoglobin-containing lesions whereas “white lesions” were the most frequent lesions “under-diagnosed” from red-free digital images. The grading error tendencies were significantly lower from the red-free digital images than from the colour transparencies (p< 0.008) (3x3 table, two-sided McNemars test).
5. DISCUSSION

Classification of DRP

When using wide-field (45º- 60º) photography, assessment of individual lesions from four retinal quadrants is convenient. In studies I, II and IV an ETDRS-based grading scale adapted for 60º photography was used. The grading scale was based on the findings of the ETDRS nr 12 (1991c) where the prognostic value of individual DRP abnormalities for further progression to PDR were evaluated. The grading scale emphasized red haemoglobin-containing lesions, IRMAs, Hs/Mas and VB. All these abnormalities are well detected from red-free photographs or digital images. Furthermore, a set of red-free black-and-white 60º reference photographs suitable for assessing DRP per retinal quadrants from wide-field photographs, was used. In line with early ETDRSs not only the number but also the size of Hs was taken in account.

Classification by four retinal quadrants, adopting the knowledge concerning the prognostic value of individual DRP abnormalities from earlier ETDRSs, was adopted also by the ETDRS group (Table 5)(Davis et al. 1998). The predictive value of the combination of individual DRP abnormalities was again estimated on the basis of the development of high-risk PDR during follow-up. Findings per one 30º field (3-7) were converted to findings per one quadrant.

In 2002 a workshop was held in the USA with invited international experts, diabetologists and ophthalmologists with a special interest in DRP, in order to create a user-friendly consensus grading-scale for DRP and DMO, suitable for every-day screening use worldwide. The proposed five-level grading-scale for DRP (Wilkinson et al. 2003) (Table 6) was based on all the knowledge obtained from earlier ETDRS and WESDR results concerning the natural course of DRP emphasizing those individual DRP lesions known to predict a poor prognosis. Of special interest in this grading scale is the modification of the concept of severe NPDR based on the 4-2-1 rule, namely instead of moderate H/Ma in four quadrants a least 20 intraretinal Hs in four quadrants irrespective of their size, is required for the “4”. The rest of the 4-2-1 rule is unchanged, VB in at least two quadrants and definite IRMA in at least one quadrant. In this classification the level of moderate DRP is wide and might include eyes with preproliferative NPDR as defined earlier (Table 7, Davis et al. 1998). Thus, in treatment-decision the use of the grading scale proposed by Wilkinson et al. (2003) may be too simplified. It also became evident that the ETDRS DRP severity scale (Table 5) (Davis et al 1998) has two important shortcomings as a predictor of the development of high-risk PDR; eyes with very severe NPDR (DRP level 53E) were at greater risk than those with mild PDR (DRP level 61) which was higher up on the scale; and the intraretinal characteristics (H/Ma, VB, IRMA), predictive of progression from NPDR to PDR were ignored in eyes with PDR, even though these characteristics were clearly related to the risk of progression from non-high-risk to high-risk PDR (ETDRS 1991a,c, Davis et al.1998).
Table 5.

Early Treatment Diabetic Retinopathy Study Scale of diabetic retinopathy
(Davis et al. 1998, reprinted with the permission of ARVO)

<table>
<thead>
<tr>
<th>Level</th>
<th>Severity</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>No retinopathy</td>
<td>Diabetic retinopathy absent</td>
</tr>
<tr>
<td>20</td>
<td>Very mild NPDR</td>
<td>Mas only</td>
</tr>
<tr>
<td>35*</td>
<td>Mild NPDR</td>
<td>Hes, CWS, and/or mild retinal Hs</td>
</tr>
</tbody>
</table>
| 43    | Moderate NPDR  | 43A Retinal Hs moderate (> Sp 1) in four quadrants or severe (≥ Sp 2A) in one quadrant  
|       |                | 43B Mild IRMA (< Sp 8A) in one to three quadrants                           |
| 47    | Moderate NPDR  | 47A Both level 43 characteristics                                           |
|       |                | 47B Mild IRMA in four quadrants                                             |
|       |                | 47C Severe retinal Hs in two to three quadrants                             |
|       |                | 47D VB in one quadrant                                                      |
| 53 A-D| Severe NPDR    | 53A ≥ 2 level 47 characteristics                                           |
|       |                | 53B Severe retinal Hs in four quadrants                                     |
|       |                | 53C Moderate to severe IRMA (≥ Sp 8A) in at least one quadrant              |
|       |                | 53D Venous beading in at least two quadrants                                |
| 53E   | Very severe NPDR| ≥ 2 level 53A-D characteristics                                             |
| 61    | Mild PDR       | NVE <0.5 disc area in one or more quadrants                                |
| 65    | Moderate PDR   | 65A NVE ≥ 0.5 disc area in one or more quadrants                            |
|       |                | 65B NVD < Sp 10A (< 0.25-0.33 disc area)                                   |
| 71,75 | High-risk PDR  | NVD ≥ Sp 10A, or NVD < Sp 10A or NVE ≥ 0.5 disc area plus VH or PRH, or VH or PRH obscuring ≥ 1 disc area |
| 81, 85| Advanced PDR   | Fundus partially obscured by VH and either new vessels ungradable or retina detached at the centre of macula |

For standard photographs (Sps) see ETDRS report nr 10, 1991
*) NPDR levels 35 and above, all require presence of Mas
<table>
<thead>
<tr>
<th>Proposed disease severity level</th>
<th>Findings observable upon dilated ophthalmoscopy</th>
<th>Derivation from ETDRS levels</th>
<th>Risk assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>No apparent retinopathy</td>
<td>No abnormalities</td>
<td>Level 10: DRP absent</td>
<td></td>
</tr>
<tr>
<td>Mild non-proliferative diabetic retinopathy</td>
<td>Microaneurysms only</td>
<td>Level 20: Very mild NPDR</td>
<td></td>
</tr>
<tr>
<td>Moderate non-proliferative diabetic retinopathy</td>
<td>More than just microaneurysms but less than severe NPDR</td>
<td>Levels 35, 43: Moderate NPDR Less than 4:2:1 Level 47: Moderate NPDR. Less than 4:2:1</td>
<td>One year early PDR: 5.4-11.9%. One year high risk PDR 1.2-3.6% One year early PDR: 26.3 %. One year high risk PDR 1.2-8.1%</td>
</tr>
</tbody>
</table>
| Severe non-proliferative diabetic retinopathy | Any of the following:  
  - More than 20 intraretinal haemorrhages in each of 4 quadrants  
  - Definite venous beading in 2+ quadrants  
  - Prominent IRMA in 1+ quadrant  
  And no signs of proliferative retinopathy | Level 53A-E: Severe to very severe NPDR, 4:2:1 rule | One year risk for early PDR: 50.2 % (severe NPDR) One year high risk PDR 14.6 % (severe NPDR) – 45.0 % (very severe NPDR |
| Proliferative diabetic retinopathy | One or more of the following:  
  - Neovascularisation  
  - Vitreous/preretinal haemorrhage | Levels 61,65,71,75,81,85: PDR, high-risk PDR, Very severe or advanced PDR |                |
Table 7.

Grading of diabetic retinopathy. Three important levels of NPDR.
(Davis MD, XXIXth International Congress of Ophthalmology, Sydney 2002, reprinted with the permission of M. Davis)

<table>
<thead>
<tr>
<th>Severity of NPDR</th>
<th>One-year risk of high-risk PDR</th>
<th>Number of quadrants of the fundus involved with specified abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Moderately severe</td>
<td>938</td>
<td>8.6</td>
</tr>
<tr>
<td>Severe</td>
<td>500</td>
<td>14.6</td>
</tr>
<tr>
<td>Very severe</td>
<td>92</td>
<td>45.0</td>
</tr>
</tbody>
</table>

* ≥ Standard photo 2A (ETDRS)
# < Standard photo 8A (ETDRS)
** ≥ Standard photo 8A (ETDRS)
† The 2-1-4 rule
‡ The 4-2-1-rule

When two or more of moderately severe NPDR criteria are present, NPDR is severe.

Detection of DRP from 60° red-free black-and-white photographs or digital images compared with 60° colour transparencies

60° Fundus photography has not been an established screening method for DRP although some reports using this screening approach have been published (Falck et al. 1993, Joannou et al. 1996). However, when photographic screening is organized on a stationary hospital basis most ophthalmic clinics are equipped with this type of camera, the use of which might offer advantages in terms of a wide field coverage. Likewise, the use of red-free technique has not earlier been an established screening approach although it has been described (Falck et al. 1993, Hirvelä and Laatikainen 1997).

Performance of varying screening methods can be measured in terms of reliability (test-retest reliability and inter-observer agreement) and validity (sensitivity, specificity, positive and negative predictive value) (Bachmann et al.1997). In our study we did not validate the 60° red-free photographic or digital imaging method calculating sensitivity, specificity, positive and negative predictive value, as we did not have the opportunity to get 30° stereoscopic seven-field photographs as a reference standard. Neither were the patients for the studies (I, II, IV) representative of the population to be screened. Therefore, the investigated methods were compared with the photographic screening method which at the point of time of the study was the predominant method, eg. 33 mm colour transparencies from, however, two 60° fields. In studies I, II and IV inter-method and inter-observer agreement for the detection of DRP for two methods and the same two retinal experts (GvW, PS) were calculated using percentages and kappa and weighted
kappa values with 95% CIs. Furthermore, the eyes with discrepant gradings for the two methods investigated were analysed in detail (I, II, IV).

Interrater agreement in the first study (I) applying the unpooled grading scale for DRP was 70% and 68% from 60° red-free black-and-white prints and colour transparencies, respectively. For five pooled main DRP levels the corresponding figures were 88% and 81%. Kappa and weighted kappa values for agreement were 0.83 and 0.96 for red-free black-and-white prints. Corresponding values for colour transparencies were 0.73 and 0.89. These figures are comparable with those presented in the fourth study (IV) when corresponding kappa and weighted kappa values for inter-observer agreement for five pooled main levels were 0.81 and 0.86 for red-free digital images and 0.85 and 0.88 for colour transparencies. All figures indicate substantial to almost perfect interrater agreement and good reproducibility of the grading method. They are also comparable with the results by Aldington et al (1995) presenting kappa values for inter-observer agreement for DRP in the EURODIAB study. Their kappa value of 0.85 for three DRP levels is congruent with those for five DRP levels by us.

Henricsson et al. (2000) reported exact inter-method agreement for eight DRP levels in 82% (wk 0.88) between DRP gradings from 50° colour slides and colour digital images. The agreement improved to 85% (wk 0.86) when additional information from digital red-free images captured with a green filter in the fundus camera was used. The photographic fields used were the same with both methods. The weighted kappa values indicated almost perfect agreement despite a relatively low image resolution (640 x 480 pixels). The authors also reported that significantly more lesions were found from red-free images but the discrepancies were not analysed in detail. Other prior results comparing DRP grades from colour transparencies and digital images have presented excellent agreement as well (George et al.1998).

Recently Hansen et al. (2004a) presented results from a Danish study in which five-field high-resolution 45° colour digital images were compared with 30° seven-field standard photography for screening of DRP. Their kappa value, 0.76, indicated substantial inter-method agreement for four severity levels of DRP. The majority of disagreements between the two methods occurred in eyes with minimal or mild DRP. The authors reported difficulties in distinguishing Hs, Mas and IRMAs from the digital images. This may be due to the fact that the images were assessed in colour.

Our studies showed that early DRP (eg. Mas) was easier to detect from red-free photographs/digital images as compared to colour transparencies. This is of importance as the number of “red-dots” has a predictive value for the progression of DRP, as well as for subsequent DMO and thus also have an influence on screening intervals (Klein et al. 1989b, Kohner 1999, UKPDS 2001a). Likewise, other haemoglobin containing lesions as Hs, IRMAs and VBs, important features of severe DRP are readily detected from red-free photographs/digital images. However the results in the fourth study (IV) also showed that some “dots” interpreted as Mas from red-free black-and white photographs or digital images, were only pigmentary lesions. “White lesions”, especially small CWS, where the only abnormalities that could be detected more easily from colour images. The grading error tendencies, when statistically tested in the fourth study (IV), were significantly lower from red-free digital images than from colour transparencies.

In the first study (I) the level of maculopathy was assessed as well. However, the detection of maculopathy with non-stereo fundus photographs, in particular DMO without hard exudates, is virtually impossible (Kalm et al.1989). Such problems exist in all photographic screening methods when a stereo pair of photographs of the macular field is not included. Visual acuity measurements may help in the interpretation of maculopathy (The Royal College of Ophthalmologists 1997). Our
study did not reveal any significant differences in the gradings for DMO between the two photographic methods. Inter-observer assessment of six pooled maculopathy levels yielded kappa and weighted kappa values of substantial to almost perfect agreement.

Photographic fields

In the second study (II) we found that the assigned severity level of DRP from two 60° fields for the two graders was more severe in 13% and 15% using red-free black-and-white photographs. Corresponding figures for colour transparencies were 14% and 16%. The inter-method agreement between one- and two-field photography, using kappa and weighted kappa coefficients, revealed almost perfect agreement for both graders and for both photographic methods (Landis and Koch 1977). No STDR were detected using two 60° fields as long as no DRP abnormalities could be detected in the 60° macular field. However, when any DRP was detected in the 60° macular field it appeared that the second optic disc-centred field could give valuable additional information showing even STDR undetected from the macular field alone.

Controversial opinions concerning one-field retinal photography for the screening of DRP have been published. Murgatroyd et al. (2004) compared digital images from one and three 45° fields using pupil dilation. The three-field photography, according to their results, did not increase sensitivity or specificity in detecting DRP. Likewise, the authors of a review by the Health Technology Assessment Programme concerning the value of digital imaging in DRP, suggested the use of single-field imaging (Sharp et al. 2003). Williams et al. (2004) recently evaluated a single-field retinal photography screening approach according to the strength of evidence in a systematic review comprising literature for the years 1968 to 2001. The authors concluded that single-field photography is not a substitute for a comprehensive ophthalmic examination but there is evidence that it can serve as a screening tool for DRP to identify patients with referral-needing DRP.

When it comes to the detection of STDR, the results of the third study (III) revealed that one and two-field 45° photography allowed for the detection of respectively 53% and 77% of the NVEs detected in two 60° fields. One 60° fovea-centred field alone disclosed 73% of the NVEs detected in two-field 60° photography. In 19% of the eyes NVEs were detected in the 60° optic disc-centred field only. Of these NVEs, 90% were also detected in the corresponding 45° field.

When comparing how NVEs were detected from the three 45° macular fields which were evaluated, (e.g. centred on the fovea (Field A), centred between the disc and the fovea (Field B) and with nasal field border along the disc margin (Field C)) compared with the 60° fovea-centred field, 73%, 62% and 79%, respectively of the NVEs were detected. Kappa coefficients for agreement, when the data were collapsed into two groups based on whether or not at least one NVE was visible, were 0.62 (95% CI 0.40-0.83), 0.38 (95% CI 0.19-0.57) and 0.80 (95% CI 0.62-0.99), respectively. When the 60° fovea-centred field revealed only one NVE, it could not be detected in 30%, 53% and 13% in fields A, B and C, respectively. Of the macular fields investigated, the field recommended by the EURODIAB (Field C) disclosed NVEs, when only one present, significantly better than that used by Klein et al (1985b).

For detection of STDR, Moss and co-workers (1989) showed that the sensitivity for detecting PDR from two 30° standard fields was 74% rising to 90% from four 30° standard fields. Joannou et al. (1996) studied how DRP were diagnosed from one and two 45° fields in comparison with one 60° field. According to their results some 30 and 11%, respectively, of serious retinal pathology may be missed in comparison with the 60° field. In a Danish study 11% of the NVEs which were
detected from standard 30° seven-field photographs were not detected from one 60° macular field (Møller et al. 2002). The authors concluded, that one-field 60° photography is insufficient as a screening procedure in patients with moderate/severe NPDR. This is in line with our results. Notably, digital images from complementary photographic fields are easily captured when needed. According to our experience, peripheral NVEs may occur when only minimal or mild DRP is detected from the 45° fovea-centred field, especially in patients with a long duration of Type I DM (Fig. 2).

**Fig. 2.** Red-free, 60° fundus photograph of a patient with DRP. A prominent NVE lesion is visible at 3 o’clock (arrow). Only mild DRP lesions are visible within 45° field borders (inner ring).

The planimetric calculations in the second study (II) revealed that one 60° macula-centred field covered 60% and two 60° fields, macula-and optic disc-centred field, 80% of the area covered by seven-field 30° photography (Gold Standard). However, there are areas around the posterior pole which are covered by the two 60° fields but are left outside the area covered by standard seven-field 30° photography. To our knowledge such comparisons have not been performed earlier.

**6. CONCLUDING REMARKS**

It is of importance to detect early, as well as referral-needng DRP, as accurately as possible. This is crucial with the increasing prevalence of DM. Furthermore, the too early referral of patients will overload ophthalmology departments. It is therefore crucial, to develop reliable and valid screening
methods and to recognize risk factors and the natural course of DRP in varying categories of DM patients in order to find eyes at risk early enough and to optimise screening intervals. The automatic detection of DRP lesions may in future be a time-saving method to detect referable DRP. Encouraging preliminary study results have been reported (Hansen et al. 2004b). Furthermore, the success rate of retinal photography in older ages should be evaluated to find out when a clinical examination is a more efficient screening method. This is crucial also from the viewpoint of cost-effectiveness. Hansen et al. (2004a) reported unfavourable outcomes of nonmydriatic photography in people aged ≥ 70 years with ungradable images in 50% of the cases. The results by Hirvelä and Laatikainen (1997) with 24% ungradable 45º monochrome black-and-white photographs taken with pupil dilation in people aged 70 years or older were more optimistic, but did not comply with the recommendations of the British Diabetes Association.

Despite guidelines recommending regular dilated eye examinations, poor compliance with these guidelines have been reported (Mukamel et al. 1999). Only 16% of the patients with DM in Upstate New York received annual screening in two consecutive years. Furthermore, Kraft et al. (1997) reported that only 52% of physicians performed in-office ophthalmoscopy, 90% without pupil dilation. Patient factors also explain why guidelines are not being followed. In the WESDR over 30% of patients who did not have regular eye examinations reported not having been told they needed one. Up to 30% of the patients told they were too busy and over 70% reported not having any examination because they had no problems with their eyes (Moss et al. 1995). Therefore, it is of importance to educate people with DM about the asymptomatic character of DRP, even the STDR, and the benefits of regular dilated eye examinations.
7. ACKNOWLEDGEMENTS

The first three (I-III) of the studies included in this thesis were performed at the Diabetic Retinopathy Photographic Screening Unit, Helsinki City Health Department and the Helsinki University Central Hospital, Finland. The fourth (IV) study was performed at St Eriks Eye Hospital and Institution for Neurosciences, Karolinska Institutet, Stockholm, Sweden.

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Last but not least, I feel sad that my beloved parents, who have passed away years ago, could not be present today. Although not physically present, you certainly both are in my mind.

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