

EXTENDED REPORT

Digital analysis of choroidal neovascularisation in consecutive fluorescein angiograms for use in longitudinal clinical trials

C Bellmann, D W Miller, K Mehlretter, F Schütt, J Jorzik, K Unnebrink, F G Holz

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See end of article for authors' affiliations

Correspondence to: Frank G Holz, MD, Department of Ophthalmology, University of Heidelberg, INF 400, 69120 Heidelberg, Germany; frank_holz@med.uni-heidelberg.de

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Background/aims: To document the natural history and to assess the efficacy of interventional therapies in neovascular age related macular degeneration (AMD), an accurate and reproducible method is required for analysis of consecutive fluorescence angiograms. The development and evaluation of an image analysis software for this purpose is described here. It allows for the quantitative analysis of changes in CNV and/or leakage area over time.

Methods: In digitised angiograms, a mouse driven arrow was used to delineate the CNV border. The ratio of the CNV area to the square of the distance between two vessels was automatically calculated by pixel count to compensate for variation in image sizes at different examination times. These results were directly transferred and stored in a database. To assess reproducibility, CNV areas in 20 patients with occult and 20 patients with classic CNV were determined independently by two readers.

Results: There was only marginal variability between observers with this method: the mean deviation was 0.01 pixels for classic CNV (95% CI –0.17 to +0.15, SD 0.35) and 0.55 pixels for occult CNV (95% CI –1.06 to –0.04, SD 1.14).

Conclusions: This practical PC based method allows for quantification of angiographic features such as CNV size in early frames and area of leakage in late frames. Limitations include non-readily defined borders in angiograms of poor image quality or indistinct borders of the hyperfluorescent areas of interest. The software is applicable to future clinical trials where the analysis of neovascular complex changes is required, for example, following therapeutic intervention.

Age related macular degeneration (AMD) is the leading cause of legal blindness in the Western world, beyond 50 years of age.^{1–4} The most frequent cause for severe visual loss associated with AMD is the growth of neovascular membranes from the choriocapillaris into the subretinal space. To analyse the natural course and effects of therapeutic measures on choroidal neovascularisation (CNV), a precise and reproducible method is desirable for evaluation of CNV changes in consecutive fluorescence angiograms. In various trials such as the Macular Photocoagulation Study (MPS), planimetric measurements were performed manually.^{5,6}

In the Radiation Therapy for Macular Degeneration (RAD) Study, a randomised prospective double masked clinical trial, the efficacy of external beam radiation therapy in neovascular age related macular degeneration (AMD) was evaluated.⁷ The patients were randomly assigned to either radiation treatment with 8×2 Gy or sham treatment (8×0 Gy). While changes in visual acuity were the main parameter, variations in subfoveal choroidal neovascular membranes were also documented in serial standardised angiograms. For the latter, a PC based image analysis software was specifically customised to quantify and analyse changes in CNV over time. This software may also be used in other clinical studies and its development and features are described here.

MATERIAL AND METHODS

For measurement of CNV variation over time, we developed image analysis software for PC compatible computers with the operating system Microsoft Windows (Microsoft Corporation, Silicon Valley, CA, USA). Commercially available software was used to generate this CNV analysis program (InpriseDelphi 4; graphics library: ImageLib CorporateSuite 4, SkylineTools, CA, USA).

Standardised fluorescein angiograms with a 30° field were obtained using a Zeiss fundus camera (Fundus camera type 450, Zeiss, Oberkochen, Germany) with Agfa RSX-100 film (Agfa-Gevaert NV, Mortsel, Belgium). For evaluation, fluorescein angiography frames from early and late phases—that is, 15 minutes after injection, were selected and scanned using a TWAIN compatible scanner. After importing images into the program, the examiner defined either a distance between two specific points of retinal vessels by drawing a red marked line or by encircling the area of the optic disc with a PC computer mouse. Subsequently, the examiner outlined the border of the CNV or area of leakage with the mouse. The ratio of the CNV area and the square of the distance between the two vessels (or area of the optic disc) were calculated by pixel count (Fig 1). The results were directly stored as a relative value to the described reference line in a dBase III Database (dBase Incorporation, Vestal, NY, USA).

For measurements of CNV progression at 6, 12, and 24 months the landmark determined at baseline (a distinct distance between two retinal vessels or the area of the optic disc) was used to calculate the area of interest after outlining. To compare baseline with consecutive measurements, the initially determined area was set to 100% and referred to for all post-treatment measurements.

For classic CNV, early frames from fluorescein angiography were chosen in which the well defined border of the neovascular complex was visible. In occult CNV, the area of leakage identified in late frames (15 minutes after intravenous fluorescein injection) was used for analysis.

Validation

For validation of the program, two readers determined areas of CNV independently by encircling them with an arrow directed

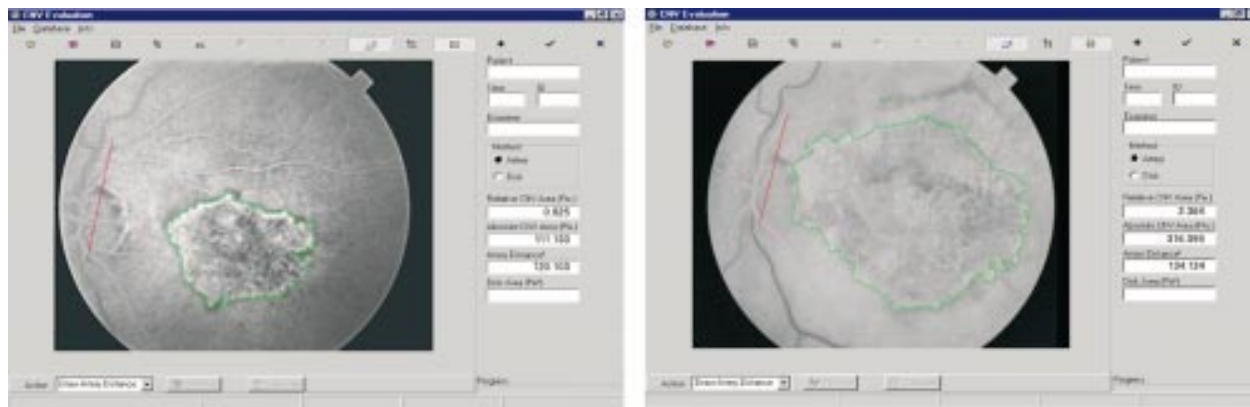


Figure 1 The examiner defined a distance between two retinal vessels by drawing a red line between them. Subsequently, the CNV was outlined in green. The ratio of the CNV area and the square of the distance between two vessels was calculated by pixel count as a relative value. This example shows an increase in the relative value over the first 12 months (relative CNV area 2.364) (right) in comparison with the baseline examination (relative CNV area 0.925) of the same patient (left).

by a PC mouse. Interobserver variability was assessed according to the method of Bland and Altman.⁸ The amount of agreement in CNV size between two observers was calculated from their differences for each patient. A difference of 0 represented perfect agreement. The 95% confidence intervals of the mean difference between observers were also reported. Differences were plotted against the mean of the two observers' readings in order to detect changes in agreement with increasing sizes of CNV (Fig 2).

RESULTS

Utilisation of the PC based software proved to be easy and practical for quantifying angiographic changes in CNV over time. For validation of the program, two independent readers measured 20 classic CNV and 20 occult CNV. Overall interobserver variability was small, the mean deviation between two

independent observers was 0.01 pixels for classic CNV (95% confidence interval -0.17 to $+0.15$, SD 0.35) and 0.55 pixels for occult CNV (95% confidence interval -1.06 to -0.04 , SD 1.14) (Fig 2).

Limitations in the use of the program included poor image quality and indistinct borders of the area of interest. It was easier to choose the distance between two points from retinal vessels as a reference distance as opposed to encircling the optic disc, since disc margins are sometimes not clearly definable on fluorescein angiograms.

DISCUSSION

The primary outcome in interventional clinical studies for neovascular AMD, such as the RAD study, is visual acuity.⁷ However, evaluation of safety and efficacy also includes effects on angiographic appearance of the neovascular subretinal complex. Deterioration of visual function is a result of various secondary mechanisms in the presence of CNV including hyperpermeability, presence of subretinal and/or intraretinal fluid and its biochemical composition, and expression of factors with an impact on the function of neuronal cells of the neurosensory retina. Various characteristics of CNV can be visualised by fluorescein angiograms. Comparison of CNV size or area of leakage from CNV in angiograms taken at different time points requires compensation for unwanted alterations in image size. In addition, easy and practical outlining with good reproducibility and reliability is desirable to facilitate analysis in clinical studies, which usually encompass large numbers of angiograms. Here, a novel PC based image analysis software yielded good results in interobserver variability and proved practical when used in the context of a large clinical trial.

Planimetric measurements of areas of interest in a two dimensional plane are commonly carried out in medicine and were initially applied in histopathological studies. In clinical ophthalmology, planimetry has been primarily of interest in determining cup-disc ratios for patients with glaucoma.⁹⁻¹¹ For retinal lesions, the Macular Photocoagulation Study Group (MPS) developed a two dimensional method to determine the size of the CNV in disc areas^{5,6,12} and subsequently to determine the size of geographic atrophies.^{13,14} In this study the foveal avascular zone (FAZ) was identified in an early frame of an angiogram by projection onto the screen of a microfilm reader at 9 \times magnification. Landmarks were drawn on an overlay, the FAZ was approximated and its anatomical centre was estimated. Afterwards, the area of the CNV was outlined on a frame of the early phase. Early phase images were then compared with mid and late phase frames to determine which areas of hyperfluorescence corresponded to

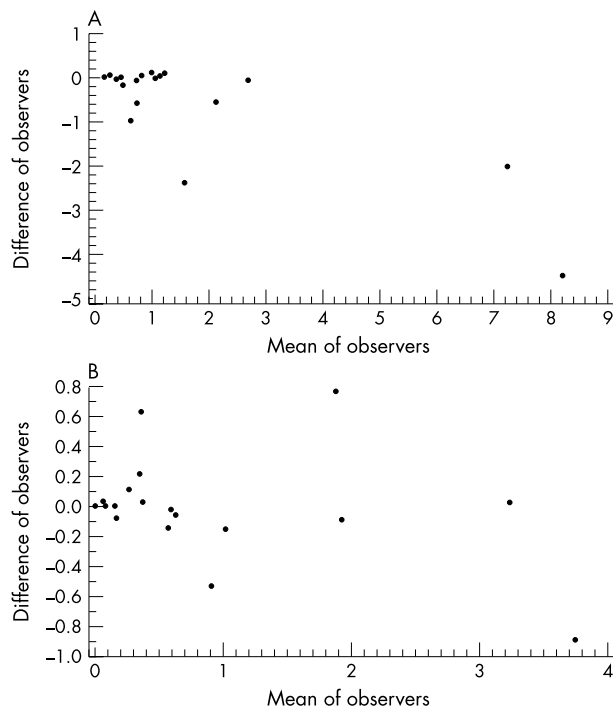


Figure 2 The mean deviation between the two independent observers was 0.01 pixels for classic CNV (95% confidence interval -0.17 to $+0.15$, SD 0.35) (B) and 0.55 pixels for occult CNV (95% confidence interval -1.06 to -0.04 , SD 1.14) (A).

neovascularisation. The figures made at baseline examination were superimposed on those from follow up examinations using the same landmarks defined in pretreatment images and allowed to compare images over time. Using a transparent template overlay in different sizes, the size of CNV was described in disc areas. One disc area was defined as a circle with a diameter of 1.5 mm (1500 μm), equal to 1.77 mm². Two disc areas were comparable with 3.5 mm², 3.5 disc areas comparable to 6.2 mm², 4 disc areas were equal to 7.1 mm², and 6 disc areas were equal to 10.6 mm².^{5,6} This technique not only allowed for a comparison of consecutive examinations but was also used to assess laser treatment. However, the magnification may have varied slightly between two cameras with the same make and model number. Retinal vessels of different photographs may also have not lined up completely, being especially apparent in patients with higher refractive errors. To minimise these problems, the same fundus camera was subsequently used for each examination. In defining the size of geographic atrophy a computer program was developed by this group, where a digitising tablet was connected to a micro-computer, with the operator tracing the atrophy outline.¹³ The computer program then calculated the area in square millimetres on the drawing itself, using a polygonal estimation of area. The obtained results were then converted into Macular Photocoagulation Study standard disc areas.

Another approach to measure the size of subfoveal CNV has been reported by Doris and coworkers.¹⁵ They outlined the CNV manually on printed fluorescein angiograms and subsequently measured the size by using an overlaying grid. The number of intersections within the area outlining the CNV was counted. However, they did not make any attempt to convert their data into exact measurements or to take into account variations in magnification.

In brief, previous methods were relatively time consuming and prone to errors in measurements for various factors like variability of image size. With the advent of image analysis software, improved methodological approaches have become available. None the less, difficulties in measuring the real size of retinal lesions are still encountered. Each of the existing fundus imaging systems has its own correction factor, which has to be taken into account for measurement. Therefore, a comparison of images made with different fundus cameras is difficult.^{16,17} Optical changes—for example, variation in refractive errors of the eye, also influence quantitative measurements. Furthermore, it appears difficult to position the fundus camera at the same angle in front of the patient at each examination.^{18,19} Considering these problems and the fact that multiple study centres with different fundus cameras may be involved in clinical studies, we developed a computer program which allowed for the evaluation of changes in CNV size at consecutive examinations under these conditions. For this purpose, both scanned angiograms on film or primarily digital images can be used. A similar method was used by Stalmans and co-workers, where a digital fundus camera only was used, making a scan of angiograms before evaluation unnecessary.²⁰

In all of the methods described above, including the one described here, the CNV was outlined manually. Therefore, the results may be influenced by subjective judgment. To assess the reliability of the results obtained from our method, we determined interobserver variability. The differences between observers for classic CNV were lower than for occult CNV, which may reflect difficulties in outlining indistinct margins of an occult CNV by observers. However, the interobserver variability was small overall.

In summary, the computer program described here allows for the quantification of fluorescence angiograms, including the size of CNV in the early phase and leakage in the late phase. This method may be used in other clinical trials in which changes of CNV characteristics over time are of interest.

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Authors' affiliations

C Bellmann, D W Miller, K Mehlretter, F Schütt, J Jorzik, F G Holz, Department of Ophthalmology, University of Heidelberg, Germany
K Unnebrink, Coordination Center for Clinical Trials, University of Heidelberg, Germany

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