EVALUATION OF ATHEROSCLEROTIC LESIONS IN CEREBRAL ARTERIES BY UNAIDED VISUAL ESTIMATION

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SUMMARY

In an international study of cerebral atherosclerosis the extent of atherosclerotic lesions in extra- and intracranial arteries was determined using unaided visual estimation. Lesions were carefully defined and observations were recorded in a standardized manner. Specimens were coded so that the evaluators were not aware of the source.

Results of reliability tests confirm previous studies which have indicated that experience and training are essential for the successful application of visual evaluation methods. In the present studies the evaluators, on the average, were able to reproduce consensus estimates to within 5 units of percent surface involvement even after a period of 6 months. The method is simple, rapid and suitable for large scale studies requiring quantitation of atherosclerotic lesions in arteries.

Key words: *Atherosclerosis – Measuring error – Cerebral arteries – Visual estimation – Reproducibility*

INTRODUCTION

The evaluation of atherosclerotic lesions in arteries obtained from autopsied cases has become an important tool in studies of atherosclerosis[1] and the methods

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commonly applied have been thoroughly reviewed[2,3]. However, grading methods have not always been standardized so that results could be compared among different studies.

The International Atherosclerosis Project (IAP), a cooperative study of atherosclerosis in autopsied persons from different populations, determined the influence of environmental conditions, race, age, sex, and diseases on atherosclerosis[1]. Because of its international cooperative nature this study solved many of the problems concerned with the comparability of methods applied to different populations[4]. Aortas, coronary arteries, carotid arteries, vertebral arteries and intracranial arteries were collected and preserved following techniques specified in a Standard Operating Protocol[5]. All the specimens collected were shipped to central laboratories in New Orleans, Louisiana, and Guatemala City, Guatemala, for further processing and uniform staining. The extent of intimal surface involved with atherosclerotic lesions was estimated visually by trained and tested observers under controlled conditions[4,5]. A detailed description of all the standardized methods used in the IAP was presented in the report from the project[5].

In this study the authors present the results of the reliability tests of the grading system developed by the IAP as applied to grading atherosclerotic lesions in the major extra- and intracranial arteries supplying the brain.

MATERIALS AND METHODS

Collection of arteries

Carotid, vertebral and intracranial arteries from autopsied cases in five laboratories (Oslo, Norway; New Orleans, U.S.A.; Kingston, Jamaica; Guatemala City, Guatemala; Santiago, Chile) were collected and shipped to a central laboratory in New Orleans. Details of dissection and preparation of arteries are given in the Standard Operating Protocol for the IAP[5] and can be summarized as follows: The carotid, vertebral[6], and main trunks of the intracranial arteries were excised at autopsy and dissected free of adventitial fat; the arteries were then opened longitudinally, fixed in formalin with the adventitial surface adhered to cardboard, identified with a case specific code number, and stored in plastic bags (Fig. 1). The arteries were later stained with Sudan IV and repackaged in plastic bags with the case identification code. Definitions of terms concerning arterial segments and lesions were those of the IAP[5], reviewed in the preceding paper[7].

Grading atherosclerosis

The gradings of the cerebral arteries were performed in four 3-day grading sessions at 2-week intervals in March and April 1966. Altogether 1541 sets of arteries from all geographic locations were intermingled and divided into four lots of approximately equal numbers, and one lot was chosen at random for each grading session. The sets of specimens in each lot were shuffled to avoid sequencing in the order of grading. During the grading the only reference for identification was the assigned

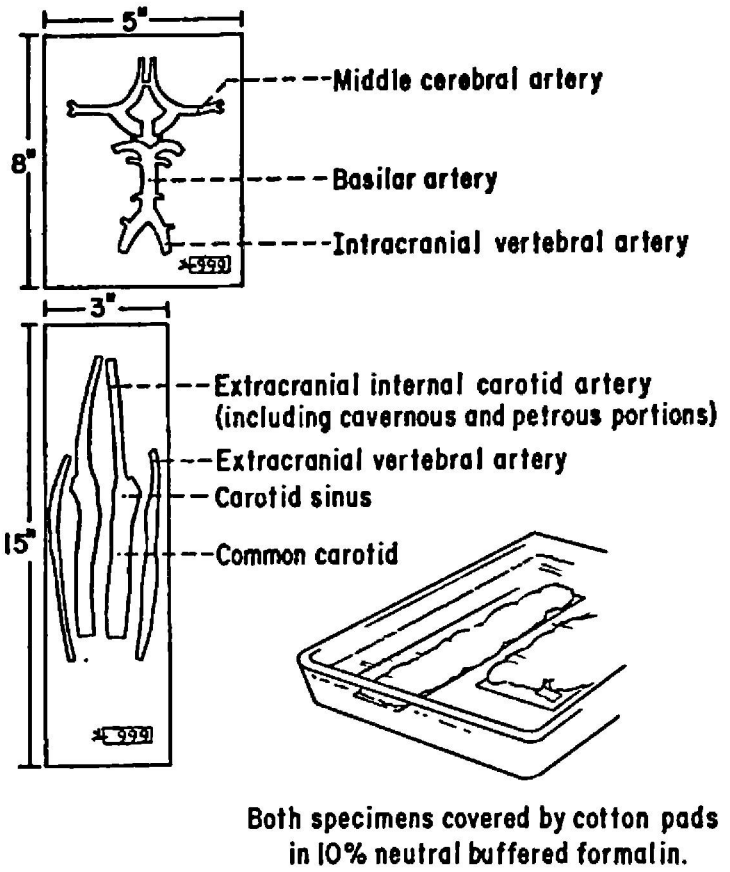

Fig. 1. Drawing of cerebral arteries dissected and prepared for fixation. Permission for reproduction of this figure from "The Geographic Pathology of Atherosclerosis" (ref. 1), granted by the International Academy of Pathology.

case code number so that the evaluators were not aware of the source, race, age, sex, disease, or any other data which may have influenced the evaluation of any given specimen.

A team of two pathologists (L.A.S. and P.A.M.) graded all the cerebral arteries, assigned by mutual accord (consensus) a single grade for each type of lesion considered in the grading system. For a short period before the first grading session the two pathologists repeatedly graded 20 specimens for practice. In all the grading sessions the neck arteries were graded first and consistently in the following order: left common carotid artery, right common carotid artery, left internal carotid artery, right internal carotid artery, left vertebral artery, and right vertebral artery. Next the intracranial arteries were graded consistently in the following order: left middle cerebral artery, right middle cerebral artery, and basilar artery.

A detailed description of the visual grading system has been published elsewhere[4,5] but, for the sake of completeness, a brief summary follows.

All visual estimates of the extent of lesion involvement in a given artery were made in two stages. First the graders recorded the percentage of the *total intimal surface* involved with lesions. Secondly, they divided the total involved area into areas affected with each of the four types of lesions considered, and recorded in each case the corresponding estimate as percent of the total intimal surface involved with lesions. For example, an artery was estimated to have 60% of its total intimal surface involved with lesions; 45% of this area was involved with fatty streaks, 35% with fibrous plaques, 15% with complicated lesions and 5% with calcified lesions.

Note that the estimated percentages for the different types of lesions add to 100% of the intimal surface involved. All recorded estimates of the extent of lesion involvement were transferred to punched cards and the statistical laboratory carried out all subsequent calculations and analyses using electronic data processing equipment. In the initial phase of analysis, the involvements by types of lesions were converted into the corresponding percentages of intimal surface involved out of the total intimal area of the artery. Using the results given in the example cited above to illustrate the conversion procedure, the percent intimal surface involved with each of the four types of lesions considered would be:

$$\frac{60 \times 45}{100} = 27\%, \text{ fatty streaks}$$

$$\frac{60 \times 35}{100} = 21\%, \text{ fibrous plaques}$$

$$\frac{60 \times 15}{100} = 9\%, \text{ complicated lesions}$$

and $$\frac{60 \times 5}{100} = 3\%, \text{ calcified lesions}$$

Note that under this transformation the parts (27, 21, 9 and 3%) total 60%, the estimated percentage of intimal surface involved with lesions. The net percent of total intimal area involved with raised lesions was obtained by adding the involvement with fibrous plaques, complicated lesions and calcified lesions. In the example above, the percent involvement with raised lesions would be: $21 + 9 + 3 = 33\%$. In accord with IAP procedures[4], the findings of this investigation will be presented in terms of total surface, fatty streak and raised lesion involvement.

Tests for reliability of grading the extent of lesions

Three tests for reliability of the gradings were performed as follows:

(1) Standard set of specimens: One of the investigators (O.D.W.) selected 21 sets of cerebral arteries from the files to be used as a standard set. The team of pathologists graded this set during each of the four grading sessions to produce, for each specimen, four independent estimates of atherosclerotic involvement.

(2) Selected specimens for duplicate gradings: The statistician also selected cases from each grading session to be regraded in the next grading session to produce, for each artery, two independent estimates of lesion involvement. In the second, third, and fourth grading sessions, a total of 69 sets of arteries was regraded. The cases to be regraded, as well as the cases from the standard set, were intermingled with the specimens prepared for each grading session. The graders were aware that repeat test cases were included among the arteries to be graded in each session, but they were not able to identify these cases.

(3) Long-term reproducibility of the gradings: A previous study[8], based on the grading of cerebral arteries, showed a high correlation in the extent of involvement between the right and left segments of bilaterally paired arteries and particularly between the left and the right common carotid arteries. Since the right common carotid artery was graded immediately following the left common carotid, it is possible that the grading sequence could have influenced the results, introducing spurious correlation. To investigate this point and at the same time to evaluate the long-term reproducibility of the grading system, a set of 68 cases was randomly selected from the file and both common carotid arteries were graded independently for the extent of lesion involvement 6 months after the original grading. The left common carotid artery was graded first with all other arterial segments covered to eliminate possible biases, and, one week later, the right common carotid artery was graded under the same grading conditions.

Statistical analysis

Analysis of variance was used to obtain estimates of the grading error and for testing the significance of the differences in mean extent of atherosclerotic lesions among the quadruplicate gradings. When the F test was significant ($p < 0.05$), a studentized range (Q) was used to identify which of the means was different. The

TABLE 1

PERCENT OF THE TOTAL INTIMAL SURFACE INVOLVED WITH ATHEROSCLEROTIC LESIONS IN 21 LEFT COMMON CAROTID ARTERIES GRADED IN FOUR SUCCESSIVE SESSIONS — INTERNATIONAL ATHEROSCLEROSIS PROJECT, MARCH–APRIL, 1966

Specimen	*Grading session*			
	1	*2*	*3*	*4*
1	25	20	25	30
2	03	05	05	02
3	35	30	60	25
4	80	90	90	80
5	95	95	99	95
6	60	65	75	65
7	90	85	80	90
8	75	70	80	80
9	40	40	40	45
10	20	30	20	25
11	40	35	35	35
12	40	40	70	40
13	45	35	40	35
14	35	25	25	25
15	20	30	20	20
16	85	80	95	85
17	30	30	20	30
18	75	70	85	65
19	40	40	40	30
20	30	25	20	25
21	03	02	03	05
Mean	46.0	44.9	48.9	44.4

concordance of grading results in the cases that were graded twice was evaluated using the correlation coefficients and the t test for correlated pairs. All the calculations in these statistical analyses were carried out following methods described by SNEDECOR AND COCHRAN[9].

RESULTS

Reproducibility of grading the standard set of specimens

In the grading system for cerebral arteries only consensus estimates of involvement were recorded. Under such a system it is not possible to estimate the "among-grader" component of error and only a net grading error, representing the sum of all error components, can be estimated from the quadruplicate consensus estimates for the standard set. Since these arteries were not graded as a separate set but were interspersed among the arteries to be graded in each grading session, the estimates of the net grading error should reflect the expected operational variability of the grading system.

The results obtained in the four independent assessments of total surface involvement in the left common carotid (Table 1) and of fatty streak involvement in the basilar artery (Table 2) are examples of the level of reproducibility that can be

TABLE 2

PERCENT OF THE INTIMAL SURFACE INVOLVED WITH FATTY STREAKS IN 21 BASILAR ARTERIES, GRADED IN FOUR SUCCESSIVE SESSIONS — INTERNATIONAL ATHEROSCLEROSIS PROJECT, MARCH–APRIL 1966

Specimen	*Grading session*			
	1	*2*	*3*	*4*
1	14	12	12	11
2	01	01	01	01
3	01	02	03	03
4	14	05	10	09
5	14	19	19	19
6	06	08	08	07
7	05	01	00	05
8	01	01	05	05
9	03	08	07	07
10	01	05	05	08
11	01	02	06	02
12	30	36	36	39
13	04	03	05	05
14	01	06	05	03
15	01	01	09	01
16	04	01	08	05
17	01	05	04	03
18	09	03	07	10
19	05	04	06	02
20	00	01	01	01
21	00	00	00	00
Mean	5.5	5.9	7.5	7.0

achieved in using the visual grading system. In both tables the range of the estimates for a specific case gives a crude indication of the variability in grading. The first example (Table 1) illustrates actual grading variability in the case of relatively high involvements, while the second example (Table 2) applies to the case of low involvements. These data show also that the repeated visual estimates tend to be of the same order of magnitude and in general are sufficiently close to one another in absolute values to suggest an adequate agreement in spite of occasional large discrepancies due to a few aberrant estimates. The quantitative estimates of the grading error which are described in the following paragraphs will justify this first general impression.

The mean values for the quadruplicate estimates of total intimal surface involved, surface involved with fatty streaks, and surface involved with raised lesions are given in Tables 3, 4 and 5, together with the estimates of grading errors in the various arteries and each type of lesion. The estimates of grading error included in Tables 3, 4 and 5 were obtained from the corresponding analyses of variance and represent the failure in assigning the same percent involvement to a given specimen on repeated gradings. All the calculated estimates of grading error are below 10 units of percent involvement, and only the error estimates for the assessment of total surface and raised lesion involvement in the common carotid artery (Tables 3 and 5) appear to be substantially greater than 5% involvement. This suggests that, on the average, the two pathologists grading the cerebral arteries were generally able to reproduce their consensus estimates to within 5 units of percent surface involvement.

Statistically significant differences among the quadruplicate estimates occurred in five of the nine arterial segments, but most of these are due to extreme estimates of involvement in the early grading sessions. These findings suggest that the graders

TABLE 3

MEANS FOR THE PERCENT OF THE INTIMAL SURFACE INVOLVED WITH ATHEROSCLEROTIC LESIONS IN 21 SPECIMENS OF CEREBRAL ARTERIES GRADED IN FOUR SUCCESSIVE SESSIONS — INTERNATIONAL ATHEROSCLEROSIS PROJECT, MARCH–APRIL 1966

Cerebral artery	*Side*	*Grading session*				*Net grading error*
		1	*2*	*3*	*4*	
Common carotid	left	46.0	44.9	48.9	44.4	6.4
	right	47.4	48.1	50.0	44.8	7.1
Internal carotid	left	25.7*	25.6*	22.5	23.2	3.6
	right	24.5*	24.5*	22.1	21.4	3.4
Vertebral	left	18.7	18.3	18.0	16.2	3.2
	right	14.6*	12.9	12.4	12.1	2.7
Middle cerebral	left	30.8	33.3	33.3	31.0	5.2
	right	27.7	30.0	28.6	29.7	3.7
Basilar		37.3*	39.2	40.3	40.0	2.6

* This mean value is different ($p < 0.05$) from one or more of the other means in the corresponding sequence of gradings.

TABLE 4

MEANS FOR THE PERCENT OF THE INTIMAL SURFACE INVOLVED WITH FATTY STREAKS IN 21 SELECTED CEREBRAL ARTERIES, GRADED IN FOUR SUCCESSIVE GRADING SESSIONS — INTERNATIONAL ATHEROSCLEROSIS PROJECT, MARCH–APRIL 1966

Cerebral artery	*Side*	*Grading session*				*Net grading error*
		1	*2*	*3*	*4*	
Common carotid	left	18.2*	13.5	13.2	14.6	5.0
	right	16.3	13.6	12.1	13.5	6.0
Internal carotid	left	3.1	3.4*	2.1	2.7	1.3
	right	2.7	3.5*	1.6	2.6	1.6
Vertebral	left	3.6	4.5	3.6	3.9	1.8
	right	3.8	3.7	3.0	3.4	1.8
Middle cerebral	left	2.7	2.6	3.2	3.0	1.8
	right	2.5	2.4	3.3	2.8	1.7
Basilar		5.5*	5.9	7.5	7.0	4.8

* This mean value is different ($p < 0.05$) from one or more of the other means in the corresponding sequence of gradings.

TABLE 5

MEANS FOR THE PERCENT OF INTIMAL SURFACE INVOLVED WITH RAISED ATHEROSCLEROTIC LESIONS IN 21 SPECIMENS OF CEREBRAL ARTERIES GRADED IN FOUR SUCCESSIVE SESSIONS — INTERNATIONAL ATHEROSCLEROSIS PROJECT, MARCH–APRIL 1966

Cerebral artery	*Side*	*Grading session*				*Net grading error*
		1	*2*	*3*	*4*	
Common carotid	left	27.8*	31.3	35.7	30.6	8.2
	right	31.1	34.5	37.9	32.7	9.9
Internal carotid	left	22.6	22.2	20.4	21.0	3.2
	right	21.9*	21.1	20.5	19.0	3.2
Vertebral	left	15.1	13.8	14.3	12.5	3.1
	right	10.8	9.1	9.5	9.0	2.7
Middle cerebral	left	28.1	30.7	30.1	28.4	5.6
	right	25.2	27.6	25.3	26.5	3.7
Basilar		31.8	33.4	32.9	33.0	3.2

* This mean value is different ($p < 0.05$) from one or more of the other means in the corresponding sequence of gradings.

were able to improve the reproducibility of their estimates in the course of the regular grading sessions. These results also suggest that of the three measurements, the estimates of raised lesions seem to be the most reproducible since only two means in the first grading session differed significantly from the corresponding means calculated from the subsequent gradings of the same arteries (Table 5). On the other hand, when the grading variability is examined in relative terms, expressing the grading error as a fraction of the corresponding mean estimates of involvement (coefficients

of variability), it becomes apparent that, in spite of a greater number of significant differences among the quadruplicate means, the estimates of total surface involvement were the least variable. The relative variability in the estimation of raised

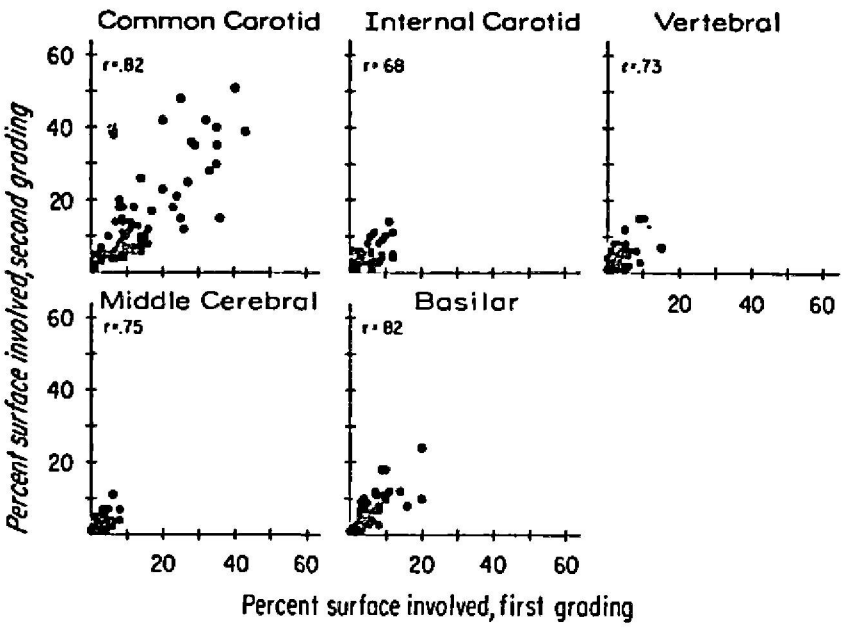

Fig. 2. Correlation between the first and the second grading of fatty streaks in selected cerebral arteries from 69 autopsied cases. Each point may represent more than one case. International Atherosclerosis Project, March–April 1966.

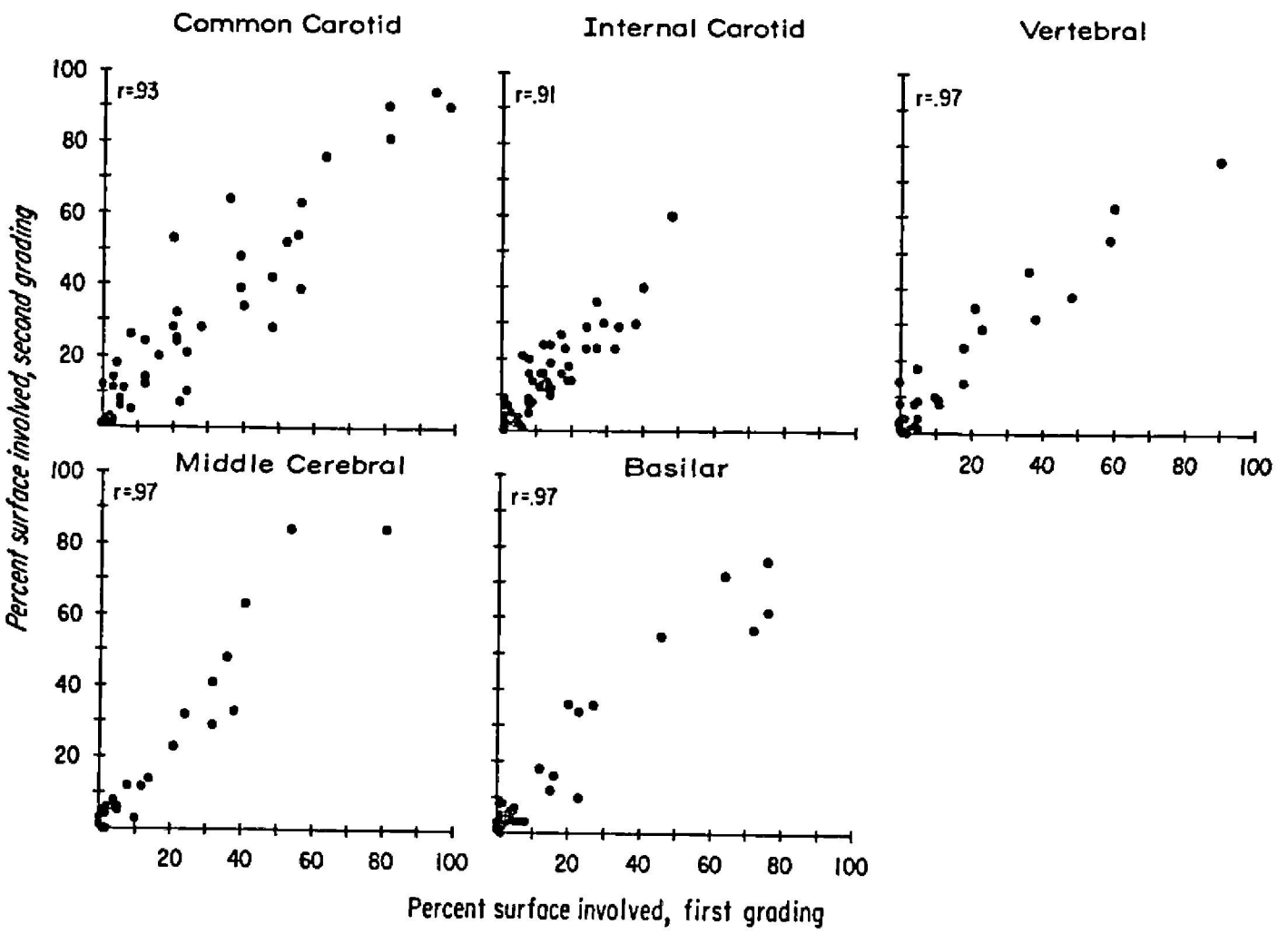

Fig. 3. Correlation between the first and the second grading of raised atherosclerotic lesions in selected cerebral arteries from 69 autopsied cases. Each point may represent more than one case. International Atherosclerosis Project, March–April 1966.

lesions was somewhat higher, while the variability in estimating fatty streak involvement was highest.

Reproducibility of the gradings in the specimens selected for independent duplicate gradings

The correlation coefficient between the first and the second gradings for the 69 sets of arteries that were graded twice (Figs 2 and 3) was highly significant ($p < 0.01$ when $r \geqslant 0.325$). For raised lesions, all correlation coefficients were 0.91 or higher, while for fatty streaks they ranged from 0.68 to 0.82, indicating again better reproducibility in the evaluation of raised lesion involvement. The correlation coefficient has limitations in this evaluation of agreement since many cases with no lesions may contribute to the high correlation coefficients. Therefore, and as a complement to the correlation analyses, the two repeated gradings were compared using the t test for correlated pairs. The results indicate that, with few exceptions, the mean difference between duplicate gradings does not differ significantly from zero, suggesting the absence of a time drift in the grading criteria during the four grading sessions tested.

Long-term reproducibility of gradings

The results in regrading the common carotid arteries 6 months after the ordinary grading sessions are shown in Fig. 4. The correlations between the first and the second grading are statistically highly significant, both for fatty streaks and raised lesions. For raised lesions the correlation coefficient was $r = 0.87$, and for fatty streaks $r = 0.55$ ($p < 0.01$ when $r \geqslant 0.325$). The results were similar for the right common carotid artery and indicate that the general reproducibility of the consensus estimates is not markedly affected by time. The lower correlation coefficients, however, indicate that the grading criteria may drift over prolonged periods of time without continued practice in grading.

Finally, in the ordinary grading as well as in the regrading 6 months later, the extent of lesions in the left common carotid artery correlates significantly with the extent of lesions in the right common carotid artery (Fig. 5). The correlation coefficient for raised lesions is greater if calculated using the results obtained in the regrading, when the evaluation was carried out independently for the arterial segments considered. This result suggests that the grading of one segment does not seem to influence the grading of other segments.

DISCUSSION

Reproducibility of method

The data presented show that, with proper training and practice and under the general conditions of the tests carried out, the visual estimation of surface involvement of atherosclerosis is sufficiently reproducible to be useful in comparing population groups. The net grading error was always under 10 units, and, in most cases, under 5 units of percent surface involvement. Statistically significant differ-

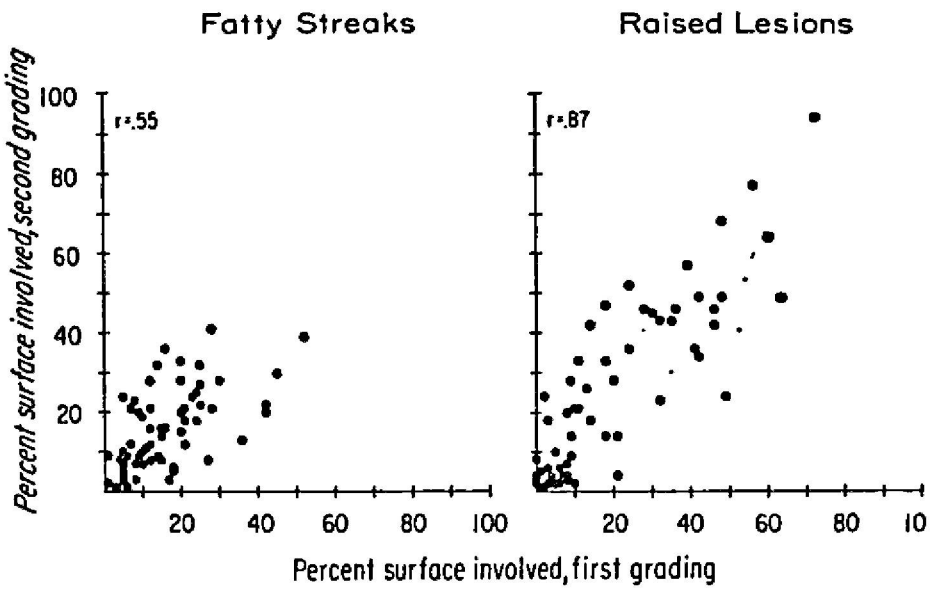

Fig. 4. Correlation between the first and second gradings for the extent of fatty streaks and the extent of raised atherosclerotic lesions in the left common carotid artery of 68 autopsied cases. The second grading took place 6 months after the original grading. Each point may represent more than one case. International Atherosclerosis Project, April–September 1966.

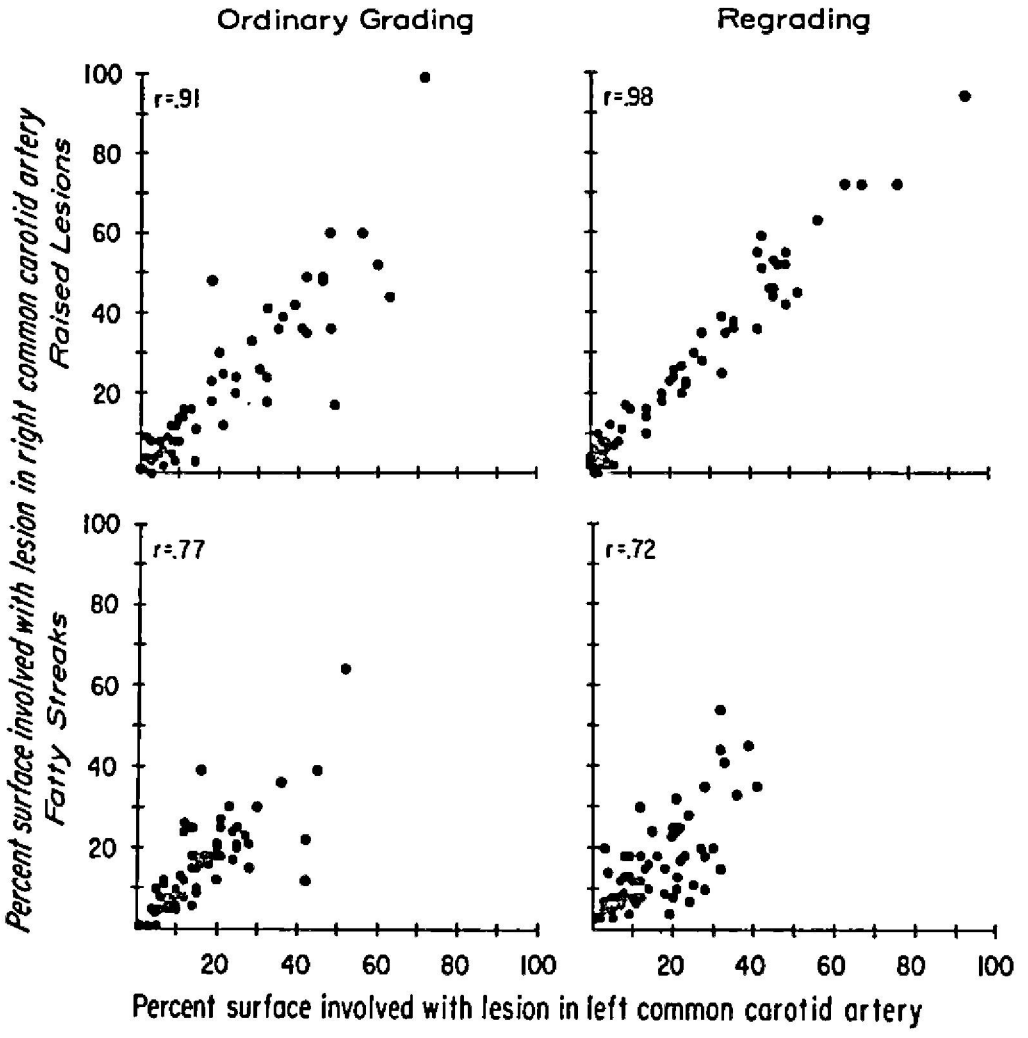

Fig. 5. Correlation of lesion involvement in the left and the right common carotid arteries of 68 autopsied cases. Each point may represent more than one case. International Atherosclerosis Project, April–September 1966.

ences in mean values among the four grading sessions in most instances were limited to the results obtained in the first grading session. Furthermore, the graders were able to reproduce original gradings several months after the last grading session. The contention, therefore, that the method is unreliable[10,11] is not supported when the

reliability tests are made with graders who are familiar with the method and trained in its application. In applying this method of evaluation definitive gradings should be carried out under controlled conditions similar to those outlined by STRONG AND EGGEN[2] and described in detail by GUZMAN *et al.*[4].

Methods of improving reproducibility

Our data support the intuitive expectation that training and experience improve the reproducibility of this method. This phenomenon occurs with almost every measurement procedure and should be particularly prominent with a method of visual estimation involving a great deal of interpretation and judgment. Knowledge that reproducibility tests are performed during routine gradings by the reintroduction of previously graded specimens may also stimulate effort on the part of the graders.

Sources of error and difficulties encountered

The graders felt that the internal carotid artery presented problems in grading because lesions are almost exclusively located in the carotid sinus and in the petrous and cavernous portions, leaving a long segment of the artery free of lesions[12].

Differences in interpretation of atherosclerotic lesions may have contributed to the different means of fatty streaks and raised lesions obtained in the first and second of the quadruplicate gradings. The study to determine whether grading one segment of a pair influences the grading of the other segment when both are graded together shows that this is not a source of error. In grading paired segments (carotid, vertebral, middle cerebral arteries) the grading error appears to differ in the left and right arteries. Differences in size between the left and the right carotid and vertebral arteries may be contributing factors in producing different grading errors. This may not be as great a factor in grading the middle cerebral arteries since these paired segments usually do not differ greatly in size.

Advantages of method

Visual estimation of percent surface involved is simple and rapid and, therefore, can be applied to large numbers of specimens. Other methods such as optical electronic scanning[13], planimetric measurement[10], or point-counting grids[14] may be more exact but they are expensive in effort and time, and consequently prohibitive for large scale applications. In the case of large studies, therefore, the visual estimation for initial survey, followed by other methods for comparison of selected groups of cases, may represent the only practical compromise.

CONCLUSION

This study confirms that reproducible estimates of atherosclerotic lesions can be made by the method of unaided visual estimation of the percentage of intimal surface involved with lesions. Therefore, by utilization of visual estimates, meaningful comparisons of atherosclerosis among collected samples of arteries can be made pro-

viding the investigators carry out definitive gradings under controlled conditions which permit the estimation of grading errors.

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