European Journal of Ophthalmology / Vol. 13 no. 1, 2003 / pp. 49-56

A method for detecting progression in glaucoma patients with deep, localized perimetric defects

G. CORALLO[1], E. GANDOLFO[2]

[1]Department of Neurological and Visual Sciences, Eye Clinic, University of Genova, Genova
[2]Eye Clinic, University of Brescia, Brescia - Italy

PURPOSE. *To define a method for early detection of progressive visual field loss, based on monitoring the "healthy" component of the visual field, in glaucoma patients whose perimetric findings show the co-existence of deep scotomata and normal sensitivity areas.*
METHODS. *We reviewed all the "central 30-2 threshold tests" stored in the oldest of our Humphrey perimeters (a 640 VFA model, in use at the Glaucoma Service of the University Eye Clinic of Genoa since 1986). Only the perimetric findings of glaucoma patients with pure, deep, localized defects were collected for this study. In accordance with several inclusion criteria, we could select only 12 series of consecutive examinations (12 eyes of 12 patients). Each series included 12 to 20 examinations and the observation period ranged from 6 years 2 months to 9 years 4 months. Some pre-defined criteria made it possible to separate the defective component of the visual field from the "healthy" one. Then two independent "mean deviations" were calculated, one related to the "healthy" area and one to the defective one.*
RESULTS. *The mean deviation related to the "healthy" component of the visual field showed very little variation (0.6 to -1.3 dB) in the four series that had no increase in defects, even at the end of the observation period. However, in 7 of the 8 series with a tendency to worsen there was a small inter-test increase (-2.2 to -2.6 dB). This finding anticipated the enlargement of the scotomata, confirmed by subsequent examinations. Only in one series did the increase of the mean deviation related to the "healthy" area coincide in time with the real deterioration of the visual field, rather than anticipating it, but the inter-test interval had by chance been much longer than in the other series. The mean deviation related to the defective areas always showed very large changes in all the series, caused by the high variability of thresholds inside scotomata. This was the explanation for the large variations revealed by the "global" mean deviation too.*
CONCLUSIONS. *Detecting progression is still one of the major problems in evaluating perimetric results. It might be easier to achieve this goal with a method for selectively monitoring light sensitivity inside the "healthy" areas of the visual field. (Eur J Ophthalmol 2003; 13: 49-56)*

KEY WORDS. *Automated perimetry, Visual field, Glaucoma, Variability, Progression*

Accepted: June 3, 2002

INTRODUCTION

Automated visual field (VF) testing is a psycho-physical method of examination for measuring light differential thresholds at a large number of locations. The reliability of the procedure depends on the patient's co-operation and many other factors that may cause intra- and inter-test variability (1-4): short- and long-term threshold fluctuations (5-8) learning effect (9-12) and fatigue effect (13-16) are the main factors

Data in this paper were partially presented at the XV AGM of the Italian Association for the Study of Glaucoma (Rapallo, Italy, March 24-25, 2000) and at the XIV International Perimetric Society Meeting (Halifax, Canada, September 6-9, 2000).

1120-6721/049-08$04.00/0

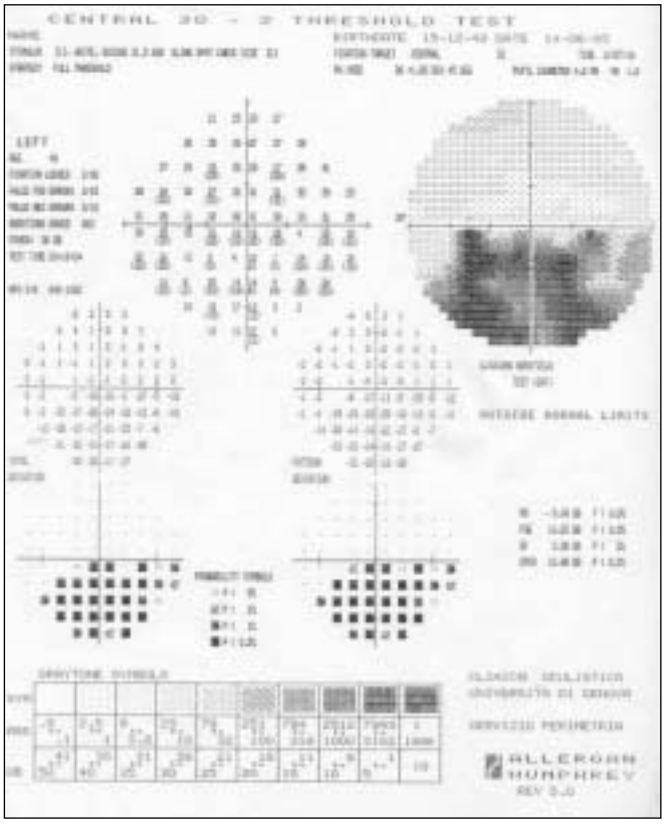

Fig. 1 - *Pure, localized defects involving both the inferior quadrants; sensitivity in the upper quadrants is within normal limits.*

involved. These make it difficult to state whether a small change in perimetric results in consecutive tests should be considered true progression or is merely a deceptive effect caused by variability (17-23). A developing cataract further increases these difficulties.

Unfortunately, the range of variability is not confined within definite, predictable limits. It is influenced by test-point status (defect or normal) and general status of the VF; in addition, it is higher in glaucoma patients and increases with eccentricity (24). Inside the defective areas, thresholds may fluctuate widely from one examination to the next, and this can simulate progression. When defects are large and deep, the global perimetric indices (25) lose their helpfulness. The "mean deviation" (MD) is inexorably influenced by the fluctuations of thresholds inside the defective areas and may even be misleading. The "pattern standard deviation" (PSD) and the "corrected pattern standard deviation" (CPSD), generally very helpful for detecting initial, mild, localized defects, become less useful when large, deep defects are present.

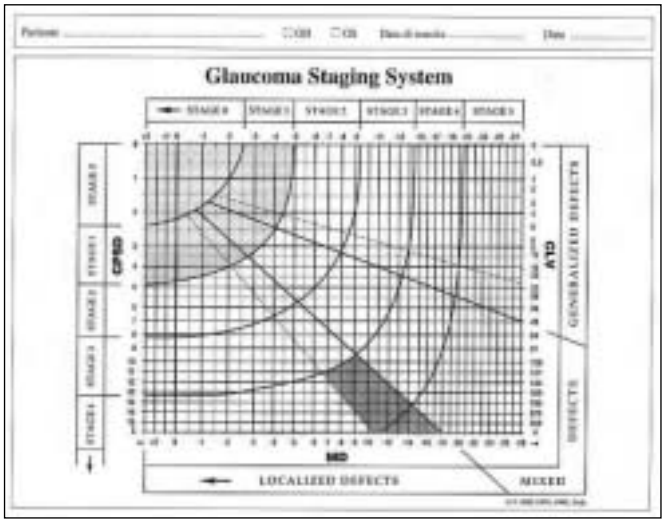

Fig. 2 - *The Glaucoma Staging System (GSS) diagram. The dark areas indicate the stages corresponding to all the perimetric findings included in this study.*

Most VFs with advanced glaucomatous damage show mixed defects, i.e. both diffuse and localized components. In a minority of cases, however, pure, localized losses are seen (Fig. 1). They are neither frequent, of course, nor exceptional. When a VF falls into that category, the effects of variability are magnified. A normal, "healthy" component can be identified in those perimetric findings, that may be a useful indicator of the true trend of VF changes.

The aim of this study was to define a method of monitoring the "healthy" component of the VF, when normal sensitivity areas and deep, large scotomata co-exist. The method is intended to distinguish between true progression of defects and false progression due to high variability inside the defective areas.

METHODS

We reviewed all the "central 30-2 threshold tests" (full threshold strategy) stored in the memory of the oldest of our Humphrey perimeters (a 640 VFA, Humphrey Instruments Inc., San Leandro, Cal., U.S.A.), in use at the Glaucoma Service of the University Eye Clinic of Genoa since 1986. A first selection was made by collecting all the series of examinations including at least 12 tests, in order to have at least 10 perimetric findings per series (the results of the first two tests were discarded, to avoid the in-

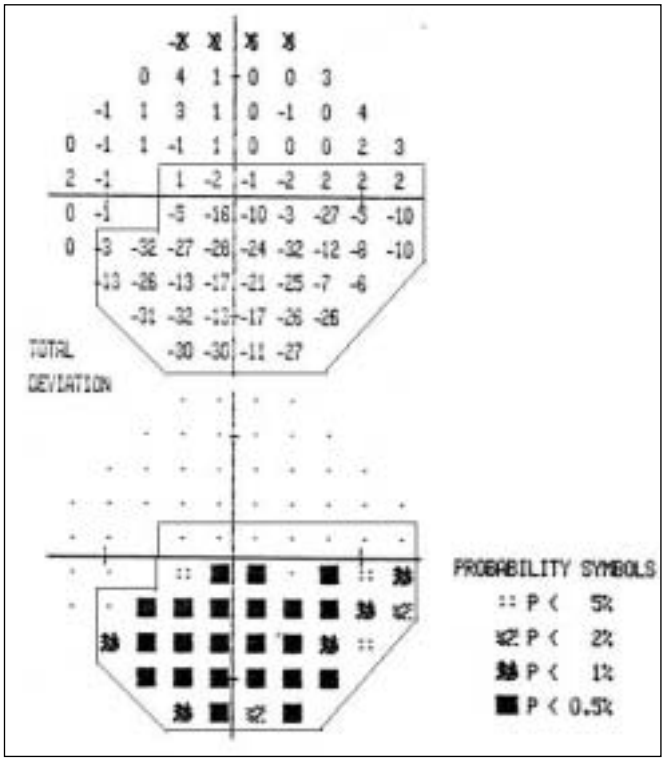

Fig. 3 - *The limits separating the "healthy" component of the visual field (VF) from the defective one (see text for the criteria adopted for separating the two zones). The four points located at the upper outer edge of the pattern were disregarded.*

fluence of the learning effect). A minimum of 10 consecutive examinations per series was considered enough for testing the feasibility of the method. Secondly, only the series satisfying the following inclusion criteria were taken into consideration:

- primary open angle glaucoma (POAG) diagnosis;
- VF findings showing the co-existence of deep scotomata and areas with normal sensitivity;
- topography of defects with fairly clear limits, making it easy to separate the defective component of the VF from the "healthy" one;
- classification as "localized defects, stage 4 or 5" according to the Glaucoma Staging System (GSS) (26)) proposed by Brusini (Fig. 2);
- good topographic stability of defects during at least the first three consecutive tests over the observation period (starting from the third examination in each series, as specified above, implying that stability had to be found at least in examinations numbers 3, 4 and 5); the defect was considered unstable when three or more pathological probability symbols in the "total deviation" plot (27) with a P value at least < 1% appeared beyond the limits of the defective area (marked out according to the criteria described below), and this finding was confirmed by the subsequent tests;
- good reliability (according to Humphrey's standards, perimetric findings showing fixation losses > 20% or false positive and false negative responses > 33% were rejected).

Applying all these inclusion criteria, only 12 series (12 eyes of 12 POAG patients) could be included in the study. The number of VFs available in each series ranged between 12 and 20. The observation period ranged from 6 years 2 months to 9 years 4 months. A method was applied to each series in order to define the limits separating the defective area of the VF from the "healthy" one. The whole line of single dots (indicating normal locations in the "total deviation" plot) surrounding the scotoma was included as a part of the defective area, mainly to avoid a misleading influence of the high variability generally present near the border of deep scotomata, when calculating the sensitivity inside the "healthy" area (Fig. 3). In other words, we considered the line of dots surrounding the scotomata in the "total deviation" plot as a "transition zone" and included it inside the defective area.

The same limits, marked out starting from the third test in each series, were then identically applied on all the subsequent tests. The upper four points at the outer edge of the pattern were excluded when they belonged to the "healthy area", because of their high variability and their tendency to be affected by artifacts due to the influence of the upper eyelid (Fig. 3).

Then, two independent MD values were calculated, one for the "healthy" area and one for the defective one. They were obtained simply by calculating the sum of the deviation values in the "total deviation" plot (indicating deviation from age-adjusted average normal sensitivity) and then dividing the result by the number of locations inside each area ("healthy" and defective). Lastly, all three MDs ("global" MD, MD of the "healthy" area, MD of the defective one) were plotted on a diagram, in order to make it easier to read the results. The number of examinations was noted on the abscissa, with the MDs (dB) on the ordinate (Figs. 4 and 5).

The appearance of three or more probability symbols with a p value at least < 1% beyond the limits of

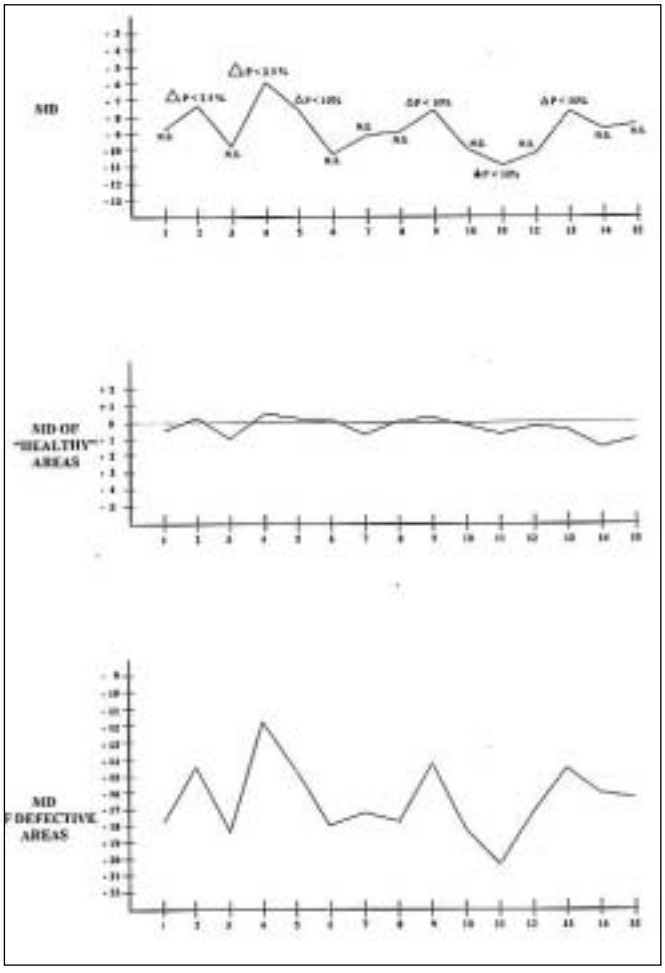

Fig. 4 - *The three mean deviations (MD: "global", for the "healthy" area, and for the defective one) of a series showing a stable trend are presented on three separate diagrams. The number of examinations is on the abscissa, and the MDs on the ordinate. A "MD slope" significance symbol from the Statpac 2 Glaucoma Change Probability statistical analysis is shown for each test (N.S. = not significant).*

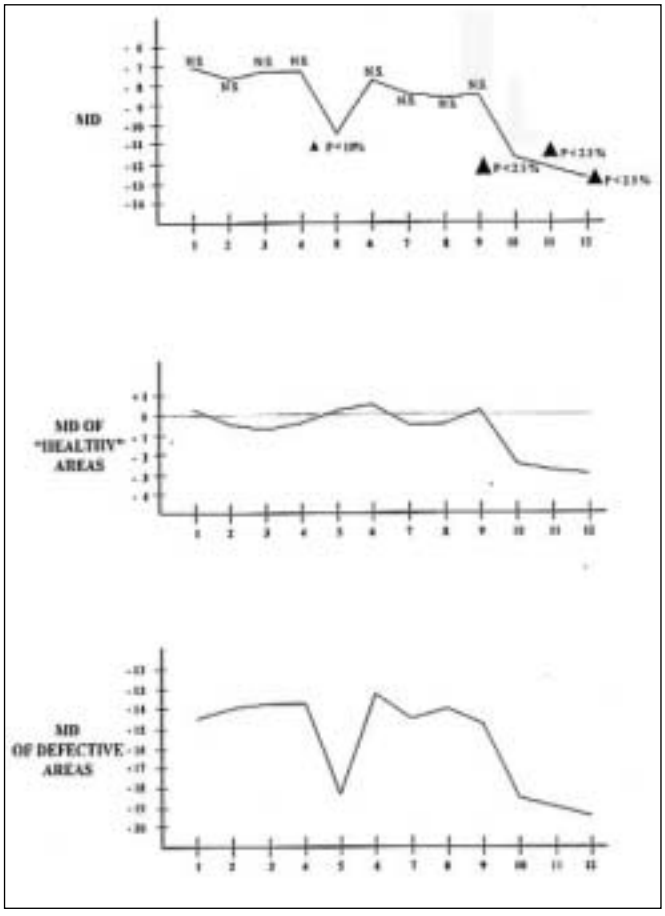

Fig. 5 - *The three mean deviations (MD: "global", for the "healthy" area, and for the defective one) of a series showing a worsening trend are noted on three separate diagrams. The number of examinations is on the abscissa, and the MDs on the ordinate. A "MD slope" significance symbol from the Statpac 2 Glaucoma Change Probability statistical analysis is shown for each test (N.S. = not significant). At test no. 10 the MD for the "healthy" area showed a small increase. This anticipated true progressive loss, which became evident from examination no. 11.*

the defective area, confirmed by subsequent examinations, was the criterion adopted to decide that a change on the perimetric map indicated a true worsening.

RESULTS

Four of the 12 series in this study showed, at the end of the observation period, a good topographical stability of defects over time (i.e. no probability symbols appeared beyond the limits of the defective area at any step of the follow-up), in spite of large fluctuations in "global" MD which sometimes seemed to indicate evident worsening of perimetric findings. The MD related to the "healthy" area of the VF appeared fairly unchanged over time in these cases (ranging between 0.6 dB and –1.3 dB with a maximum inter-test increase of 0.9 dB, considering all four series).

At a certain stage of the observation period, eight series showed evident worsening, according to the above mentioned criteria. A small inter-test increase (the range was between -2.2 and -2.6 dB) of the MD related to the "healthy" area was found in seven of these eight. It anticipated the true progression of defects, which became evident at the next test (Tab. I).

TABLE I - MEAN DEVIATION (MD) FOR THE "HEALTHY" AREA OF THE VF IN ALL THE SERIES COLLECTED

SERIES Number	**EXAMINATION Number**																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	-0.3	0.2	-0.7	0.2	0.1	-0.1	-0.6	0.1	0.3	-0.2	-0.5	-0.2	-0.3	-0.8	-0.6					
2	0.1	-0.2	0.2	0.3	0.1	-0.1	-0.2	0.1	-0.1	-0.3	-0.2	-0.3	-0.2	-0.1						
3	-0.2	-0.3	0.0	0.1	-0.3	0.2	0.6	0.1	0.2	-0.1	0.3	0.2	0.3							
4	-0.5	-0.4	-0.6	-0.2	-0.8	-1.3	-0.7	-0.5	-0.6	-0.2	-0.4	-0.3	-0.7	-0.4	-0.5	-0.8				
5	0.2	-0.2	-0.3	-0.2	0.1	0.3	-0.3	-0.3	0.1	**-2.5**	*-3.8*	*-4.5*								
6	-0.6	-0.3	-0.4	-0.5	-0.2	-0.7	-0.8	-0.4	**-2.6**	*-3.6*	*-3.8*	*-4.2*	*-4.4*	*-4.8*						
7	-0.8	-0.7	-0.9	-1.2	-1.4	-1.2	-0.7	**-2.9**	*-3.8*	*-4.6*	*-4.9*	*-5.1*	*-5.5*	*-5.4*	*-5.7*	*-6.1*	*-6.5*	*-6.8*	*-7.0*	*-7.3*
8	-0.3	0.0	0.1	**-2.4**	*-3.2*	*-3.6*	*-3.9*	*-4.8*	*-5.2*	*-5.5*	*-5.8*	*-6.2*	*-6.6*	*-7.0*	*-7.1*	*-7.3*				
9	-0.9	-0.8	-1.1	-1.0	-1.3	-0.9	-0.6	**-2.8**	*-4.6*	*-5.2*	*-5.6*	*-7.8*								
10	-0.6	-0.3	-0.6	-0.4	-0.2	**-2.5**	*-3.2*	*-4.1*	*-5.3*	*-5.5*	*-6.3*	*-6.9*	*-8.0*	*-8.6*						
11	-0.4	-0.1	-0.3	-0.6	-0.2	-0.5	-0.4	-0.5	**-3.7***	*-4.3*	*-4.5*	*-5.4*	*-5.8*							
12	-0.2	-0.6	-0.1	-0.2	**-2.5**	*-3.6*	*-3.8*	*-4.3*	*-4.9*	*-6.1*	*-6.3*	*-6.6*	*-6.8*							

The first four series showed good stability and the MDs showed very little variation. Series 5-12 showed a worsening trend, which was pre-announced by a small increase in MD (-2.2 to –2.6 dB) - marked with bold characters. Italics indicate the MDs corresponding to true impairment of the VF. Only series no. 11 showed a significant MD increase (*), coinciding in time with the impairment of the VF, but the interval from the previous examination was much longer than in the other cases.

This mild increase of the MD related to the "healthy" area was not detected simply by looking at the "total deviation" plot: the grid of dots, indicating no significant changes, appeared much the same. Only one of the eight series that worsened showed an increase in MD related to the "healthy" area which coincided in time with the appearance of some pathological probability symbols inside this area on the "total deviation" plot, but the interval from the previous examination had by chance been much longer in that case (14 months), compared to the average for all the other series (eight months).

DISCUSSION

Variability is a major problem affecting the reliability of perimetric procedures. Automation of perimetry has eliminated all the variability depending on the examiner's skill or even patience. Nevertheless, a lot of factors can still induce variability: short- and long-term threshold fluctuations, learning effect, fatigue effect, etc. Moreover, the range of variability does not fall within definite, predictable boundaries, but is influenced by a number of factors such as test-point status (defect or normal) and general status of VF; it is higher in glaucoma patients and increases with eccentricity. The fact is that variability itself is variable!

Inside defective VF areas thresholds may show large differences from one examination to the next. These heavily affect the MD, sometimes giving the impression of improbable improvements, though more often of a noticeable worsening of VF. In such cases, a change of medical therapy or even a surgical strategy may be based on the apparent deterioration of a VF which is already seriously damaged. It is often very difficult to decide whether differences from one examination to the next are true changes or simply due to variability. Global indices are not helpful in these cases. Even the statistical analysis programs in the most up-to-date instruments may lead to false interpretations.

A particular type of glaucomatous VF defects involves pure, deep, localized losses, co-existing with normal sensitivity areas. Although these findings are not frequent – mixed defects are much more common – they do occur and offer the opportunity of monitoring the "healthy" component of the VF as a more sensitive indicator of the true trend of the VF. Variability inside the "healthy" areas, in fact, is normally very limited. As a consequence, even small changes in these areas should be considered significant. However, the problem is how to monitor the "healthy" com-

ponent of the VF. The most simple and even obvious method might involve looking at the "total deviation" plot, evaluating any changes in the grid of dots (the "healthy" area), and possibly counting them. In our opinion this method, which is probably the most common in clinical practice, is inaccurate. In the numerical map of the "total deviation" plot, indicating deviations from age-adjusted average normal sensitivity, deviations fluctuating from positive (2 to 3 dB; higher values are suspect for poor patient's co-operation) to negative (-4 to –6 dB, depending on different eccentricities) are all marked by the same symbol, a little dot, on the map of the "total deviation" plot. This means that at a single location, sensitivity may oscillate between 6 and 9 dB, but that location is still always marked with a dot, giving the impression that there has been no change at all. The MD, like the other global perimetric indices, is not helpful, because of its wide variations caused by fluctuation of thresholds inside defective areas. As affirmed by Anders Heijl (personal communication) "variability is higher in pathological locations than in normal locations. In defect positions, the threshold may reach any value by chance alone. Therefore, deterioration of normal locations has more significance than in pathological locations".

Taking inspiration from this sentence, we made an attempt to isolate and monitor over time the normal component of the VF, separating it from the defective one. Using the method described above to calculate the specific MD for the "healthy" component of the VF, it is easier to detect small changes inside that area. We arbitrarily included in the defective area the whole line of dots surrounding it (we explained the reasons for this above). This procedure produces a small "dilution effect" by the MD for the defective area, but we feel this is not important because our purpose is to focus on the "healthy" component of the VF. Calculating the MD inside the defective one has scant relevance because of the misleading effect of high threshold fluctuations. This is also the reason why we think that only enlargement of the scotoma, confirmed by subsequent tests, can be considered a sure expression of true worsening of defects in VF_s like those described.

Deciding whether a scotoma has become deeper, on the other hand, is very difficult and sometimes even impossible, because of the high threshold fluctuations. Fig. 1 gives an example of pure, localized loss. This perimetric finding can be classified as "localized defect, stage 4", according to Brusini's GSS. The limits separating the "healthy" and the defective areas are particularly clear in this case. The defects show an altitudinal topography, suggesting that vascular factors perhaps contribute to optic disc damage. It must be noted that not all the series included in this study showed similar topography (most defects were limited to one quadrant or less). However, all the series in this paper belonged to POAG patients who had a history of ocular hypertension and documented glaucomatous optic disc anomalies.

The defects presented in Figure 1 are large, deep, not absolute, with the exception of only a few points. If genuine absolute scotomata were present, it might be objected that the changes in the "global" MD should reflect only the changes in thresholds inside the "healthy" area, since areas with no sensitivity obviously show no fluctuations. Therefore, monitoring the "global" MD alone should be sufficient in these cases. In our opinion, even in a case with a pure, circumscribed, absolute scotoma, the method we propose may play a role, because thresholds surrounding the scotoma, in the "transition zone", are generally very unstable. Our method includes this "transition zone" in the defective area, so the "healthy" component of the VF is represented only by genuine "healthy" points.

It might also be objected that 12 series are too few. In absolute terms this is true, but no more could be collected after scanning the hard disk of our Humphrey 640 VFA (a total of about 11,000 files, of examinations executed with many programs and strategies), because of the need to respect numerous, very selective inclusion criteria. This is the reason why the sample is so small, and might erroneously induce one to believe that the type of defect considered is more rare than it actually is. Look now at the diagrams in Fig. 4, which summarize a series of 15 VF examinations over about ten years. The course of the three MDs - "global", for the "healthy" area, and for the defective area - is easily investigated. The "delta" symbol indicating the "MD slope" significance from the STATPAC 2 "Glaucoma Change Probability" statistical analysis (28) has been noted for each test. We can see the significant MD worsening (after a number of unlikely improvements) at examination number 11. At a stage like this there may have been a change

in therapeutic strategy. But later examinations show a MD recovery and after 15 tests over about ten years, the MD is surprisingly similar to its starting value. As the diagrams clearly display, all the fluctuations in the "global" MD were induced by large variations of thresholds exclusively inside the defective area. The MD related to the "healthy" one showed almost no variation in this series, with a very stable trend.

The diagrams in Figure 5 refer to a series of 12 VF examinations over about seven years. Test number 5 suggests a false worsening, not confirmed by the next four examinations. It was caused by an occasional, large fluctuation of the MD related to the defective area (the corresponding MD for the "healthy" one appeared fairly unchanged, in comparison with previous examinations). At test number 10 the MD for the "healthy" area showed a small increase (about 2.5 dB). This anticipated the appearance of true VF worsening, that became evident at the next examination. Similar findings were seen in seven of the eight series that showed a worsening trend. Only in one of these did the increase of the MD for the "healthy" area coincide in time with the appearance of pathological probability symbols inside that area (it is obvious that when such a finding makes its appearance, the role of our method has ended). However, this is the case where 14 months had elapsed since the previous examination whereas the mean interval in this series and in others was about seven months.

In conclusion, the method we propose for monitoring "healthy" areas seems to differentiate between true progression and false changes due to variability. It may therefore provide a better basis for oriented therapeutic strategies over time in glaucoma patients with advanced perimetric damage.

Reprint requests to:
Guido Corallo, MD
Department of Neurological and Visual Sciences
University Eye Clinic
Ospedale San Martino, Pad. 9
Largo Rosanna Benzi, 10
I-16132 Genova, Italy
guidocorallo@libero.it

REFERENCES

1. Bebie H, Fankhauser F, Spahr J. Static perimetry; accuracy and fluctuations. Acta Ophthalmol 1976; 54: 339-48.
2. Koerner F, Fankhauser F, Bebie H, Spahr J. Threshold noise and variability of field defects in determinations by manual and automatic perimetry. Doc Ophthalmol Proc Ser 1977; 14: 53-9.
3. Parrish RH II, Schiffman J, Anderson DR. Static and kinetic visual field testing; reproducibility in normal volunteers. Arch Ophthalmol 1984; 102: 1497-502.
4. Lewis RA, Johnson CA, Keltner JL, Labermaier PK. Variability of quantitative automated perimetry in normal observers. Ophthalmology 1986; 93: 878-81.
5. Flammer J, Drance SM, Fankhauser F, Augustiny L. Differential light threshold in automated static perimetry; factors influencing short-term fluctuation. Arch Ophthalmol 1984;102: 876-9.
6. Flammer J, Drance SM, Zulauf M. Differential light threshold; short- and long-term fluctuation in patients with glaucoma, normal controls, and patients with suspected glaucoma. Arch Ophthalmol 1984; 102: 704-6.
7. Flammer J, Drance SM, Schulzer M. Covariates of the long-term fluctuation of the differential light threshold. Arch Ophthalmol 1984;102: 880-2.
8. Chauhan B, Leblanc R, Drance SM, Wijsman K, Cruz A. Effect of the number of threshold determinations on short-term fluctuation in automated perimetry. Ophthalmology 1991; 98:1420-4.
9. Werner EB, Adelson A, Krupin T. Effect of patients experience on the results of automated perimetry in clinically stable glaucoma patients. Ophthalmology 1988; 95: 764-7.
10. Wild JM, Dengler-Harles M, Searle AET, O'Neill EC, Crews SJ. The influence of the learning effect on automated perimetry in patients with suspected glaucoma. Acta Ophthalmol 1989; 67: 537-45.
11. Heijl A, Lindgren G, Olsson J. The effect of patient experience in normal subjects. Arch Ophthalmol 1989; 107: 81-6.
12. Marchini G, Pisano F, Bertagnin F, Marraffa M, Bonomi L. Perimetric learning effect in glaucoma patients. Glaucoma 1991; 13: 102-6.
13. Johnson CA, Adams CW, Lewis RA. Fatigue effects in automated perimetry. Appl Optics 1988; 27: 1030-7.
14. Marra G, Flammer J. The learning and fatigue effect in automated perimetry. Graefes Arch Clin Exp Ophthalmol 1991; 229: 501-4.
15. Fujimoto N, Adachi-Usami E. Fatigue effect within 10°

visual field in automated perimetry. Ann Ophthalmol 1993; 25: 142-4.
16. Hudson C, Wild JM, O'Neill EC. Fatigue effects during a single session of automated static threshold perimetry. Invest Ophthalmol Vis Sci 1994; 35: 268-80.
17. Werner EB, Bishop KI, Koelle J, et al. A comparison of experienced clinical observers and statistical tests in detection of progressive visual field loss in glaucoma using automated perimetry. Arch Ophthalmol 1988; 106: 619-23.
18. Schulzer M. Errors in the diagnosis of visual field progression in normal-tension glaucoma. Ophthalmology 1994; 101: 1589-94.
19. Fitzke FW, Mc Naught AI. The diagnosis of visual field progression in glaucoma. Curr Opin Ophthalmol 1994; 5: 110-5.
20. Birch MK, Wishart PK, O'Donnell NP. Determining progressive visual field loss in serial Humphrey visual fields. Ophthalmology 1995; 102: 1227-35.
21. Smith SD, Katz J, Quigley HA. Analysis of progressive change in automated visual fields in glaucoma. Invest Ophthalmol Vis Sci 1996; 37: 1419-28.
22. Katz J, Gilbert D, Quigley HA, Sommer A. Estimating progression of visual field loss in glaucoma. Ophthalmology 1997; 104: 1017-25.
23. Katz J, Congdon N, Friedman DS. Methodological variations in estimating apparent progressive visual field loss in clinical trials of glaucoma treatment. Arch Ophthalmol 1999; 117: 1137-42.
24. Werner EB, Saheb N, Thomas D. Variability of static visual threshold responses in patients with elevated IOPs. Arch Ophthalmol 1982; 100: 1627-31.
25. Flammer J. The concept of visual field indices. Graefes Arch Clin Exp Ophthalmol 1986; 224: 389-92.
26. Brusini P. Clinical use of a new method for visual damage classification in glaucoma. Eur J Ophthalmol 1996; 6: 402-7.
27. Heijl A. The Humphrey Field Analyzer, construction and concepts. In: Heijl A, Greve EL, eds.: Sixth International Visual Field Symposium. Doc Ophthalmol Proc Ser 42. Dordrecht: Dr W Junk, 1985; 77-84.
28. Morgan RK, Feuer WJ, Anderson DR. Statpac 2 glaucoma change probability. Arch Ophthalmol 1991; 109: 1690-2.