Stereological Evaluation of Tumor Regression Rates in Lung Cancer Using CT Via the Cavalieri Method

Akciğer Kanserinde BT ve Cavalieri Yöntemi ile Tümör Regresyon Oranının Stereolojik Olarak Değerlendirilmesi

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Abstract

Objective: The aims of this study were to analyze the relevant methods of computed tomography (CT) and stereology with respect to the estimation of tumor volume and to determine whether the response rates measured by the stereological method correlate with those of conventional morphometric techniques in lung cancer.

Materials and Methods: The study group was composed of 32 patients, including 25 males and 7 females. All the subjects included were non-small celled lung cancer patients (NSCLC), and they were all treated with either chemotherapy (n=12) or chemotherapy plus radiotherapy (n=20) for locally advanced disease (Stage III A and Stage III B). All patients underwent contrast enhanced CT of the thorax before and after treatment. Tumor diameters were measured according to stereological methods, the World Health Organization (WHO) criteria and the Response Evaluation Criteria in Solid Tumors (RECIST).

Results: With all three methods (stereological method, RECIST and WHO), an improvement was observed in the mean tumor size. The response rates were 11.8 ± 117.5% (stereological method), 27.4 ± 38.8% (RECIST), and 38.7 ± 68.1% (WHO). Although the response rates in RECIST and WHO criteria were statistically significant (P=0.02 and P=0.045 for RECIST and WHO, respectively), the response rates with stereological measurements were not statistically significant (P=0.21), showing that response rates obtained by the Cavalieri method differ from those obtained through WHO and RECIST. The comparison between response rates obtained with each method shows that the stereological response rate was not correlated with the response rate in either RECIST or WHO, (r=-0.15, P=0.59 and r=-0.27, P=0.33 for RECIST and WHO, respectively), while there was good correlation between the WHO and RECIST response rates (r=0.87 and P<0.001).

Conclusion: The Cavalieri principle is more suitable for the evaluation of tumor volumes in response to treatment in the management of advanced malignancies, in particular in patients with tumors of irregular shape or when the determination of treatment response is not clear.

Keywords: Computerized tomography, Lung cancer, Stereology, Volume measurement

Özet

Amaç: *Akciğer kanserinde tümör volümünün belirlenmesinde bilgisayarlı tomografi (BT) ve sterelojinin kullanıldığı yeni bir yöntemi tanımlamak, uygulamak; stereoloji ile ölçülen tedaviye yanıt oranlarını konvansiyonel morfometrik yöntemlerle karşılaştırmak.*

Gereç ve Yöntem: *Çalışmaya 25'i erkek, 7'si kadın toplam 32 hasta alındı. Hastaların tümü kemoterapi (n=12) veya kemo-radyoterapi alan lokal ileri hastalığı (Evre III A veya Evre IIIB) olan küçük hücreli dışı akciğer kanseri (KHDAK) idi. Hastalara tedavi öncesi ve sonrası kontrastlı toraks BT çekildi. Tümör çapları stereolojik yöntemle, dünya sağlık örgütü kriterlerine (WHO) ve solid tümörlerde yanıt değerlendirme kriterlerine göre (RECIST) ölçüldü.*

Bulgular: *Her üç yöntemle de (stereolojik, RECIST ve WHO) ortalama tümör boyutunda azalma gözlendi. Yanıt oranları stereolojik yöntemle % 11,8 ± 117,5, RECIST'e göre % 27,4 ± 38,8% ve WHO'ne göre % 38,7 ± 68,1% idi. Yanıt oranları RECIST and WHO kriterlerine göre istatistiksel olarak anlamlı (sırasıyla P=0,02 ve P=0,045) iken stereolojik yöntemle ölçüldüğünde anlamlı değildi (P=0,21). Bu da Cavalieri ile yapılan ölçümün WHO ve RECIST ile yapılan ölçümden farklı olduğunu göstermektedir. Her bir yöntemin yanıt oranları birbirleriyle karşılaştırıldığında stereolojik yanıt değerlendirmesi ne RECIST ne de WHO ölçümüyle korele değildi (sırasıyla r=-0,15, P=0,59 ve r=-0,27, P=0,33). WHO ve RECIST ölçümleri kendi aralarında anlamlı korelasyon gösteriyordu (r=0,87 ve P<0,001).*

Sonuç: *İleri evre malignitelerin tedavisinde yanıt değerlendirmede, özellikle tümör düzensiz konturlara sahipse veya tedavi yanıtı değerlendirmede güçlük varsa Cavalieri yöntemi daha uygun bir yöntem gibi gözükmektedir.*

Anahtar Kelimeler: *Akciğer kanseri, Bilgisayarlı tomografi, Stereloji, Volüm ölçümü*

Introduction

Lung cancer is the leading cause of cancer-related death in both men and women, despite extensive knowledge of the risk factors involved in the development of this disease. More Americans die from lung cancer than from colorectal, breast and prostate cancers combined [1]. The overall 5-year survival rate for lung cancer is only 10% in Europe and 15% in the United States. Progress in the development of curative treatments for this disease during the last 20 years has been modest [2].

Despite advances in the early detection of lung cancer [3-5], less than 20% of individuals suffering from this disease are diagnosed in stages in which curative surgery is an option [6]. While surgical resection offers the best chance of a cure for lung cancer, particularly for non-small cell lung cancer, only a small proportion of patients are eligible for surgery, and the majority of them must rely on nonsurgical and adjuvant therapies [7]. Although overall survival should be used as the primary end-point for the analysis of these patients, a broad spectrum of end-points other than survival often need to be taken into consideration, such as symptom relief, response to therapy, impact on quality of life and disease recurrence [8]. The evaluation of tumor response to treatment is critical in the management of advanced malignancies, and the response-survival relationship might vary according to the method of tumor response assessment [9].

The measurement of tissue or organ volumes can be performed without bias by applying Cavalieri's principle to histological slices of a set of consecutive serial sections, visualized using computerized tomography (CT) or another suitable imaging technique [10-13]. Most of the existing studies evaluating tumor morphology use conventional morphometric techniques, and only a few stereological studies have been performed to estimate the volume of different tumor regions on CT images using the Cavalieri method [12-15]. There are presently no studies using the Cavalieri method to estimate tumor volume in lung cancer. The purposes of the present study were to apply and adapt relevant methods of CT and stereology for the estimation of tumor volume in lung cancer and to determine whether the response rates measured by the stereological method are correlated with those of conventional morphometric techniques.

Materials and Methods

Study Design:

This study consisted of a retrospective evaluation of the CT of the thorax of lung cancer patients.

Patient Population:

Patients with lung cancer who received followed-up care at the departments of Chest Disease and Medical Oncology between January 2006 and March 2008 were considered for this study (n=60) through evaluation of their medical charts. Patients who were considered eligible (n=32) were inoperable, non-small celled lung cancer (NSCLC) patients, with locally advanced tumors and no distant metastasis. All patients in the study underwent contrast-enhanced CT of the thorax before and after being treated with chemotherapy or chemotherapy plus radiotherapy.

Table 1. Estimation of total volume, CE of an axial lung sec

Section no;	Pi*	Pi x Pi	Pi x Pi+1*	Pi x Pi+2*
1	7	49	70	84
2	10	100	120	150
3	12	144	180	156
4	15	225	195	195
5	13	169	169	208
6	13	169	208	234
7	16	256	288	256
8	18	324	288	288
9	16	256	256	176
10	16	256	176	256
11	11	121	176	165
12	16	256	240	224
13	15	225	210	180
14	14	196	168	224
15	12	144	192	156
16	16	256	208	208
17	13	169	169	169
18	13	169	169	221
19	13	169	221	143
20	17	289	187	0
21	11	121	0	0
	287	A	B	C
Total		4063	3890	3693

* Pi, Pi, point count; Pi+1, point count of following section; Pi+2, point count of section after following section

CT Scan Protocol:

Multidetector computed tomography was performed with a 16-detector-row CT scanner (Aquillon; Toshiba Medical Systems, Tokyo, Japan). Scans were obtained with collimation of 10 mm and pitch ratio of 1:1.5 in all patients. Iodinated contrast medium (90 mL; Omnipaque; Amersham Health, Cork, Ireland) was injected intravenously at 4.5 mL/s.

Determination of Tumor Volumes by Stereology:

Consecutive axial slices of approximately 10 mm were obtained using a random starting position and encompassing the entire tumor area for the scanning images. Approximately 16 slices of CT images were obtained per patient. A minimum of 6-8 consecutive slices imaging the tumor area were used to estimate tumor volumes. Volumetric measurements were acquired using Stereo Investigator software (version 6.0, Microbrightfield, Colchester, VT). The calculation of the lung tumor volume was done using the Cavalieri principle (Fig 1) [11, 16].

The Cavalieri Principle:

The determination of the volume of any structure with an arbitrary shape and size can be obtained using the Cavalieri principle [16]. This method is based on the principle that an unbiased estimate of the volume of an interesting object must be obtained by sectioning the object in a series of parallel planes separated by a fixed distance (t) [10, 17, 18]. In our study, we used modified point counting grids for the area estimation of section profiles (a/p=1 μm2 intervals). The point density of the counting grid was designed to obtain an appropriate coefficient of error (CE) for the serial CT transects of our study. Coefficient of error and coefficient of variation (CV) were estimated based on the formula by Gundersen and Jensen [16].

The test grid with a systematic array of points is placed on a PC screen and superimposed on images of the sections of the areas of interest (total tumor areas) for each individual under study. The volumes of the tumor for each section were estimated by the following formula:

$$\hat{V} = t \times \frac{a}{p} \times \sum_{i=1}^{m} P_i$$

V is the volume of the object of interest (tumor) in one section plane, t is the section thickness, a/p is the interpoint area, and P is the number of points hitting the tumor in that section. After the same formula is applied for each of the sections, the total volume to be estimated is obtained with the following formula:

$$\hat{V}_{\underleftarrow{total}} = V_1 + V_2 + \ldots V_n$$

Error predictions for the Cavalieri estimation

The point density of the counting grid was designed to obtain an appropriate coefficient of error (CE) for the images of the serial sections. CE and coefficient of variation (CV) were estimated according to Gundersen and Jensen's formula [16]:

$$Noise = 0.0724 \times (b/\sqrt{a}) \times \sqrt{n \times \sum P}$$

Noise is the value of information on the complexity of the examined cut surface area of the specimen; $b/\sqrt{a}$ is equivalent to the mean boundary length of the profiles divided by the square root of their mean area, n is the examined section number, and P the number of points hitting the whole section:

$$Var_{SRS}(\sum_{i=1}^{n} a) = (3 \cdot (A - Noise) - 4 \cdot B + C)/12$$

$$Var_{SRS}(\sum_{i=1}^{n} a)$$

Indicates variance of total area in the systematic random sampling (SRS). These data provide information on the number of sections required to obtain an appropriate variation for the samples under study. A, B, and C are the total numerical values for the data in the related column of Table 1.

$$TotalVar = Noise + Var_{SRS}$$

$$CE(\sum P) = \frac{\sqrt{Total\ Var}}{\sum P}$$

CE is the last calculated value. The generally accepted highest limit of CE is 5% [16]. An example of the estimation of total volume (CE) of a tumor sectioned axially in our study is shown in Table 1.

$$\text{Noise} = 0.0724 \times (b/\sqrt{a}) \times \sqrt{n \times \sum P} = 0.0724 \times 5 \times \sqrt{21 \times 287} = 28.103$$

$$\text{Var}_{SRS}\left(\sum_{i=1}^{n} a\right) = (3 \times (A - \text{Noise}) - 4 \times B + C)/12 =$$

$$(3x(4063 - 28.103) - 4x3890 + 3693)/12 = 19.80758$$

$$\text{TotalVar} = \text{Noise} + \text{Var}_{SRS} = 28.103 + 19.80758 = 47.91058$$

$$CE(\sum P) = \frac{\sqrt{\text{Total Var}}}{\sum P} = \frac{\sqrt{47.91}}{287} = 0.024$$

$$\text{Volume} = t \times a/p \times (\sum P) = 15000\mu m\ x1\mu m^2 \times 287 = 4.305x10^6 \mu m^3$$

$$\text{Total Volume} = 4.305x10^6 \mu m^3 \times \text{Section}_{\text{Number}} x \text{Magnification}_{\text{Image}}$$

$$= 4.305x10^6 \mu m^3\ x21x25/3 = 753375000 \mu m^3 = 0.753 cm^3$$

Other Measurements:

Tumor diameters were also measured according to the WHO (World Health Organization) [19, 20] and RECIST (Response Evaluation Criteria in Solid Tumors) criteria as previously described [21]. The measurements were made with a radiologist and a clinician working together.

Statistical Analysis:

Statistical analyses were conducted using SPSS 11.0 for Windows. The comparative analysis of the results before and after treatment for each method was done using the Wilcoxon test. The Pearson correlation was used to compare the response rates for the three different methods. The data were expressed as mean ± SD unless otherwise indicated. P<0.05 was considered

Table 2. The results of measurements before and after treatment and response rates via three different methods

	Measurement method		
	RECIST (cm)	WHO (cm^2)	Cavalieri (cm^3)
Before treatment	8.7 ± 4.5	42.3 ± 30.8	1.5 ± 3.5
After treatment	5.3 ± 2.6	23.1 ± 37.6	1.1 ± 3.3
Response rate as %	24.7 ± 38.8	38.7 ± 68.1	11.8 ± 117.5
P value	0.02	0.045	0.21

statistically significant.

Results

5% (stereological method), 27.4 ± 38.8% (RECIST), and 38.7 ± 68.1% (WHO) (Table 2). The response rates obtained with the RECIST and WHO criteria were statistically significant (P=0.02 and P=0.045 for RECIST and WHO, respectively); however, the response rates obtained using stereological measurements were not statistically The study group was composed of 32 patients, including 25 males and 7 females with an average age of 56± 36 years (range: 38-74 years). All the patients were NSCLC and treated with either chemotherapy (n=12) or chemotherapy plus radiotherapy (n=20) for locally advanced disease (Stage III A and Stage III B). Four patients had two lesions (T4), and the remaining ones (n=23) had a single lesion on their thorax CT scan.

An reduction in the mean tumor size was obtained with all three methods applied and consisted of 1.5 ± 3.5 cm3 vs. 1.1 ± 3.3 cm3 (stereological method); 8.7 ± 4.5 cm vs. 5.3 ± 2.6 cm (RECIST); and 42.3 cm2 ± 30.8 vs. 23.1 ± 37.6 cm2 (WHO). The response rates were 11.8 ± 117significant (P=0.21), showing that there is a difference between the response rates obtained by the Cavalieri method and those obtained using WHO and RECIST criteria. The comparison between the response rates obtained with each method revealed that those obtained using the stereological method did not correlate with the response rates obtained with either RECIST or WHO, (r=-0.15, P=0.59 and r=-0.27, P=0.33 for RECIST and WHO, respectively), while the response rates obtained with WHO and RECIST showed higher correlation between them (r=0.87 and P<0.001).

In addition, while most of the patients (n=20) showed different response rates according to the evaluation method used, in a few of them (n=12), the results were similar with all three methods.

Discussion

Lung cancer is the leading cause of cancer-related death in both women and men throughout the world. Tumor volume is an important prognostic factor for the progression-free survival interval and for the risk of malignant transformation [22-25]. The measured rate of tumor regression can have important implications in the improvement of local tumor control, optimum timing of therapy, and in minimizing the risk of adverse events of chemotherapy and radiation damage on surrounding tissues.

Evaluation of tumor response to treatment is a critical issue in the management of advanced malignancies. Although most studies have relied on conventional morphometric techniques to evaluate tumor morphology, some studies using stereology to estimate the volume of different tumor regions on CT images using the Cavalieri method have been reported [12-15].

The goal of the present study was to determine the tumor regression rate retrospectively using three different methods: the

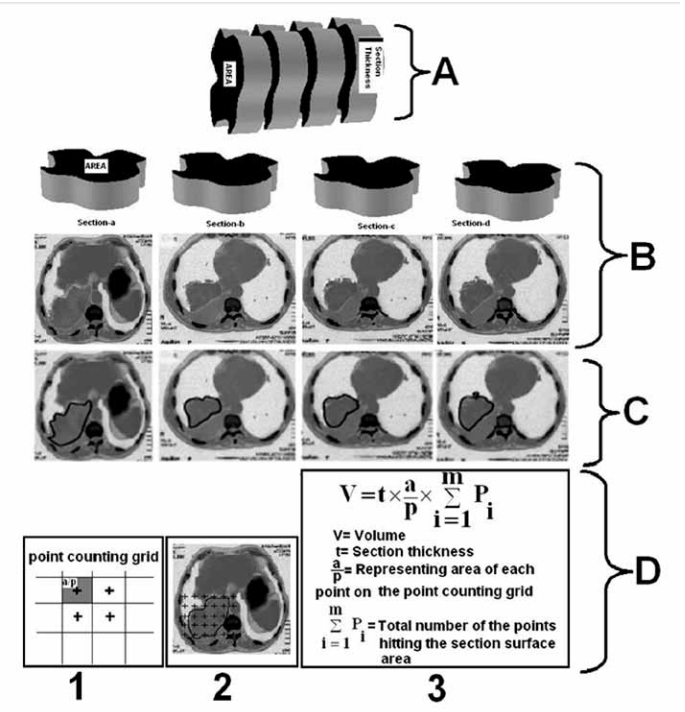

Fig. 1 — A: Parallel slices, obtained from a solid structure.
B: Axial slices of thorax CT of patients with NSCLC.
C: Traced lines of tumors in Stereological Software 6.0.
D: Stereological process of this study. First Inset (1), A point counting grid, as used in the study. Second Inset (2), A point counting grid, superimposed on the same images shown in C. Third Inset (3), Formula of the Cavalieri method used in volume estimation.

Cavalieri volume estimation principle [17], WHO criteria [19, 20] and RECIST [21] criteria, on the same CT images of the thorax, obtained before and after treatment of non-small cell lung cancer (NSCLC) patients.

The Cavalieri estimation, WHO, and RECIST methods are commonly used to evaluate the structures of interest in 3-dimensional, 2-dimensional and/or 1-dimensional space, which provides the researcher with volumetric data, surface area, and length of the structure under study. The mathematical data derived from the volumetric measurement in three dimensions cannot be compared with data obtained through measurement of the surface area based on two dimensions or with the length measured in one dimension.

The Cavalieri method is one of the most popular stereological approaches for estimating a volume of interest, and it consists of the generation of mathematically unbiased estimates of the geometric properties of three-dimensional structures from randomly generated two-dimensional slices of the object [26]. This method has the following advantages for the researcher: i) The structure under study requires no preconditioning since the Cavalieri method is a design-based approach but not a model based method; ii) The actual features of the structure, such as section thickness, are taken into consideration; and iii) The sampling or estimating procedure can be easily modified to obtain an appropriate coefficient of variation [27]. The reliability and efficiency of the Cavalieri method for the determination of volume have been proven repeatedly [27], and its validity has been demonstrated

in comparison to the fluid displacement method [28].

Applying the Cavalieri principle in studies aimed at obtaining quantitative data on irregularly shaped three-dimensional objects offers advantages such as the resulting quantitative data, the application of strict sampling procedures, easily reproducible data, and a well-established theoretical background, making the reliability of the data easy to test [29-31].

The average of the response rates of all the patients (n=32) included in the study showed a regression after treatment, as evaluated by all methods used (Table 2). The mean response rates in those patients showing a minimum of two measurements with different tendencies (n=20) (Cavalieri volume estimation principle, mean regression rate: 25 %; WHO, mean regression rate: 13 %, and RECIST, mean regression rate: 11 %) were significantly different from each other. The mean response rates in patients with three measurements showing similar tendencies (n=12) (Cavalieri volume estimation principle, mean regression rate: 67 %; WHO, mean regression rate: 76 %, and RECIST, mean regression rate: 56 %) were also significantly different from each other. However, since some patients showed significantly different response rates, these findings may indicate a discrepancy between the methods used.

The determination of tumor dimensions is difficult in a tumor of irregular shape, which can complicate the reliable assessment of its diameter. The tumor size data obtained may therefore be inaccurate, making both the WHO and RECIST criteria liable to result in a wrong estimation or in the inability to estimate the tumor size in some patients, as was shown in our study.

Although some reports compare uni-dimensional, bi-dimensional, and volumetric approaches (32, 33), these studies are still in progress, and additional work needs to be completed before they can be used as gold standards compared with stereology (34). In addition, stereological measurements using automated software are currently available and can be performed easily and rapidly, although still slower than measurements using CT workstations. However, a recent study shows that the use of stereology for the estimation of volume is more sensitive than CT (34). Stereological measurement can therefore provide additional useful data to supplement CT measurements, especially in borderline and controversial cases.

The Cavalieri method may be more suitable in the estimation of tumor volume in response to treatment in advanced malignancies, in particular in tumors of irregular shape or in cases in which the treatment response is difficult to determine. However, further studies using this method in the assessment of tumor progression/regression rates are needed.

Conflict interest statement The authors declare that they have no conflict of interest to the publication of this article.

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