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RAPID STEREOLOGICAL METHOD FOR ASSESSING THE AREA FRACTION (A_A) OF PROSTATIC CARCINOMA IN TURP SPECIMENS

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ABSTRACT

Incidental carcinoma of the prostate (Stage A) is reported in 10% of TURP (Transurethral resection) specimens. The aims of this study were first, to assess the accuracy of the area fraction (A_A) of prostatic cancer defined as the percentage of chips against stereology (gold standard technique) and second to evaluate a newly described rapid stereology technique based on drawing tube and image analysis system. Fifty formalin fixed fully embedded TURP specimens from patients with prostatic carcinoma were used. Patients were divided into three groups in relation to the A_A of carcinoma. Group 1 (20) the A_A was 0 - 5%, group 2 (18) the A_A was 5.1-35% and group 3 (12) the A_A was 35.1-100%. H&E stained slides were used to assess the A_A by three different techniques. Regression line analysis and the Altman and Bland correlation were used for the comparison between the methods. The overall accuracy of A_A (0-100%) of both routine and new methods was good in comparison with stereology. In group 1 the percentage of chips method was less sensitive than stereology (p=ns). The new method compared well with stereology ($P<0.0001$) technique in all three groups of patients. The Altman and Bland correlation showed overestimation of the percentage of chips which was more marked at the level of upper value for group 3 with overlap between groups 1 and 2. The new method showed minor underestimation for the lower values for group 1 and small overestimation for the upper value in group 3. In general the new method compared well with stereology technique in patients with carcinoma of prostate. It is concluded from this study that routine counting of chips with tumour was less sensitive than stereology for the accurate assessment of A_A. The new drawing tube method compared well with stereology, and it was fast and accurate for assessing A_A of tumour in TURP specimens in patients with incidental (stage A) and advanced (stage B) carcinoma. The Altman and Bland correlation was superior to the coefficient of variation for assessing the differences and agreement between two methods.

Key words: accuracy, limits of agreement, prostatic cancer, statistics, stereology.

INTRODUCTION

Quantitated tumour volume is reported to be an important factor in predicting the biological behaviour of prostatic carcinoma (McNeal et al 1986 and 1988, Epstein et al 1988, Partin et al 1989), pelvic lymph node involvement and distant metastases (Donohue et al 1977, McNeal et al 1990). Incidental carcinoma of the prostate (Stage A) is reported in

approximately 10 per cent of TURP specimens (Lowe and Listrom 1988, Cantrell et al 1981, Epstein et al 1988, McNeal et al 1988). Stage A carcinoma was divided into two biological subclasses (Jewitt et al 1975): Stage A1 when 5 per cent or less of the specimen was involved by carcinoma (low grade, focal) and Stage A2 when more than 5 per cent of the specimen contained carcinoma or the tumour showed histologically Gleason's combined score of 8 to 10 (high grade, diffuse) (Humphrey and Walther 1993). Reappraisal of the classification of patients with stage A has been reported (Sheldon et al 1980). The differences in the pathological characteristics and prognosis of patients with stage A1, A2 and B disease have been reported (Golimbu et al 1978).

Several methods have been used to quantify the amount of carcinoma in TURP specimens. These include focus counting (Parfitt et al 1983), estimation of cancer percentage (Donohue et al 1977), the percentage of chips containing tumour (Humphrey and Vollmer 1988) computer assisted morphometry technique (Foucar et al 1990) and dissecting microscope at very low magnification (X8) (Delahunt and Heng Teoh 1990).

In this study the author describes an objective computerised stereology method to assess the area fraction (A_A) of carcinoma in TURP specimens. The percentage of chips containing tumour was assessed against a stereology technique.

In addition, the author used the Bland and Altman plot to assess correlation and agreement between these methods.

MATERIALS AND METHODS

Fifty formalin-fixed totally embedded TURP specimens were used to assess the area fraction (A_A) of carcinoma. The total weight of specimens was 654.4gms, mean 13.09gms, minimum 2gms and maximum 60gms. The A_A was assessed in three different ways using haematoxylin and eosin (H&E) stained slides (5μm thickness). The simple method of estimating the percentage of chips involved by carcinoma was obtained in each TURP specimen and this was correlated with the stereology method (gold standard technique).

A Nikon Labphot 2 microscope with drawing tube was used with an image analysis system (Prodit 5.2 The Netherlands). The image analysis system has a selection of computer-stored grids to be superimposed over a video image of the section. To assess the area fraction of carcinoma by the drawing tube method, the outlines of carcinoma areas were marked by fine ink marker on histological slides. The slide was then placed on light viewing box at a known distance from the drawing tube to give final magnification of nine times the original on a computer screen. A grid of 42 points was used at a distance of 415.60 micrometers between points for this analysis.

To assess the A_A of carcinoma by a stereology technique a X 1 Nikon lens was used. This gives a final magnification of 65 times the original on the video screen. Magnification was kept constant throughout the assessment. A grid of 24 points at distance of 461.36μm was used for this analysis.

Estimation of the area fraction by stereology

In two dimensional structures (a tissue section is not strictly two dimensional but for the purposes of this study it has been used as such) it is possible to focus the attention on phase 1 (cancer) and consider the remaining structure as phase 2 (benign glands and stroma). The area fraction A_A can be estimated by placing a grid probe at random spaces and counting the number of points that hit each area. In this study the A_A is estimated from this basic stereological relation:

$$A_A = N_C / (N_C + N_O) \quad (1)$$

where N_C is the mean number of points that hit the cancer per unit area and N_O the mean number of points per unit area that hit the remaining structure. The estimation of the area fraction A_A in two dimensional structures gives a good assessment of the volume fraction V_V according to the usual relation:

$$A_A = V_V \quad (2)$$

Statistical analysis

Data analysis was performed using a computer programme. The relationship between the percentage of chips involved and the area fraction of carcinoma stereology was determined by linear regression analysis. The intraobserver variation for assessing the A_A of carcinoma by drawing tube method was determined by calculating A_A in five slides containing carcinoma from two patients. This gave a co-efficient of variation of 4.6% (reproducibility 95.4%). After obtaining the correlation between these methods for all patients, they were then divided into three groups according to the A_A of tumour obtained by stereology (gold standard technique). Group 1 includes all patients (20) with A_A of 5% or less. This corresponds clinically to patients with incidental carcinoma (stage A1 disease). In Group 2 (18 patients) the A_A was above 5% but less than 35% (patients with clinical disease and less advanced from group 3). In the third group (12) the A_A was above 35%, corresponding clinically to patients with advanced cancer. Regression line analysis was performed for each group between the percentage of chips method v stereology and the new drawing tube method v stereology.

Comparison between these methods was also assessed by Bland and Altman plot (Altman and Bland 1983, Bland and Altman 1986) for each group of patients separately and for all patients (50 patients). Statistically significant level was set at $P<0.05$.

RESULTS

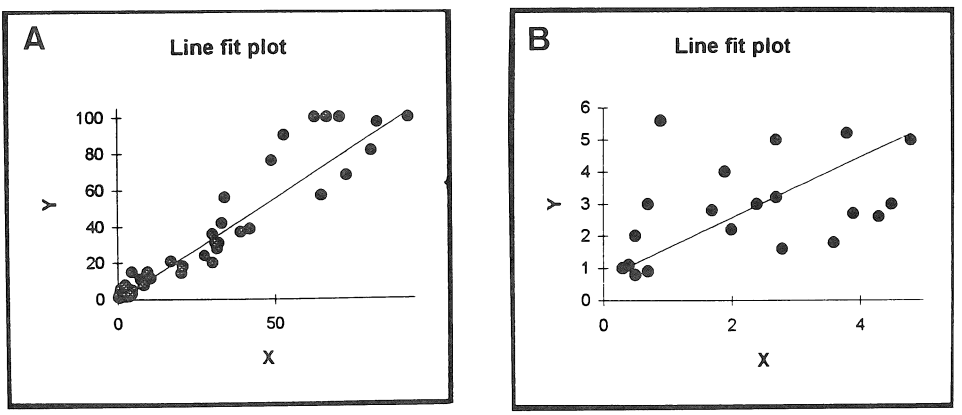

Fig. 1. a) Regression line analysis for comparison between standard stereology (x) and percentage of chips (y) for 50 patients with carcinoma; R = 0.96. b) The correlation between the stereology (x) and the percentage of chips (y) for patients with stage A1 disease; R = 0.43.

The relationship between the percentage of chips and stereology is shown in Figure 1a (all patients) and figure 1b (patients with group 1).

The correlation between the two methods was significant ($p<0.0001$) for all patients, but not significant (p=ns) for group 1 patients (Table 1). Figure 2a shows the correlation between the drawing tube method and stereology. Figure 2b shows the correlation between these two methods in group 1 patients.

Table 1. The co-efficient of correlation of regression line analysis with p value between methods. Value in brackets represents the 95% of confidence limits.

A_A range (groups)	No of patients	Stereology V Percentage of chips	Stereology V Drawing tube method
0 - 5	20	R = 0.43 (0.02-0.73) P = 0.06	R = 0.91 (0.78-0.96) P <0.0001
5.1 - 35	18	R = 0.83 (0.56-0.94) P <0.0001	R = 0.96 (0.89-0.99) P <0.0001
35.1 - 100	12	R = 0.67 (0.15-0.90) P = 0.02	R = 0.98 (0.92-0.99) P <0.0001
Total (1 - 100)	50	R = 0.96 (0.92-0.97) P <0.0001	R = 0.99 (0.99-1.00) P <0.0001

The correlation coefficients were 0.98 and 0.91, respectively, and were statistically highly significant ($P<0.0001$). The comparison between the percentage of chips and stereology using the Altman and Bland method of comparison are shown in Figures 3, 4 and 5 and in Table 2.

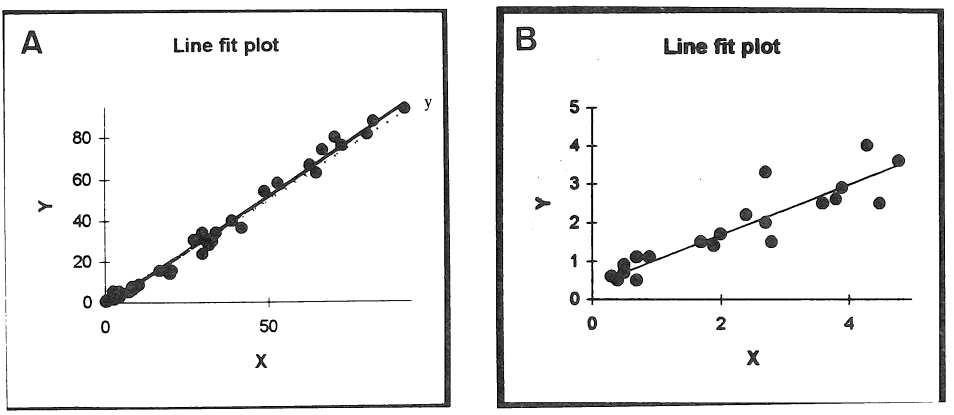

Fig. 2. a) Regression line analysis for comparison between stereology method (x) and drawing tube method (y) in all patients (50); R = 0.98. b) The correlation between stereology (x) and drawing tube method (y) in patients with stage A1 disease. R = 0.91.

Table 2. The results of Altman and Bland correlation between methods. Values in brackets represent 95% confidence limits.

A_A range (groups)	No of patients		Stereology V percentage of chips	Stereology V drawing tube method
0 - 5	20	lower limit	- 3.72	- 0.98
		bias	- 0.57	0.4
			(-1.32 to 0.18)	(0.07 to 0.73)
		upper limits	2.58	1.78
5.1 - 35	18	lower limit	-16.13	-3.87
		bias	-1.49	1.85
			(-5.62 to 2.65)	(0.24 to 3.47)
		upper limits	13.15	7.58
35.1 - 100	12	lower limit	-48.52	-10.55
		bias	-13.87	-2.73
			(-25.09 to -2.63)	(-5.26 to -0.19)
		upper limits	20.78	5.1

For all patients in this study (Figures 3a, 3b) there was a tendency for the spread of difference to increase as the average increased. The limits of agreement will be overestimated for the higher value (bias 4.39). For patients with stage A1 (group 1) (Figures 4a, 4b) there was also a tendency for the spread of difference to increase as the average increased.

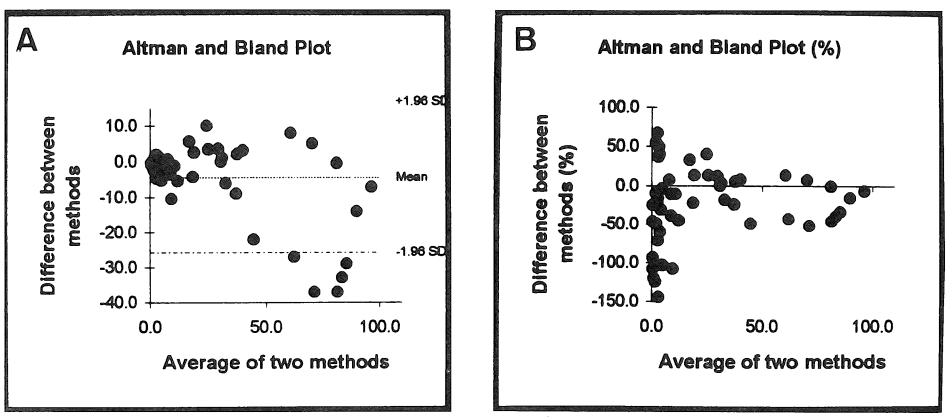

Fig. 3. a) Correlation between stereology (1) and percentage of chips (2) for all (50). x = (method 1 + method 2)/2; y = difference method 1 - method 2. b) Correlation between stereology and percentage of chips x = average {[log 10 (stereology) + log 10 (percentage)]/2} y = Difference log 10 (stereology) - Log 10 (percentage).

The limits of agreement will be overestimated for the higher value (bias 0.57). For patients with group 2 (Table 2), the upper value will be over estimated by 5.62 and the lower value by 2.65. In patients with group 3 the limits of agreement will be overestimated for the value with bias of 13.87. Comparison between drawing tube method and stereology using Altman and Bland plots for the three groups of patients is shown in Table 2. In group 1

(Figures 5a, 5b) the main difference was towards the lower value and was overestimated by 0.4. In group 2 (Table 2) the spread of difference increased only at upper limits with underestimation for upper value (bias 1.85). In group 3 patients (Table 2) the difference was minimal and there was overestimation for upper value (bias 2.73).

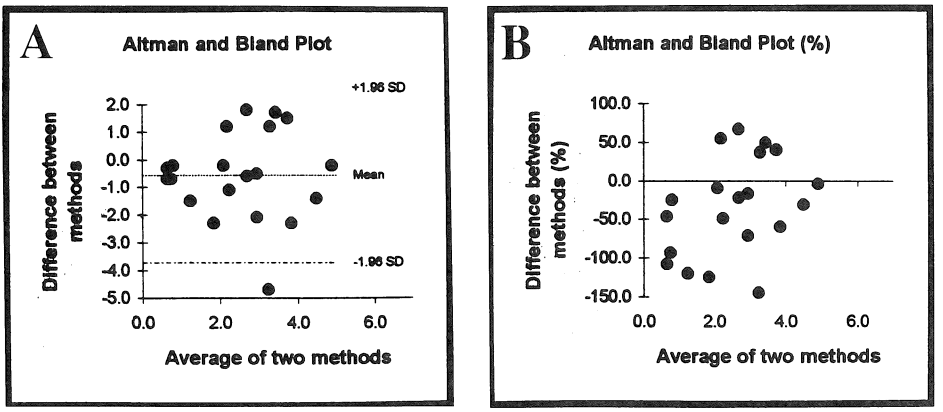

Fig. 4. a) Correlation between stereology (1) and percentage of chips (2) for patients with stage A1 disease (20 patients); x = (method 1 + method 2)/2; y = difference method 1 - method 2.
b) The log difference plot for patients with stage A1 disease x = average {[log 10 (stereology) + log 10 (percentage)]/2}; y = Difference log 10 (stereology) - Log 10 (percentage).

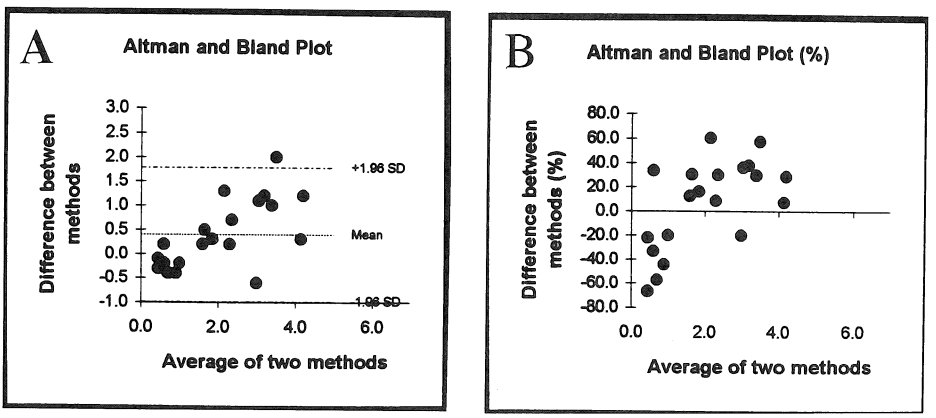

Fig. 5. a) The difference between stereology (1) and drawing tube method (2) for patients with stage A1 disease (20); x = (method 1 + method 2)/2; y = Difference method 1 - method 2.
b) The log difference between stereology and drawing tube method;
x = average {[log 10 (stereology) + log 10 (new method)]/2};
y = Difference log 10 (stereology) - Log 10 (new method)

DISCUSSION

Quantitative assessment of the volume of prostatic cancer in TURP (Epstein et al 1988, Foucar et al 1990) and in prostatectomy specimens (Humphrey et al 1990, McNeal et al 1990, Yatani et al 1986) is one of the most important methods for predicting the biological behaviour of tumours (McNeal et al 1986, Epstein et al 1988, Partin et al 1989, Mohler et al 1992). Yatani et al (1986) estimated the tumour volume in total prostates obtained at autopsy and found that the percentage area of prostatic carcinoma correlated well with the grade of tumour. Nuclear cytology, cell borders, growth pattern and perineural invasion added little to the prognosis. These results are supported by Humphrey and Vollmer (1988) who reported that the ratio of chips involved by carcinoma to the total ratio of chips in patients with carcinoma who underwent TURP was closely associated with tumour stage. Currently assessing the volume percentage of prostatic chips containing tumour is one of the most common methods used routinely (Delahunt and Heng Teoh 1990, Svanholm et al 1990). The interobserver and intraobserver reproducibility of this method is very high (Svanholm et al 1990b) the reproducibility of carcinoma grade (Svanholm et al 1985 and 1990a), type (Svanholm et al 1989b) and histopathological diagnosis (Svanholm et al 1989a) were less than that reported for assessing the volume percentage of carcinoma. However, there are several problems concerning the assessment of volume percentage of tumour using this method. First, there is marked variation in the size of chips. Second, the irregularity in the shape and thickness of chips lead to further increase in variability of the surface area on the histological slide. Third, there is also marked variation (up to one hundredfold) in the area of carcinoma in these chips. Therefore, the assessment of carcinoma by counting the percentage of chips is a crude method as the measuring unit is markedly variable. On the other hand, assessing the cancer volume by stereology is reliable and accurate (gold standard) but time consuming and expensive (Yatani et al 1986). In addition, stereology requires an experienced operator and an image analysis system that is not available in all histopathology laboratories.

In this study slides were assessed by three different techniques, stereology, simple modified stereology and the percentage of chips. The study has shown good correlation between the percentage of chips and stereology ($P<0.0001$). However, when the data was split into three groups, the correlation co-efficient was $R = 0.43$ for group 1, $R = 0.83$ for group 2 and $R = 0.67$ for group 3. These were considerably lower than the R value for all patients [undifferentiated by group] ($R = 0.96$). This phenomenon has been observed previously by Altman and Bland (1983) who suggested a new method for assessing the correlation between two methods. Unlike the percentage of chips, the correlation co-efficient between the new method and stereology was high for all patients as well as for each group separately.

In this study, the Altman and Bland method of comparison was used. In patients with stage A1 disease (group 1), the Altman and Bland correlation showed a wide scatter of points around the means of the percentage of chips and stereology with bias by 0.57 to the higher value of percentage of chips. This indicates that the assessment of area fraction of tumour by the percentage of chips may lead to overestimation of the volume of cancer. Marked overestimation was observed between these two methods for group 3 patients. In general the Altman and Bland correlation showed that the assessment of area fraction of carcinoma by the percentage of chips method leads to overestimation in all groups of patients (1,2 and 3).

When comparing the new method and stereology, the Altman and Bland correlation showed differences towards the lower value (underestimation) for group 1. In group 2 the Altman and Bland correlation showed underestimation towards the upper value. The lower and upper limits for patients with group 2 were much smaller than those obtained by the percentage of chips. For group 3 the new method showed small overestimation with bias of 2.73 (in comparison to 13.87 for the percentage of chips method).

The newly described method combines both the accuracy of stereology and simplicity of grid counting method (Humphrey et al 1990) that has been used for assessing the volume of carcinoma and prostatic intraepithelial neoplasia. In this method, the tissue area is transmitted to computer screen and the image enlarged nine times the actual area. This increases the sensitivity of assessing the area fraction in comparison with the grid counting technique. Other advantages over the grid counting method are that the operator can increase the size of the image measured on the computer screen and apply any type of preferred grid. In the hands of the author, each slide took less than 2 minutes to be assessed. The method can also be used for assessing the area fraction of carcinoma and prostatic intraepithelial neoplasia in total prostatectomy specimens. The limitation of this method is the high cost of equipment not available in most histopathology laboratories.

Although the number of cases analysed in this study was too small to assess prognostic value of area fraction of tumour in relation to the clinical groups of stage A1 (group 1), and B (groups 2 and 3) disease, there is, however, indication that overestimation of the area fraction assessed by the percentage of chips method would lead to overlap between groups 1 and 2 patients. Similar results have been reported by Cantrell et al (1981). These authors found that morphometrically determining percentage of tumours was the most accurate method of determining the extent of disease. Determination of the area fraction of carcinoma by the new method confirms these findings. Assessing the A_A of tumour by the new method in patients with advanced and symptomatic disease (group 3) was more superior to the percentage of chips method. It is well known that patients with high volume of tumour have poor prognosis (McNeal et al 1986 and 1988, Epstein et al 1988, Partin et al 1989).

It is concluded from this study that counting the number of chips involved by cancer is a crude method for assessing the actual percentage of cancer area (area fraction). Stereology is an accurate but time consuming technique. The new modified stereology (drawing tube) method was fast, reliable and correlated well with the stereology method by both the regression line analysis and the Altman and Bland correlation. For assessing the A_A of tumour in TURP specimens, the percentage of chips may lead to small overestimation in patients with stage A1, overlap between group 1 and 2 patients and greater overestimation for patients with advanced disease (group 3) that may be clinically significant for assessing the prognosis in patients with carcinoma of the prostate. The new method is recommended for assessing the area fraction of carcinoma in TURP and prostatectomy specimens. The method of comparison by regression line and Altman and Bland correlation showed that the new stereological method and stereology were reliable for estimating the area fraction of carcinoma in TURP specimens.

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