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Easy Method of Assessing Volume of Prostate Adenocarcinoma from Estimated Tumor Area: Using Prostate Tissue Density to Bridge Gap Between Percentage Involvement and Tumor Volume

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Aim To determine prostate carcinoma tumor volume in routine pathology practice by using prostate tissue density.

Methods Prostate tissue density was determined experimentally by using pycnometry in 57 unfixed prostate tissue fragments of different size. The percentage of prostate involvement was converted to tumor volume using the equation $V = m/\rho$ (g/mL). Additionally, all tumor foci were outlined in 46 prostates. A high grade component was also designated. The percent of prostate involvement by the tumor and separately by the high-grade component was determined using the fine grid method (0.9 mm resolution) in all cases. Pathologist's estimated square area method was applied for comparison in 27 cases. All tumor foci were evaluated for Gleason grades.

Results Prostate tissue density (ρ) was 0.98 or $\approx$ 1.0 (g/mL). Quicker estimated square area method was fully comparable to more laborious fine grid method for determination of percent of prostate involvement. The percentage of prostate involvement by the tumor as measured by the grid method was not significantly associated with the Gleason sum of the tumor. However, the total tumor volume that was calculated from the percent tumor involvement, mass of the prostate, and tissue density was positively associated with the Gleason sum ($P = 0.035$, linear-by-linear association).

Conclusion Our results show that prostate tissue density can be used to determine prostate carcinoma tumor volume in routine pathology practice.

Previous reports on the natural history of prostatic adenocarcinoma have shown that the total tumor volume and the percentage of poorly differentiated tumor component are reliable indices of evolving malignant potential (1,2). Also, a strong association has been reported among tumor volume, the Gleason grade, seminal vesicle involvement, capsular penetration, and lymph node metastases (2-4). Complex intraprostatic distribution of the dominant tumor and the simultaneous presence of multiple incidental tumors within a single prostate make it difficult to determine the tumor extent in the prostate (5-9). Because of this difficulty, pathologists usually report on the anatomical extent within the prostate and try to give a crude estimate of the percentage of prostate involved by the tumor. It is recommended by both the College of American Pathologist (CAP) and the World Health Organization (WHO) that all pathology reports should include some measure of tumor size (10). The use of the percentage of gland involved by cancer was recommended probably be-

cause of the ease of application and likely acceptance by most pathologists (10,11). However, there is no unanimous agreement whether volume (1,2,12) or percent of prostate involvement by carcinoma (13,14) is a better predictor of the pathological stage or clinical outcome of the patients with prostatic adenocarcinoma. In this study, we bridged between the two clinically very important measures of tumor extent by providing the value of tissue density of prostate gland, which enables the use of a simple equation for conversion of one value into the other ($V = m/\rho$ (g/mL), where V = volume, m = mass, and ρ = tissue density). We also provided direct evidence that a relatively crude estimate of the percent of prostate involvement by the tumor is clinically sufficiently as accurate as the broadly accepted grid-method.

Materials and Methods

Pycnometry

Prostate tissue density (ρ) was determined from multiple measurements using pycnometry (15,16). We tested 57 unfixed prostate tissue fragments from several consecutive radical prostatectomy specimens. They were of different sizes and shapes, and their means varied from 0.15 g to 4.85 g (Fig. 1A). The measured values were recorded at two decimal points in grams and milliliters. All readings of the mass and volumes were performed by two independent observers who were blinded from the results of the measurements obtained by the other observer. The mass of the tissue was determined using a scale with a resolution of 0.001 g, but the results were rounded in grams (g) to two decimal points, the reading error of 0.0005 g was not regarded as significant since it did not change the value of a calculated propagated volume error (ΔV). The volume of expressed distilled water was recorded in milliliters (mL) in a small measuring cylinder, with the reading error of the measuring device of 0.05 mL. Random error was determined using calibrated floats/beads of 0.2 mL and 0.7 mL and was found to be less than the reading error. The mass of each measured fragment was divided by its volume and the mean tissue density, recorded as a final result for the use in calculations of prostate tumor volume.

Tumor Volume

Tumor volume was calculated from the tumor mass and density of the prostate tissue: $V = m/\rho$ (mL). Propagation error was calculated from the reading error established for pycnometry and was $\Delta V = 0.07$. Thus, all calculated volume values could be expressed as $V = n \pm 0.07$ mL, where n is calculated tumor volume.

We also compared different measurements of tumor size (total tumor volume, percent of prostate involvement by the tumor, and pathologist-estimated percent involvement) with the Gleason score of the tumors.

Forty-six prostatectomy specimens included in the study were retrieved from the archives of the pathology department, the Norwegian Radium Hospital, Oslo, Norway, following the guidelines of the regional/hospital Ethics Committee.

Histological Examination

Careful tracing of the prostate carcinoma was done on each slide in all 46 prostates by outlining the tumor with a marker pen. The tumors were graded using the Gleason grading system (17). All Gleason grades in all tumor foci on each slide were recorded for each case.

Grid-Method and Estimated Square Area Method

Calculations of the percent of prostate tumor involvement were based on measurements performed by using two previously described methods with some modifications: 1) grid method (18) and 2) estimated tumor area method using a ruler (19). The grid ratio value was calculated by dividing counted points of intersection of the grid, which overlaid the tumor, by the total points of the intersection overlaying the prostate tissue. The points were counted on images of glass slides projected by Focomat (Leica, Wetzlar, Germany) on the grid placed on the projection plate. The grid was composed of 1.0 cm squares. The purpose of the Focomat projection was to achieve a higher resolution of the grid than reported previously (18). The final resolution of the grid was 0.9 mm in contrast to 3.0 mm described by Hamphrey et al (18). The percentage of prostatic tissue involved by the tumor was also determined using the estimated square area method as described previously, with minor modification (19). Briefly, on each slide, the area of the prostate was determined by recording the greatest dimensions of the tissue on the slide, as well as the greatest dimension perpendicular to it. These two values were multiplied

for each slide. The sum of the areas of the prostate tissue from each slide represented the total prostate area. The tumor area was measured in the same manner, summed to represent the total tumor area and then the percent involvement was calculated. The measurements were performed for all tumor foci on all slides, including not only dominant but also all incidental tumors and all measurement were recorded separately for each tumor focus.

Statistical Analysis

Paired sample *t* test was used to compare grid-percent measurement with pathologist's estimated square area method. Linear-by-linear association was used to test for association between percent of prostate involvement and calculated total volume of the tumor with the Gleason sum. Pearson's correlation test was used to calculate correlation between measured weight and volume of the prostate tissue samples. Statistical analyses were performed by using Statistical Package for Social Sciences, version 12.0 (SPSS Inc., Chicago, IL, USA).

Results

Tissue density of the prostate was 0.98 g/mL (Fig. 1A). No significant difference was found between percentage prostate involvement as measured by grid-method or by pathologist's estimated square area method ($P=0.07$, paired t test, $t=-1.93$) (Fig. 1B).

Tumor volume calculated from the percent prostate involvement as measured by the grid method and prostate tissue density (Fig. 2) was positively associated with Gleason sum ($P=0.035$, linear-by-linear association). However, this was not the case for the percentage prostate involvement by the tumor as measured by the grid-method ($P=0.059$, linear-by-linear association).

A diagram illustrated in Figure 2 could be used as a guideline for the pathologist how to

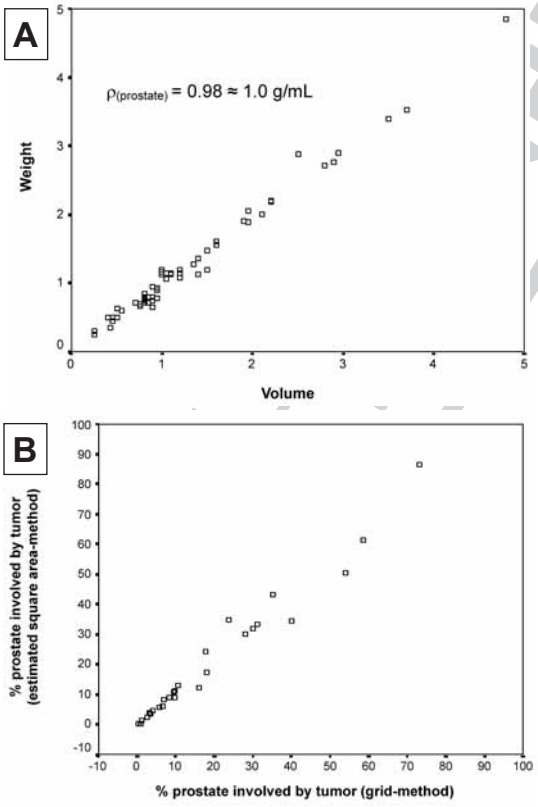

Figure 1. Determination of prostate volume. (**A**) Actual measurements of the 57 prostate tissue samples. Calculated prostate tissue density was about 1.0 g/mL. (**B**) Estimated square area by the pathologist is in excellent agreement with results obtained with high-resolution grid percentage measurement.

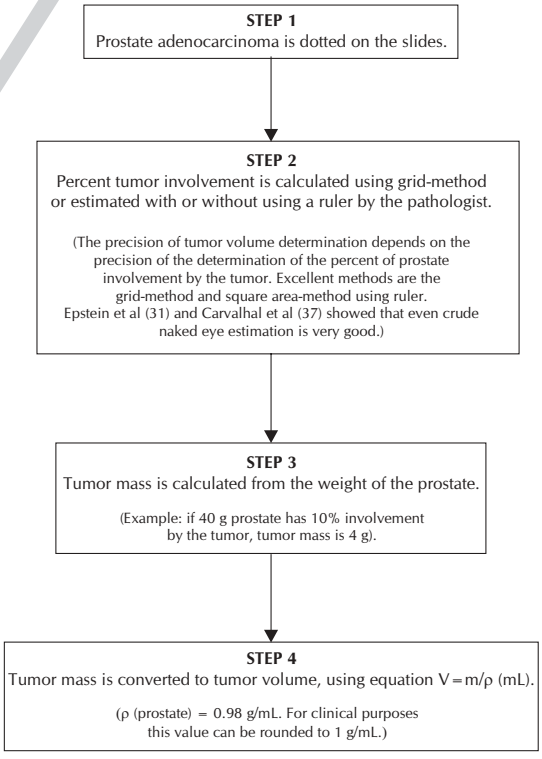

Figure 2. Four steps in determination of tumor volume in the prostate gland are illustrated. The value of the mass of the tumor in grams becomes the value of tumor volume in mL if $\rho_{(prostate)}$ is rounded to 1 g/mL.

determine tumor volume of the prostate carcinoma in four simple steps.

Discussion

Carcinomas less than 4 mL in volume were shown to be protected from extensive capsule penetration, positive surgical margins, seminal vesicle invasion, and lymph node metastases. Conversely, cancers larger than 12 mL in size were a nearly homogeneous group, in which all of the adverse determinants tended to be positive (20,21). In these studies, both the total tumor volume and the high-grade tumor volume were found to be important and independent predictors for histological progression of the prostate cancer. However, the percent of prostate involvement was also recommended by some authors as a good measure of tumor progression since its determination does not necessarily require sophisticated computer software (14,18,22,23).

Our study showed that it was possible to easily bridge the gap between the percent tissue involvement and tumor volume in a routine diagnostic pathology setting. Estimated square area method is easy to use and requires dotting of the tumor contours on the glass slide, a simple ruler, and a few basic calculations (addition and division). Remarkably, it highly correlated with the very precise, but more laborious fine-resolution (0.9-mm) grid-method. Furthermore, it was previously shown that the grid method was as good or probably an even better method than manual or automatic segmentation in area estimation of complex structures, e.g. carcinomatous tissue in a prostate (24,25). However, neither calculation of estimated square area method nor calculation of tumor volume require sophisticated computer equipment and software because they involve the use of simple equations by a pathologist. Epstein et al found that even naked eye examination of the glass slides after the pathologist had circled all identifiable foci of carcinoma with a marking pen correlated well with a morphometric measurement (14) and Carvalhal et al (22) showed that naked eye estimation of the percent of prostate involvement even without circling the tumor areas correlated with the parameters of clinical outcome. In theory, any method that provides the percent of prostate involvement with clinically acceptable precision could be used for calculations of tumor mass and the subsequent conversion to tumor volume.

The additional information that we provide in our study, the prostate tissue density (ρ), enables us to do this simple conversion. As shown in Figure 2, the mass of the tumor is easily calculated from the mass of the total prostate (seminal vesicles need to be removed before the fresh specimen is weighed) once percent of prostate involvement is measured or estimated. The same method can be used to determine high-grade volume if areas involved by the high-grade volume are outlined on the slides. It appears that this conversion is warranted, because we found that there was significant association between such calculated tumor volume and the Gleason sum, whereas no such association could be shown for the percent of prostate involvement as measured by the grid-method, the exact measurement that was used to determine the calculated tumor volume. Schmid and McNeal suggested using an abbreviated standard procedure for accurate tumor volume estimation (26). Their abbreviated procedure consists of dotting the tumor area on the slides, then using a 4-mm grid method to calculate approximate tumor area from the number of grid squares within the ink-dotted cancer boundary in every other section of the specimen. The volume was than calculated by multiplying each cancer area by an appropriate thickness factor and by a factor of 1.5 to account for tissue shrinkage during processing. The purpose of grid measurements in our study was entirely different, because we only used the grid method to determine the percent involvement. The percent involvement was then used only to determine tumor mass from the mass of the prostate. Tumor mass was easily calculated from accurate, unfixed prostate weight and then tumor mass was easily converted to tumor volume by using prostate tissue density and applying the simple equation $V = m/\rho$.

Our measurements showed that the prostate tissue density is 0.98 g/mL and for all practical purposes, this can be rounded up to 1.00. Benign and malignant prostate tissue has very low fat content, so it is not surprising that the tissue density is close to distilled water density and generally to tissue density of a lean body mass (27). A Pub Med literature search revealed only two previous studies using pycnometry to determine tissue density of a particular organ other than fat, muscle,

or lean body mass, and both of these were applied on the brain tissue (15,16). The aim of those studies was to determine the specific gravity of the brain tissue or percent water content as an index of the degree of brain edema with a much higher requirement for analytical precision. However, methods more precise than those used in our study would not be needed or desirable using the prostate tissue with final clinical goal of conversion of percent of prostate involvement to tumor volume. There is no strict definition of what clinically significant error is in prostate carcinoma volume measurements. Schmid and McNeal stated that an error of tumor volume estimation in prostate cancer of ±20% is clinically acceptable (26). For clinical purposes, the value of prostate tissue density could be rounded to 1 g/mL because if this value is used instead of 0.98, the largest difference of the calculated volume would be +2% (+0.08 mL in the smallest and +0.77 mL in the largest tumor in our study), in addition to an inherent reading error propagation of ΔV of 0.07 in each tumor, both of which are very small and clinically acceptable (26). Epstein et al (14) calculated the density of prostatic chips on 10 consecutive specimens for the purpose of conversion of morphometric measurements into mass in specimens of unknown weight. The authors found no variation among 10 specimens and the density was calculated to be 0.5 g/mL. Even though the authors concluded that their calculation should be accurate, their result suggests that prostate tissue density is less than that of the fat tissue ($\rho_{[fat]} = 0.9$) and surely as such they cannot be accurate. The method used for the determination of tissue density was probably the cause of the erroneous result.

In summary, we conclude that: 1) estimated square area method is as good as fine-resolution grid-method for the determination of the percent of prostate involvement by the tumor; 2) tumor mass can be calculated from the percent of prostate involvement; 3) tumor volume can be calculated from tumor mass and tissue density of the prostate; 4) tissue density of the prostate is 0.98 g/mL and could be rounded to 1 g/mL for clinical purposes; 5) whereas different measures of the tumor extent (percentage prostate involvement and tumor volume) are highly associated with each other, only the total tumor volume was associated to the Gleason score of the tumor; and 6) the final result of the conversion of the percent of prostate involvement by the tumor into tumor volume is as good as the method used for measuring the percent involvement, since reading errors propagated from the tissue density measurements and rounding of tissue density value to 1 are minimal and according to the current knowledge, clinically irrelevant.

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