The Peritoneal Membrane in Peritoneal Dialysis Patients: Estimation of Its Functional Surface Area by Applying Stereologic Methods to Computerized Tomography Scans

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Abstract. The surface area of the peritoneal membrane in contact with dialysate is an important determinant of solute transport across the peritoneum. Yet there is no method for its estimation in peritoneal dialysis patients. In this study, stereologic methods were applied to computerized tomography (CT) imaging of the peritoneal membrane to estimate the peritoneal membrane surface area. The method was first validated by implementing stereologic methods on a phantom of known surface area. The phantom was a distorted bottle filled with contrast media. Series of thin helical CT sections were performed, and random sections were obtained after reconstruction. A transparent counting grid was placed over the random sections. The surface area was estimated using 9, 18, and 36 random sections. To calculate the coefficient of variation (CV) of the method, 20 different combinations of 9, 18, and 36 random sections were used. With 36 random sections, the error in estimation of the bottle’s surface area was $-9.4\%$ to $+8.8\%$. The CV was 5.0%. Decreasing the number of sections used to 18 and 9 yielded a CV of 7.8 and 12.3%, respectively. This method was then applied to the peritoneal membrane, which was visualized by instilling dialysate containing contrast media into the peritoneal cavity of peritoneal dialysis patients. The estimated peritoneal membrane surface area of six patients was $0.55 \pm 0.04 \text{ m}^2$. This novel method permits the measurement of the peritoneal membrane surface area with a high degree of accuracy.

Peritoneal dialysis uses the peritoneal membrane as an endogenous exchange membrane to remove solutes and water from the body fluids of patients with end-stage renal disease. The efficiency of this system is variable, with the solute transport up to three times more effective in some patients than in others (1). Factors determining the solute transport are membrane permeability and its functional surface area, i.e. the area in contact with dialysate (2). The peritoneal membrane surface area (PMSA) has been estimated only in post mortem studies (3–5). There is as yet no method for measuring the functional PMSA in vivo. The study of peritoneal physiology would benefit from a method of PMSA estimation. The present study describes such a method, which applies stereology to computerized tomography (CT) imaging of the peritoneal membrane.

The peritoneal space can be visualized with CT scanning by instilling into the peritoneal cavity a mixture of dialysate and contrast media through the dialysis catheter. This imaging procedure, which has been used to visualize dialysate leaks in the clinical setting (6,7), exhibits the boundaries of the peritoneal space. These boundaries represent the mesothelial aspect of the peritoneal membrane in contact with dialysate. The surface area of these boundaries is the functional PMSA.

Stereologic techniques enable the quantitative estimation of three-dimensional structures from two-dimensional sections, regardless of their shape (8). Surfaces in three dimensions are seen as lines in two dimensions. Therefore, a method that quantifies the lines on the two-dimensional sections will enable estimation of the corresponding surface area. These methods are usually used on physical slices, such as geologic, anatomic, or histologic specimens. We present their application to the estimation of the functional PMSA as visualized by CT scanning.

Materials and Methods

The method was first validated by implementing stereologic methods (8) on CT sections of a phantom of known surface area. In a later stage, it was applied to CT sections visualizing the peritoneal cavity.

Estimation of the Surface Area of a Phantom

The phantom was a distorted plastic bottle filled with a solution containing 100 cc of contrast media (a sodium and meglumine ioxithalamate 35% solution; Telebrix 35, Guerbet, Aulnay-s/Bois, France) diluted with 1900 cc of water (Figure 1). The bottle was distorted to create an object with an irregular shape simulating the shape of the peritoneal space. The surface area of the bottle was...
estimated by covering the bottle’s surface with cellophane paper of known weight per surface area and weighing the paper covering the surface. Helical scans of the jar were performed on a Helicat II scanner (Elscint, Haifa, Israel). Scan parameters were: slice width 3.2 mm, slice interval 1.6 mm (50% overlap), pitch 1. Image parameters were: window center 0, window width 350. After scanning, the images were reconstructed, and 72 uniform random sections (8), i.e., sections with no preferred orientation, were obtained (Figure 2).

A transparent counting grid formed of test lines (multipurpose test system) (8) was placed over the CT images (Figure 3). The test lines’ length after correction for the scale was 13.0 mm. The bottle’s surface area (SA_{bottle}) was estimated by counting the number of intersections of the test lines with the bottle’s boundaries and the number of lines falling over the reference volume. This was done on 72 sections. The reference volume was the bottle volume, calculated as the volume of the solution filling the bottle. The ratio estimate of the SA_{bottle} (S_{v\_bottle}) was generated using the following relationship (8):

\[ S_{v\_bottle}(\text{cm}^2/\text{cm}^3) = \frac{2 I}{L}, \]

where \( I \) is the number of intersections with the surface of interest, and \( L \) is the total length of test lines falling within the reference volume, after correction for the prints’ scale.

The ratio estimate of the SA_{bottle} was converted to an absolute figure (est SA_{bottle}) by multiplying it by the reference volume (V):

\[ \text{est} \ SA_{bottle}(\text{cm}^2) = S_{v\_bottle} \times V_{bottle}. \]

To determine the optimal number of random sections to be used, the precision of the estimate of the SA_{bottle} was calculated for each of 20 random combinations of 9, 18, and 36 sections drawn from the
sections of the whole peritoneum were obtained (Figure 4). Reconstructed and sets of 36 systematic isotropic uniform random window center 0, window width 350. After scanning, the images were overlap), pitch 1.0 in the other patients. Image parameters were: slice width 3.2 mm, slice interval 1.6 mm (50% overlap), pitch 1.5 in thin patients; slice width 2.7 mm, slice interval 1.4 mm (50% overlap), pitch 1.0 in the other patients. Image parameters were: window center 0, window width 350. After scanning, the images were reconstructed and sets of 36 systematic isotropic uniform random sections of the whole peritoneum were obtained (Figure 4).

Estimation of the Intraperitoneal Dialysate Volume

The intraperitoneal dialysate volume was calculated using radioiodinated serum albumin (RISA) as a volume marker with a correction applied for its elimination from the peritoneal cavity (9), using four dialysate samples taken before, twice during, and at the end of CT scanning. The repeated sampling was rendered necessary because of the variation in intraperitoneal fluid volume during the CT scanning, which lasted approximately 40 min. The dialysate volume during CT scanning was calculated as the mean of the four measurements. The peritoneal space volume at the time $t$ of each sampling ($V_{D(t)}$) was calculated as follows:

$$V_{D(t)} = V_A(t) \times CF(t),$$

where $V_A(t)$ is the apparent volume of dialysate calculated from the dilution of the RISA at time $t$, and $CF(t)$ is a correction factor at time $t$.

$$CF(t) = 1 - \frac{V_A(T) - (V_{out} + V_{res})}{V_A(T)} \times \frac{t}{T}.$$

where $V_A(T)$ is the apparent volume of dialysate estimated from dilution of the RISA at the termination of the dwell (i.e., at time $T$), $V_{out}$ is the volume of dialysate drained after the termination of the dwell, $V_{res}$ is the residual dialysate volume after drainage, calculated from the equation: $V_{res} \times C_{risa} (T) = (V_{rinse in} + V_{res}) \times C_{risa (rinse out)}$, where $C_{risa (rinse out)}$ is the concentration of RISA at completion of the dwell, $V_{rinse in}$ is 1 L of fresh dialysate 1.36% (without RISA), and $C_{risa (rinse out)}$ is the concentration of RISA in the drained rinsing fluid.

Estimation of the PMSA of Peritoneal Dialysis Patients

Six chronic ambulatory peritoneal dialysis (CAPD) patients participated in the study. They were all men, ranging in age from 41 to 76 yr, on CAPD for a period of 1 to 3 yr. The studies were performed in the morning after drainage of the fluid from the peritoneal cavity. A solution containing 1200 cc of Dianeal glucose 1.36% (Teva Medical, Ashdod, Israel), 750 cc of NaCl 0.45%, and 100 cc of contrast material (Telebrix 35, Guerbet, Aulnay/s/Bois, France) was prepared. Five micrograms of radiolabeled albumin $^{125}$I was added to estimate the peritoneal dialysate volume (9). This was done after priming the bag with 1 cc of a salt-free 20% albumin solution to minimize adhesion of the radiolabeled albumin to the surfaces of the plastic material. The solution was infused through an infusion set. The patients rolled from side to side, and 200 cc were drained out and mixed. Five cc were then sampled for radioactivity count, and the remainder of the solution was instilled back into the peritoneal cavity. Series of helical scans of the patient were performed from the level of the diaphragm to the level of the symphysis pubis. Scan parameters were: slice width 2.7 mm, slice interval 1.4 mm (50% overlap), pitch 1.5 in thin patients; slice width 3.2 mm, slice interval 1.6 mm (50% overlap), pitch 1.0 in the other patients. Image parameters were: window center 0, window width 350. After scanning, the images were reconstructed and sets of 36 systematic isotropic uniform random sections of the whole peritoneum were obtained (Figure 4).

The coefficient of variation (CV) of the estimates obtained by using 20 different random combinations of 9, 18, and 36 sections was determined as: $CV = SD/mean$.

Stereologic Measurements

A transparent counting grid formed of test lines (multipurpose test system) (8) was placed over the CT images (Figure 4). The test lines’ length, after correction for the scale, was 13.9 mm for patients 1 to 3, 13.5 mm for patients 4 and 5, and 13.0 mm for patient 6. The PMSA was estimated by counting the number of intersections of the test lines with the boundaries of the peritoneal fluid and the number of lines falling over the reference volume. The intraperitoneal dialysate volume was used as the reference volume. The ratio estimate of the PMSA ($S_{v pmsa}$) was generated using the relationship:

$$S_{v pmsa} (m^2/m^3) = 2/l/L,$$

where $l$ is the number of intersections with the surface of interest, and $L$ is the total length of test lines falling within the reference volume, after correction for the prints’ scale.

The ratio estimate of the PMSA was converted to an absolute figure (est PMSA) by multiplying it by the peritoneal volume ($V_{perit}^5$):

$$\text{est PMSA}(m^2) = S_{v pmsa} \times V_{perit}.$$  

Informed consent was obtained from all participants. The study was approved by the local ethics committee.

Results

The Bottle’s Measurements

Results for the bottle’s measurements ($S_{v bottle}$ and est $S_{A bottle}$), as estimated from the analysis of 20 sets of 9, 18, and 36 random sections, are shown in Table 1 and Figure 5. The true bottle’s surface area was 815 cm$^2$. The error in the measurement of $S_{A bottle}$ ranged from $-19.7\%$ to $+25.6\%$ for nine sections (20 measurements), from $-18.7\%$ to $15.8\%$ for 18 sections (20 measurements), and from $-9.4\%$ to $+8.8\%$ for 36 sections (20 measurements). The CV of the method for the estimate of $S_{A bottle}$ was $12.3\%$ for 9 sections, $7.8\%$ for 18 sections, and $5.0\%$, for 36 sections.
The Peritoneal Membrane Measurements

The time-averaged volume of the peritoneal solution for the six patients was 2270 ± 100 cm³. The $S_{v, pmsa}$ was 2.43 ± 0.21 m²/m³. The PMSA was 0.55 ± 0.04 m², ranging from 0.49 to 0.62 m² (Figure 6). After correction for body surface area, the PMSA was 0.51 ± 0.07 m²/1.73 m².

Discussion

The present study describes a method for estimating the PMSA using CT imaging and stereologic principles. This method enables for the first time the measurement of the functional PMSA in vivo in CAPD patients.

In a previous study, published in 1993 (10), stereologic techniques were applied to the measurement of surface areas from sections obtained with CT imaging. The authors measured the pleural surface area by using parallel CT sections and a three-dimensional grid (11). This type of grid was necessary because of the parallelism of the sections. However, when applied to a structure of such a complex geometry as the peritoneal space, the use of this spatial grid, with its system of test lines in three dimensions, is time consuming and more susceptible to human error. A simpler solution is to implement the classical stereologic methods (8). This is feasible because the peritoneal space is relatively isotropic, i.e., it has no preferential orientation in three dimensions. Such a method, which has not yet been applied to the estimation of surface areas on CT sections, requires the generation of random sections. This method, validated in the present study by comparing the estimates of the surface area of a distorted bottle with its true surface area, has a satisfactory degree of precision. The precision of stereologic methods depends on the number of sections used for the estimation of the dimension studied, be it length, surface, or volume. Our validation studies demonstrate that the use of 36 random sections yields a CV of 5.0%.

This method is relatively noninvasive and is the only method applicable to CAPD patients. In animal and in human post mortem studies, the peritoneal surface has been measured after evisceration. Early studies by Wegner have found a peritoneal surface area of 1.78 m² (3). An observation by Putiloff (reported in reference (4)) reports a value of 2.08 m². More recent studies by Esperanca and Collins in six adults (4) and by Rubin et al. in eight adults (5) have found lower values of 1.03 and 0.78 m². The lower PMSA estimate reported in the present study could be anticipated because the method used estimates the functional peritoneum in contact with dialysate and not the anatomical peritoneum measured in the previous studies. Indeed, Flessner (12) has shown in a rat model that only 25 to 30% of the anatomical peritoneum is functional.

Table 1. The ratio estimates of the bottle’s surface area ($S_{v, bottle}$), the estimates of the bottle’s surface area (est $SA_{bottle}$), the percentage error ($\Delta SA_{bottle}$), and the coefficient of variation (CV) of the method using 20 random combinations of 9, 18, and 36 random sections of the distorted bottle

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. of Random Sections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9</td>
</tr>
<tr>
<td>$S_{v, bottle}$ (mean ± SD) (cm²/cm³)</td>
<td>0.75 ± 0.09</td>
</tr>
<tr>
<td>$S_{v, bottle}$ (range) (cm²/cm³)</td>
<td>0.57 to 0.89</td>
</tr>
<tr>
<td>Est $SA_{bottle}$ (mean ± SD) (cm²)</td>
<td>857 ± 105</td>
</tr>
<tr>
<td>Est $SA_{bottle}$ (range) (cm²)</td>
<td>655 to 1024</td>
</tr>
<tr>
<td>Real $SA_{bottle}$ (cm²)</td>
<td>815</td>
</tr>
<tr>
<td>$\Delta SA_{bottle}$ (range)</td>
<td>−19.7% to +25.6%</td>
</tr>
<tr>
<td>CV</td>
<td>12.3%</td>
</tr>
</tbody>
</table>

Figure 4. (A and B) Two random sections of the abdomen in a peritoneal dialysis patient. The peritoneal cavity is filled with a dialysate-contrast media solution. A grid has been superimposed. 1 grid line = 13.0 mm.
In summary, we have presented a novel method that offers an accurate and relatively noninvasive technique of estimating the functional surface area of the peritoneal membrane in peritoneal dialysis patients.

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References


Figure 5. The percentage of error and the coefficient of variation (CV) of the method used to estimate the surface area of the distorted bottle. Twenty random combinations of 9, 18, and 36 random sections were used to calculate the CV. The dotted line represents the true bottle’s surface area.

Figure 6. The peritoneal membrane surface area of six peritoneal dialysis patients.

30% of the membrane participates in the transperitoneal exchange, thus supporting our findings.

Because the rate of mass transfer is dependent on the area of the peritoneum in contact with the dialysis fluid and on a permeability factor (2,13), it is clear that knowledge about the surface area of the peritoneum participating in the exchange process is necessary to integrate morphologic data with physiologic measurements.

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