Estimation of the Tumor Volume and Volume Ratio on Computed Tomography in Patients with Renal Cell Carcinoma: A Stereological Study

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In this study, we describe and adapt the relevant methods of computed tomography (CT) and stereology to estimate renal cell carcinoma (RCC) volume and volume ratio and compare the RCC volume estimations with the tumor stage. The study included 126 (82 men, 44 women) patients with RCC. The patients were evaluated by CT. The volume and volume ratio of the entire RCC was estimated by the following formula of Cavalierie's principles. According to TNM (tumor, nodes, metastasis) classification, there were 56 (44.4%), 30 (23.8%), and 40 (31.7%) cases in the stage T1, T2, T3, respectively. The results of the volume measurements which obtained from the Cavalier method were assessed according to the stages and were found as 125.52 ± 102.18 (25–394) cm^3, as 346.25 ± 112.55 (181–545) cm^3 and 694.88 ± 405.46 (142–1546) cm^3 in stage T1, T2 and T3, respectively. The volume ratios between the stages were compared statistically and a significant difference were found between the stage T1 and stage T2, stage T2 and the stage T3 and stage T1 and stage T3, respectively. The average tumor volume ratios was found as $28.44\% \pm 14.37\%$ (8.69%–61.26%), $55.42\% \pm 12.73\%$ (25.78%–73.86%), and $72.48\% \pm 17.15\%$ (48.80%–97.15%) in stage T1, T2 and T3, respectively. The present evaluation of RCC volume can be done on any complete set of CT images, where plane scan distance and magnification factor is known, which already take place on to CT images.

Key words —— Cavalieri principle, volume estimation, volume ratio, computed tomography, stereology, renal tumor

INTRODUCTION

The analysis of medical images using modern stereological methods enables one to make precise unbiased estimates of geometric quantities such as volume and surface area. The volume of structures can be estimated using the Cavalier principle of stereological methods, which as originally described by Bonaventura Cavalier, applies a standard computed tomography (CT) to slice the structure under study.[1,2] Starting at a random point and moving from one end of the structure to the other, a series of parallel plane sections at a constant distance is created.[2,3] The cut surface areas of the sections are estimated; multiplying the total cut surface area by the mean section thickness provides an estimation of the volume of the object under.[2,4] In addition to the specific software, the cut surface areas of each section or slab are estimated using point counting grids.[1,5] Stereology is a term applied to a wide variety of geometric estimation techniques that, seek to describe the three-dimensional (volume) characteristics of some structure based on two-dimensional section of the structure.

Renal cell carcinoma (RCC) constitutes 3% of all solid tumors in humans, and surgery is the only effective curative treatment. Many prognostic indexes are defined for RCC; however, several studies showed that several clinicopathologic features

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such as tumor size, histological subtype, pathologic stage, nuclear grade assessment have some predictive value.[6, 7] Tumor size has proven to be important prognostic factor for RCC, particularly at the ends of the spectrum. To a large extent this is due to a strong correlation between tumor size and pathologic tumor stage.[7] Sequential CT section RCC volumes are then summed to determine the whole RCC volume.

The purpose of this study is to describe and adapt the relevant methods of CT and stereology to estimate RCC volume and volume ratio and compare the RCC volume estimations with the tumor stage.

MATERIALS AND METHODS

Estimation of Volume by the Cavalier Principle —— It is known that the volume of the regular shaped objects can be or estimated by the formulae: $V = txa$ (formulae 1), where t is the height and a is the base area of object. Similar to this principle, using the Cavalier principle, an unbiased estimate of the volume of an object of arbitrary shape and size may be obtained efficiently and with a known precision.[8, 9] The method requires sectioning the structure with a series of parallel planes. To avoid bias, the first section must be placed at a uniform and random position in a constant interval of length t, *i.e.*, to start the scanning always at, for example, 1 cm from the right tip of the object will introduce an unknown amount of bias in general. Moreover, the series of sections must encompass the object entirely. The direction of cutting does not affect the unbiasedness property, but will affect the estimation precision in general.[8, 10] Thus an unbiased estimate of volume can be obtained by multiplying the total area of the section cut surfaces through the structure on all the sections, *i.e.*, $\text{est}_1\ v = tx(a_1 + a_2 + \cdots\cdots + a_n)\ \text{cm}^3$ (formulae 2), where $(a_1 + a_2 + \cdots\cdots + a_n)$ denote the section areas in cm^2 and t is the sectioning interval in cm for the n consecutive sections.[8] Some automatic machines or software can measure the contour of the object to obtain the cut surface area of section. However, several studies have shown that point counting techniques represent a more reliable and efficient approach than planimetric technique for obtaining the required cut surface areas of section.[5, 8, 11] The point counting grid, which has some point sets at distinct densities on a transparent sheet, can be used to estimate the cut surface area of the sections.[8, 10, 12–14] The point counting method consists of overlying each selected section with a regular grid of test points, which is randomly positioned. Test system orientation does not affect unbiasedness, but certain orientation improves the estimation precision. For this reason, the tests system should be superimposed on the section three times and the mean number of points hitting the objects should be used to estimate cut surface area of the section. A test point is a (+) shaped lines and it is said to hit the object if the upper right hand corner of the intersection of the cross lines representing them on the test system lies inside the object (Fig. 1A). After each superimposition, the number of test points hitting the structure of interest on the sections is counted, and the unbiased estimator becomes: $\text{est}_2\ v = txa/px(P_1 + P_2 + \cdots\cdots + P_n)\ \text{cm}^3$ (formulae 3), where $(P_1 + P_2 + \cdots\cdots + P_n)$ denote the point counts

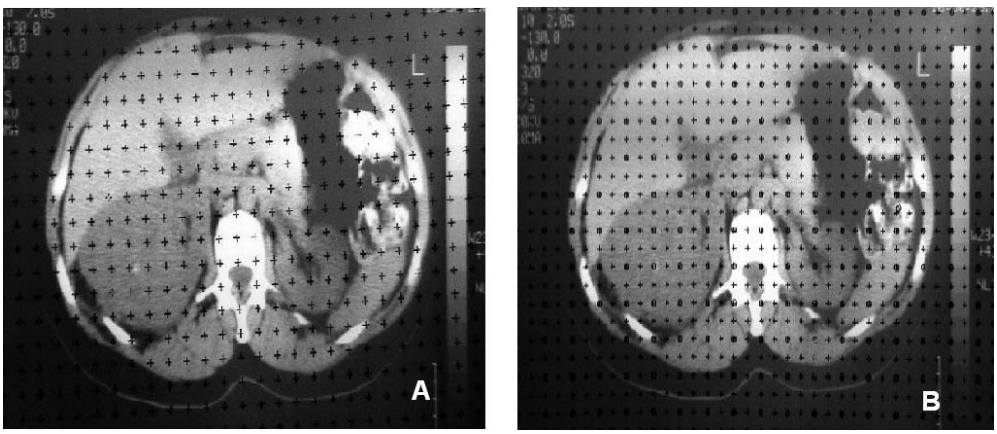

Fig. 1. A Stereological Test System Superimposed over CT Sections through the Kidney, to Estimate RCC Tumor Volume (A) and Tumor Ratio (B)

and a/p represent the area associated with each test point, corrected for any change of scale in the images as it is printed on the hardcopy films. The area of each section a_i is now estimated by $(a/p)\times P_i$. The subscript 2 in $\text{est}_2\ V$ indicates that a two-stage process, namely sectioning and point counting, estimates the volume. To avoid the over estimation due to CT scanning, adding a correction limit the bias of over projection of the RCC slices through the scan plan, the Cavalier principle provides a simple estimator.[4] Thereby, the formulae can be changed as follows: $\text{est}_2\ v = txa/px(P_1+P_2+\cdots\cdots+P_n-P_{max})$ cm^3 (formulae 4), where $P_{\max}$ is the maximal number of points counted on a single scan plane of the subject.[8]

In the Cavalier principle, a researcher obtains a data called coefficient of error (CE) to evaluate the reliability of the point density of the grids and sectioning intervals. Since the cut surface areas of consecutive sections are not independent quantities, conventional statistical formulae of CE cannot be applied to determine the variance of their sum. Many of the researchers developed some formulas to obtain the CE for the Cavalier estimation method. Those formulas not only provide the CE but also give information on the required number of slices and density of the point counting grid. The generally accepted highest limit of CE is 10%.[3, 15]

Component Volume and Calculation of the Volume Ratio —— The number of the randomized points meeting the projections over the sectional images of a component involved in any structure is directly proportional with the volume of that structure and hence with the areas of the projections emerged in the sections. By using this relationship, as a result of placing randomly an area measurement scale containing well-known frequented points over the sectional images, calculating total point count over the projections of the relevant component and multiplying this value with the average section thickness gives an unbiased calculation of the component volume.[16, 17] In order to find the volume ratio of the components, a scale containing the appropriately frequented points is thrown randomly over the relevant sectional image. Here the thing to do is to determine the number of the points meeting the projections of the components $P(y)$ and (including the components) the entire reference volume $P(\text{ref})$. When these two values are rated with each other $P(y)/P(\text{ref})$, the volume amount of the so-called component compared to the reference volume, in other words the volume ratio of the component $Vv(y, \text{ref})$ is calculated as unbiased.[16, 18] As for the volume ratio of the component, a compound pointed area measurement scale is used (Fig. 1B). Hence for the calculation of the volume ratio the following formulae is used.[16, 17]

$$Vv(Y, \text{ref}) = \frac{P(Y)}{4 \times P(\text{ref})}$$

Here, $Vv(Y, \text{ref})$ expresses the volume ratio of the Y component within the reference volume; $P(Y)$ shows the total number of the small points meeting the projections of the Y component emerged in the sections; whereas $P(\text{ref})$ expresses the total big point number meeting the reference volume. Considering the four folds of the $P(\text{ref})$ value at the denominator is due to the relationship between the point teams.

The study included 126 patients with RCC who underwent radical nephrectomy at our institution between January 1998 and August 2006. The patients were evaluated by intravenous pyelography (IVP), coloured Doppler duplex ultrasonography and CT. The scanning was of specimens were performed by a CT scanner with the following parameters: kV : 120, mAs : 100 in axial. The slice thicknesses were 10 mm on every section. The images of the sections were printed on films in square frames of $8 \times 6.4\,\text{cm}^2$ side length. A square grid test system with $d = 0.4$, 0.15 and 0.15 cm between test points, *i.e.*, 0.16, 0.0225 and $0.0225\,\text{cm}^2$ representing area per point was used to estimate the sectioned surface area of the slices of axial plane. The representing area per point in the grid was corrected with the reduction ratio of printed sections. The films were placed, in turn, on a light bow and the distinction of each specimen was done with guide of the scanogram of the section series. The transparent square grid test system was superimposed uniformly, randomly covering the entire image frame. The volume and volume ratio of the entire RCC were estimated by the following formulae of Cavalierie's principles. Calculation of RCC volume, CE of estimates and volume ratio and other related data were simply performed using Microsoft Excel as a spread sheet. After initial setup and preparation of the formulae in a small macro program, the point counts were entered for each scan and the user did the calculations automatically. Clinical staging was made according to 1997 TNM (tumor, nodes, metastasis) classification.[7]

All statistical tests were done with on computer, using the biostatistical data base program, Statisti-

cal Package for Social Sciences (SPSS). Relations between tumor volume and tumor volume ratios according to stages were compared using the Paired sample *t* test. Pearson's product moment correlation coefficient was calculated for correlation analysis.

RESULTS

Of the total 126 cases involved in the study, 82 were men (65.0%), 44 were women (34.9%). Their average age was 59.5 ± 10.1 (27–74 years). In the classification according to TNM, there were 56 (44.4%) cases in the stage T1, 30 (23.8%) cases in the stage T2, and 40 (31.7%) cases in stage T3. The results of the volume measurements obtained from the Cavalier method were assessed according to the stages and were found as 125.52 ± 102.18 (25–394) cm^3 in stage T1, as 346.25 ± 112.55 (181–545) cm^3 in stage T2, and as 694.88 ± 405.46 (142–1546) cm^3 in stage T3 (Fig. 2). When the total entire groups were considered, the average volume was found as 356.08 ± 343.37 (25–1546) cm^3. The volume ratios between the stages were compared statistically and a significant difference was found between the stage T1 and stage T2 ($p < 0.001$), between the stage T2 and the stage T3 ($p < 0.001$) and between the stage T1 and stage T3 ($p < 0.001$). The smallest volume ratio was detected as 8.69% whereas the biggest volume ratio was detected 97.15%. The average tumor volume ratio was found as 28.44% ± 14.37% (8.69%–61.26%) in stage T1, 55.42% ± 12.73% (25.78%–73.86%) in stage T2, and 72.48% ± 17.15% (48.80%–97.15%) in stage T3 (Fig. 3). The mean CE for the RCC estimates was 2%. The range of CE values changed from 1 to 9%.

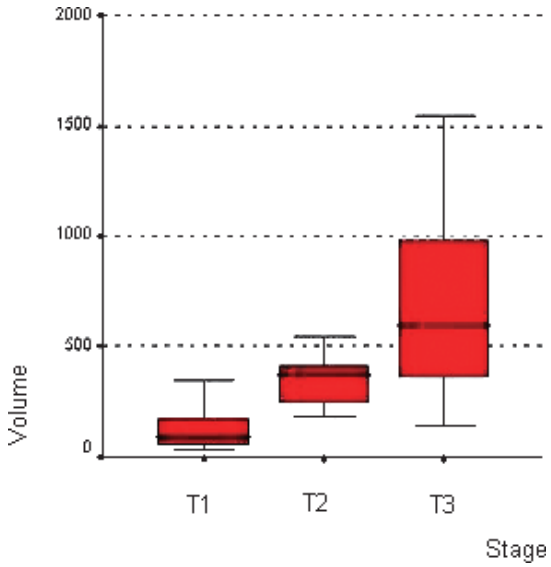

Fig. 2. Distribution of the RCC Volumes According to the Stages

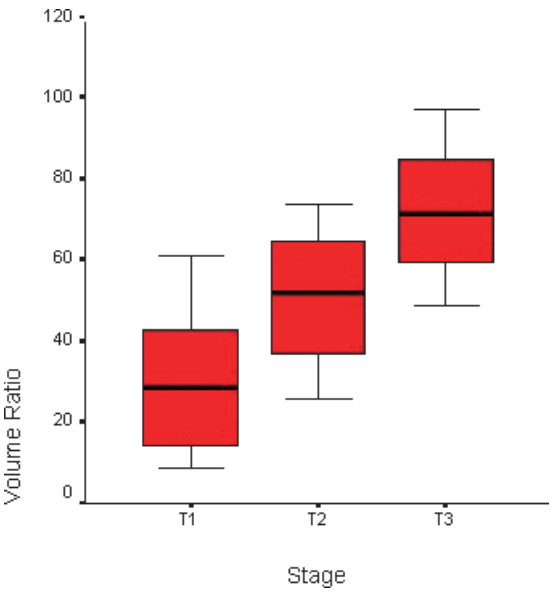

Fig. 3. Distribution of the RCC Volume Ratios According to the Stages

DISCUSSION

In morphometric studies, the volume of an organ or an organ component, the volume of the variable components in a structure and the volume ratios of these components to each other or to whole structure are frequently used and consist the important parameters. In order to calculate the total volumes of the organs or lesions or the volumes of the components, several series of methods are used.[19–22] If the structure that is in the region of interest and the volume of which is to be calculated has a macroscopic structuring that can be isolated from the other organ and structures surrounding such as liver, lung, kidney or spleen, instead of calculating the volume of this, it can be directly measured (hydrostatic weight measurement). In the situations like this a frequently used method is to throw the structure into a graded cylinder filled with water and to measure the amount of overriding water. However, mostly the structures of interest are in close relationship with the components around and isolating these structures and directly measuring the volume

is generally impossible. At the same time this is impossible for the clinical practices.[15, 18] In this situation, the Cavalier method can be applied. This method is the most frequently used volume calculation method in the stereologic methods.[15]

A two-dimensional section through a tree-dimensional kidney results in an irreversible loss of qualitative information and a reversible quantitative change of information.[23] Routine physical examination of the kidney cannot produce accurate information about the actual volume of the organ. However, the exact volume is required for the assessment RCC volume over time as an indicator of therapeutic effectiveness, medical treatment and surgical applications. The tumor volume measurement must, therefore, be quantitative and reproducible and this can only be achieved using imaging techniques. The computed tomographic presentation of anatomy in section slices of known thickness provides a unique opportunity for quantitative measurement of anatomic volumes. Such measurements have been described by previous authors and essentially involve the summation of volumes subtended in individual sequential CT sections of known slice width.[24–26] Studies examining the reliability of volume estimation using CT images have revealed that some data was not different from the actual volume.[3, 26, 27] The given values of the RCC volume are unbiased since the slice hits the RCC randomly followed by systematic sections with a known one. The point counting is unbiased, since the set of systematic points is placed randomly on the CT images. However, there are some bias sources for the estimation of RCC volume using CT technique. Systematic sampling methods with non-random start for the sectioning induce a bias of remainder term, well-known in the context of numerical integration, which may decrease but never disappears as the number of sections is increased. It is essential to start from a random point and scan the whole kidney and tumor. Failure to ensure a random start for the whole series of scan sections will cause bias, which cannot be corrected; no matter how precisely the section areas are measured.

The mean CE for the RCC estimates was 2%. The range of CE values changed from 1 to 9%, which are in an acceptable range for the RCC volume estimates and we can say that the density of the point counting grids in the present study could be used safely for the estimation of the RCC volume on CT scans.[3, 15, 28]

One of the important prognostic factors in kidney tumors is the stage of the tumor. Generally, TNM classification is used in staging. However in this classification only the size of the tumor is taken as the basis. The studies including the volume calculations of the kidney and the kidney tumor by the monitoring techniques are rare.[24, 26] In the autopsy studies performed by Reid[26] the right kidney was calculated as 125 gram (gr), the left kidney as 160 gr, the volume (hydrostatic weight measurement) of the right kidney as 120 cm^3, the left kidney as 154 cm^3, whereas in the calculation by using Cavalier method of the CT sections, the right kidney was calculated as 125 cm^3, and the left kidney as 157 cm^3. In the same study in a case with the kidney tumor the left kidney was found as 110 cm^3, the right kidney as 185 cm^3, and the tumor volume as 230 cm^3. The results of this study show that the volumes of the kidney and the kidney tumors could be accurately measured by the Cavalier method. However in this study since the size and the stage of the tumor was not stated but only the value as the volume has been given, no information about its conformity with its stage is available.

In our study, the tumor volumes were found as 125.52 ± 102.18 (25–394) cm^3 in the stage T1, as 346.25 ± 112.55 (181–545) cm^3 in the stage T2, and as 694.88 ± 405.46 (142–1546) cm^3 in the stage T3. When all groups are considered the average volume was found as 356.08 ± 343.37 (25–1546) cm^3. The volumes between the stages were compared and a significant difference was realized between the stage T1 and the stage T2 ($p < 0.001$), between the stage T2 and the stage T3 ($p < 0.001$) and between the stage T1 and stage T3 ($p < 0.001$) and it was realized that there was a strong correlation between TNM classification and the volume found by the Cavalier method. It is seen that the more advanced is the stage; the bigger is the volume amount. Under the light of these results the volume measures giving the certain results together with the size used in TNM system can be used.

The ratios of the components involved in a structure to the total volume are the parameters that provide important information. If the total volume value of the structure involving the component is known, the ratio of the volume it takes, in other words, the total volume of the component the volume ratio of which is known can be calculated by this. The volume ratios were examined and as an average the smallest and the biggest volume were detected as 8.69% and 97.15%, respectively. The average tumor volume ratio was found

as 28.44% ± 14.37% (8.69%–61.26%) in the stage T1, as 55.42% ± 12.73% (25.78%–73.86%) in the stage T2, as 72.48% ± 17.15% (48.80%–97.15%) in the stage T3. The volume ratios between the stages were compared statistically and a significant difference was found between the stage T1 and the stage T2 ($p < 0.001$), between the stage T2 and stage T3 ($p < 0.001$) and between the stage T1 and stage T3 ($p < 0.001$). On the other side, the tumor volume ratio shows us that the advanced is the stage of the tumor; the bigger is the area which the tumor covers.

Two methods were performed to in this study: volume and volume ratio measurement. Although both were found to be correlated with the stage of RCC, in early stages (stage T1 and T2) the standard deviation (SD) values for volume were lesser than the SD values in stage T3. It has been also, observed that, for stage T3 RCC the volume ratio SD values were lesser than the volume SD values. This data suggested that, especially for the advanced RCC (stage T3) volume ratio but not only volume measurement should be considered for the final decision, due to the higher SD values for volume measurement which may under stage or over stage some RCC.

In conclusion, our purpose was to develop an easy way to evaluate the RCC volume on ordinary CT scans without having to change the routine procedure for making such scans in every radiological centre. It is not necessary to standardize the CT further in order to determine the RCC volume. The present evaluation of RCC volume can be done on any complete set of CT images, where plane scan distance and magnification factor is known, which already take place on to CT images. The method is inexpensive and fast, since point counting is carried out within 5–10 min per subject. However, it should not be forgotten that further cases and studies are needed in this subject.

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