Psoas muscle area is not representative of total skeletal muscle area in the assessment of sarcopenia in ovarian cancer

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Abstract

Background  Computed tomography measurements of total skeletal muscle area can detect changes and predict overall survival (OS) in patients with advanced ovarian cancer. This study investigates whether assessment of psoas muscle area reflects total muscle area and can be used to assess sarcopenia in ovarian cancer patients.

Methods  Ovarian cancer patients (n = 150) treated with induction chemotherapy and interval debulking were enrolled retrospectively in this longitudinal study. Muscle was measured cross sectionally with computed tomography in three ways: (i) software quantification of total skeletal muscle area (SMA); (ii) software quantification of psoas muscle area (PA); and (iii) manual measurement of length and width of the psoas muscle to derive the psoas surface area (PLW). Pearson correlation between the different methods was studied. Patients were divided into two groups based on the extent of change in muscle area, and agreement was measured with kappa coefficients. Cox-regression was used to test predictors for OS.

Results  Correlation between SMA and both psoas muscle area measurements was poor (r = 0.52 and 0.39 for PA and PLW, respectively). After categorizing patients into muscle loss or gain, kappa agreement was also poor for all comparisons (all κ < 0.40). In regression analysis, SMA loss was predictive of poor OS (hazard ratio 1.698 (95%CI 1.038–2.778), P = 0.035). No relationship with OS was seen for PA or PLW loss.

Conclusions  Change in psoas muscle area is not representative of total muscle area change and should not be used to substitute total skeletal muscle to predict survival in patients with ovarian cancer.

Keywords  Sarcopenia; Body composition measurement; Psoas area; Computed tomography; Overall survival; Ovarian cancer

Introduction

Sixty percent of patients diagnosed with epithelial ovarian cancer have primary metastatic disease with a corresponding 5-year survival of only 28%.1 Therapeutic options for this advanced disease (International Federation of Gynecology and Obstetrics, FIGO stage IIb–IV) are either primary debulking surgery followed by adjuvant chemotherapy or induction chemotherapy followed by interval debulking surgery. The outcome of debulking surgery is by far the most important prognostic factor for patients with advanced ovarian cancer, and surgery should always be aimed at achieving complete removal of macroscopic tumour.2,3 At the same time, recent investigations have led to the discovery that skeletal muscle area (SMA) changes detected on computed tomography (CT) may be closely related to ovarian cancer survival as well.4,5 In our own ovarian cancer cohort of patients treated with induction chemotherapy and interval debulking surgery,
we have shown that patients who were able to gain or maintain muscle area during chemotherapy had a significantly better overall survival (OS) than patients who lost muscle area.\(^4\) What became apparent in this study was that a measurement over time was essential to identify muscle loss or sarcopenia. A cross-sectional single time point measurement could not detect change and was thus unable to predict survival.\(^4\) The importance of sarcopenia has scarcely been studied in ovarian cancer, and these results have yet to be confirmed in international prospective trials. However, similar results have been found for other cancer types; stable or increasing muscle mass has been reported to relate to a prolonged survival in non-small cell lung cancer, pancreatic cancer, and colorectal cancer, while a low muscle mass at baseline showed no prognostic significance.\(^6–8\)

Cross-sectional CT measurement of the total SMA at the level of the third lumbar vertebra (L3) has proven to give a reliable representation of total body muscle mass and has therefore been adopted worldwide for the detection of sarcopenia in cancer patients.\(^9,10\) As an alternative for measuring all skeletal muscle visible at L3, one can opt to evaluate the psoas muscle alone. A scientific rationale for using the psoas is not provided by any authors using this muscle for evaluation of sarcopenia, but we speculate that it might have been selected due to ease of identification or possibly because of its functional role as a hip flexor muscle. In case of a decrease in weight-bearing exercise due to physical unfitness or hospitalization, the psoas muscle is expected to decrease in volume, which can be used as a potential measure of muscle loss. Although imaging software is still needed, measuring the psoas area (PA) alone is easier and less time consuming. This method has been used to predict surgical complications in different cancer types with contrasting results. PA has shown a correlation with post-operative complications in individual studies on colorectal cancer, colorectal liver metastases, kidney cancer, bladder cancer, cholangiocarcinoma, and hepatocellular cancer,\(^11–16\) while this effect was not seen in other pancreatic cancer, endometrial cancer, biliary cancer, sarcoma studies.\(^17–20\) Interestingly, only few cancer studies were able to show a correlation between PA and survival.\(^21–23\) In a small number of non-cancer studies, decreased psoas muscle area has also been correlated with higher rates of morbidity\(^24–26\) and mortality\(^25,27–29\) in patients undergoing cardiothoracic, gastro-intestinal, and spinal surgery. To simplify evaluation of muscle area, an even quicker and easier novel method has been reported. By multiplying the length and width of the psoas muscle (PLW), the psoas area can be directly calculated without the need for specialized software. Jones et al. studied 100 patients with colorectal cancer and reported a good correlation between the standard and new method to measure the psoas muscle as well as a good correlation between measurements of the SMA in comparison to the standard PA method.\(^11\)

Evaluation of change in muscle mass over time is important for ovarian cancer prognosis; patients who are identified adequately could possibly benefit from nutritional or physical interventions. Standard CT measurements of SMA have been able to detect changes and predict OS in patients with ovarian cancer undergoing induction chemotherapy and interval debulking. This study aims to investigate whether two methods to assess the psoas muscle area, PA and PLW, reflect the total muscle area and give a reliable representation of sarcopenia in ovarian cancer with the same accuracy as SMA assessment.

**Methods**

This study has been approved by the local Medical Ethics Committee and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The requirement to obtain informed consent was waived by a Medical Ethics Committee.

**Eligible patients**

All patients with advanced ovarian cancer (FIGO 2013 stage IIB–IV) who were treated with induction chemotherapy and interval debulking in the Maastricht University Medical Centre (Maastricht, the Netherlands) between 2000 and 2015 were enrolled in this retrospective study. Patients were eligible for inclusion if they met the following criteria: (i) at least two routine abdominal CT scans were performed, the first before the start of induction chemotherapy and the second before interval debulking (typically after three to four cycles of chemotherapy); (ii) the quality of both CT scans was sufficient to perform measurements of muscle area; (iii) relevant clinical data could be retrieved from the patients’ medical records; and (iv) follow-up was at least 6 months after diagnosis. Part of this population was used in prior investigations from which the results have been published previously.\(^4\)

Patients were divided in age groups ≤60, 61–70, and >70 years according to their age at diagnosis. Surgical outcome was categorized into complete (no visible evidence of macroscopic residual disease), optimal (macroscopic residual disease ≤1 cm), or incomplete (macroscopic residual disease >1 cm). OS was defined as the period of time between the initial CT and a patient’s death from any cause as reported in national registries. Patients who were still alive at the time of analysis were censored at a fixed date.

**Computed tomography analysis**

A single axial image corresponding to the L3 vertebral body was selected for each CT. For both SMA and PA, SliceOmatic software (v5.0, Tomovision, Montreal, QC, Canada) was used to quantify skeletal muscles within predefined validated
boundaries of -29 to +150 Hounsfield Units. For SMA, the entire SMA consisting of the abdominal muscles, psoas muscles, and paraspinal muscles was demarcated. For PA, only the psoas muscle area (right and left) was selected. Following demarcation, the surface areas were quantified automatically in cm$^2$. For PLW, the length and width of the right and left psoas muscle were measured by hand and multiplied to compute the psoas surface area. Right and left psoas muscle were summated to estimate the total psoas length*width area in cm$^2$. Two trained observers (IR and JU) blinded to patient details and clinical outcomes individually applied the three methods to all CT scans (Figure 1). Afterwards, measurements were averaged between the observers. Mean assessment time was recorded for a random sample of patients ($n = 10$) for each method. This assessment time only included the time in which the actual measurements were performed after the correct axial image at the level of L3 had already been selected and the right HU boundaries had been defined.

**Statistical analysis**

Interobserver correlation between observer 1 and 2 was evaluated for each method with the Pearson correlation coefficient ($r$) and with the intraclass correlation coefficient (ICC). Pearson’s $r$ measures linear correlation between two variables and equals 1 in case of perfect positive correlation, 0 in case of no correlation at all, and -1 in case of total negative correlation. ICC can be used to assess both consistency and absolute agreement between quantitative measurements made by multiple observers measuring the same quantity. Further statistical analyses were conducted with the averaged measurements between observers. Intermeasurement correlations between the three methods to measure muscle tissue were also studied with the Pearson correlation coefficient. Additionally, a Bland–Altman plot was created to investigate the existence of any systematic differences between the two assay methods measuring psoas muscle area: PA and PLW. If the mean value of the difference between assay methods is significantly different from zero, this indicates a systematic difference between measurements.

The percentage change in muscle area between the pre- and post- chemotherapy CT scan was calculated per 100 days for each method. A measurement error of 2% was taken into account based on previously reported accuracy of CT for muscle analysis. Patients were divided into two groups based on the extent of muscle area change: ‘Loss’ in case of >2% decrease per 100 days and ‘Gain’ when any increase or ≤2% decrease was seen. Muscle area changes between -2% and +2% were considered as muscle stability and were included in the ‘Gain’ group. Subsequently, the three methods to quantify muscle area were compared categorically through the use of contingency tables. Cohen’s Kappa coefficient ($\kappa$) was computed to measure agreement between SMA and PA, SMA and PLW, and PA and PLW. In case of perfect agreement, $\kappa$ gives a value of 1.

Finally age, FIGO stage (‘FIGO stage IV’ vs. ‘FIGO stage II and III combined’), surgical outcome (‘complete’ vs. ‘incomplete and optimal combined’), and muscle change determined by SMA, PA, and PLW (‘loss’ vs. ‘gain’) were tested as effect modifiers in a univariable proportional hazards Cox-regression model at a significance level of 10%. Hazard ratios (HRs) were calculated with 95% confidence intervals (95%CI). Using backwards stepwise elimination, significant predictors were combined in a multivariable model in which a significance level of 5% was applied. All analyses were performed with the statistical software package SPSS v20.0 (IBM Corp, Chicago, IL).

**Results**

**Baseline characteristics**

In total, 190 patients with advanced ovarian cancer treated with induction chemotherapy and interval debulking were identified. Forty patients were excluded due to unavailability or insufficient quality of one or both CT scans, due to missing clinical data, or due to debulking being performed for

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**Figure 1** Muscle area measurement methods

![Figure 1](image-url)
recurrent disease. After exclusion, 150 patients and 300 CT scans were available for analysis. One hundred twenty-three of these 150 patients were used in previous investigations by our research group. Patient characteristics are presented in Table 1. Median follow-up for censored patients was 904 days ($n = 56$) with a minimum of 209 days. Specification of muscle area change resulted in a median SMA loss of 5.8% per 100 days, a median PA gain of 1.4% per 100 days, and a median PLW loss of 0.9% per 100 days. Mean assessment time was 110 s per patient, 27 s per patient, and 16 s per patient for SMA, PA, and PLW, respectively.

**Interobserver and intermeasurement correlations**

Interobserver correlation results for the different measurements on the pre-chemotherapy CT scan are given in Figure 2. For assessment of SMA and PA, agreement was almost perfect with Pearson’s $r$ values of 0.96 and 0.99, respectively. Interobserver agreement for PLW was 0.85. Mean SM measured by observer 1 was 110.7 with a standard deviation (SD) of 15.4 and mean SM measured by observer 2 was 112.2, SD 15.8. Mean PA was 13.7 (SD 3.2) for observer 1 and 13.9 (SD 3.1) for observer 2. Mean PLW was 15.9 (SD 110.2) for observer 1 and 14.4 (SD 4.3) for observer 2. ICC assessing consistency and absolute agreement between observers was 0.96 and 0.96 for SM, 0.99 and 0.99 for PA, and 0.85 and 0.80 for PLW, respectively. Interobserver agreement was also measured for post chemotherapy scans which resulted in similar Pearson’s $r$ values of 0.99, 0.98, and 0.80 for SMA, PA, and PLW, respectively.

Intermeasurement correlation between SMA and PA was 0.52 and 0.56 for pre- and post-chemotherapy scans, respectively. Correlation between SMA and PLW was 0.39 and 0.44 for pre- and post-chemotherapy scans, respectively. Correlation between PA and PLW was 0.83 and 0.84 for pre- and post-chemotherapy scans, respectively. Scatter plots for correlation between the methods applied to the pre-chemotherapy scan are shown in Figure 3. The mean difference between PLW and PA measuring psoas muscle on the pre-chemotherapy scan was 1.35 with a SD of 2.33, which was significantly different from zero ($P < 0.001$) and indicates that the two assay methods are systematically producing different results. The corresponding Bland–Altman plot created with 95% confidence intervals is shown in Figure 4.

**Table 1** Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>All patients ($n = 150$)</th>
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<tbody>
<tr>
<td>Age in years, median ± SD (range)</td>
<td>67 ± 9.8 (39–86)</td>
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<tr>
<td>≤ 60 years, n (%)</td>
<td>40 (26.7)</td>
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<tr>
<td>61–70 years, n (%)</td>
<td>51 (34.0)</td>
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<tr>
<td>&gt; 70 years, n (%)</td>
<td>59 (39.3)</td>
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<tr>
<td>FIGO tumour stage</td>
<td></td>
</tr>
<tr>
<td>II, n (%)</td>
<td>2 (1.3)</td>
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<tr>
<td>III, n (%)</td>
<td>91 (60.7)</td>
</tr>
<tr>
<td>IV, n (%)</td>
<td>57 (38.0)</td>
</tr>
<tr>
<td>Days between CT scans, median ± SD (range)</td>
<td>82.5 ± 22.4 (47–190)</td>
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<tr>
<td>SMA in cm$^2$</td>
<td></td>
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<tr>
<td>Pre-chemotherapy, median ± SD</td>
<td>110.2 ± 15.4</td>
</tr>
<tr>
<td>Post-chemotherapy, median ± SD</td>
<td>104.4 ± 14.3</td>
</tr>
<tr>
<td>PA in cm$^2$</td>
<td></td>
</tr>
<tr>
<td>Pre-chemotherapy, median ± SD</td>
<td>13.3 ± 3.1</td>
</tr>
<tr>
<td>Post-chemotherapy, median ± SD</td>
<td>13.4 ± 2.9</td>
</tr>
<tr>
<td>PLW in cm$^2$</td>
<td></td>
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<tr>
<td>Pre-chemotherapy, median ± SD</td>
<td>14.9 ± 4.3</td>
</tr>
<tr>
<td>Post-chemotherapy, median ± SD</td>
<td>14.5 ± 4.0</td>
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<tr>
<td>Muscle area changes in % change per 100 days</td>
<td></td>
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<tr>
<td>SMA, median ± SD</td>
<td>–5.8 ± 9.9</td>
</tr>
<tr>
<td>PA, median ± SD</td>
<td>+1.4 ± 21.1</td>
</tr>
<tr>
<td>PLW, median ± SD</td>
<td>–0.9 ± 15.7</td>
</tr>
<tr>
<td>Outcome interval debulking</td>
<td></td>
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<tr>
<td>Complete, n (%)</td>
<td>69 (46.0)</td>
</tr>
<tr>
<td>Optimal, n (%)</td>
<td>55 (36.7)</td>
</tr>
<tr>
<td>Incomplete, n (%)</td>
<td>26 (17.3)</td>
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<tr>
<td>OS in days, median ± SD</td>
<td>711 ± 753</td>
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</tbody>
</table>

SD, standard deviation; FIGO, International Federation of Gynaecology and Obstetrics; SMA, skeletal muscle area; PA, psoas area; PLW, psoas length*width; OS, overall survival.

**Survival analysis**

Age, FIGO stage IV, complete interval debulking and muscle loss measured with SMA were significant predictors of OS at a significance level of 10% in univariable Cox-regression analysis (Table 3). No relationship with OS was seen for measurement of psoas muscle loss with PA nor with PLW. In multivariable analysis FIGO stage IV (HR 1.730 (95%CI 1.129–2.652), $P = 0.012$), complete interval debulking (HR 0.381 (95%CI 0.246–0.589), $P < 0.001$) and loss of SMA (HR 1.698 (95%CI 1.038–2.778), $P = 0.035$) were predictive of OS. Median OS was 665 days for patients who lost SMA compared with 914 days for patients who maintained or gained SMA ($P = 0.017$).

**Discussion**

**Key findings**

The objective of this study was to investigate whether assessment of psoas muscle area reflects total muscle area and can be used to assess sarcopenia in ovarian cancer patients undergoing induction chemotherapy and interval debulking.
Two different quantification methods of the psoas muscle area were compared with the reference method of measuring total SMA. We found a weak correlation between SMA and PA measurements and an even weaker correlation between SMA and PLW. When categorizing the findings into muscle area loss or gain, high rates of discrepancies were also found between both SMA and PA or PLW. The correlation between the two assessment methods of psoas area was reasonable but also resulted in a high number of discrepancies when categorizing patients. From these data, we can conclude that measurement of psoas muscle area either with software delineation of surface area or with manual measurement of length and width does not give a reliable representation of skeletal muscle loss when compared with measurement of total skeletal muscle mass. The regression analysis confirmed these results; PA and PLW were not helpful in the prediction of OS whereas SMA proved to be an important individual factor in both univariable and multivariable Cox regression-analysis corrected for age, tumour stage, and surgical outcome. A clear difference in median OS was seen between patients who lost SMA and patients who maintained or gained SMA (665 days vs. 914 days).

Many studies have used PA instead of SMA for assessment of sarcopenia in various cancer types. Although in some studies a correlation between PA and post-operative complications was seen, the majority have failed to prove a relationship between PA-assessed sarcopenia and survival. Only few have actually assessed the agreement between PA and SMA within their population; Jones et al. studied 100 patients with colorectal cancer and reported a Spearman correlation of 0.8 for PA and SMA and a Spearman correlation of 0.94 for PA and PLW, which could not be reproduced in our cohort. A possible explanation for this discrepancy might be the difference in statistical analysis. Whereas we used the Pearson correlation coefficient, Jones et al. used Spearman’s rank correlation coefficient. Pearson’s method is used for linear relationships and was applicable to our data, while Spearman’s method can be applied to non-parametric data. The outcome produced by the two methods can vary according to the character of the data. It is unclear why the authors chose to use Spearman instead of Pearson
correlation. However, when we tested Spearman’s correlation in our data, results were not different from what we found with Pearson’s correlation and they were again not comparable to the high correlation found in Jones’ study. Another plausible difference between our studies is the software that was used: ImageJ vs. SliceOmatic. However, because both software programs measure and quantify tissue by outlining the muscle area and similar measurements are expected by both methods, this does not fully explain the difference in intermeasurement correlation. In addition to the former study, Taguchi et al. found a Spearman correlation of 0.75 for PA and SMA in 64 patients with urothelial carcinoma which was also markedly higher than the Pearson correlation of 0.52 we found in our population. In this study, a slightly different method of assessing PLW was used in which the length and width of the psoas muscle were compared separately and not as a combined measure. The reported Spearman correlation of 0.81 for PA and psoas width was comparable to our reported Pearson correlation of 0.83 for PA and PLW.

We believe that L3 psoas muscle area measurements are not representative of total L3 SMA. A plausible reason why the psoas muscle is less representative than the total muscle at L3 is that the psoas muscle is prone to be focally affected by degenerative diseases of the lumbar spine. Lumbar degenerative disc and facet joint disease can cause local atrophy of the trunk muscles and psoas muscle loss is hence not specifically related to cancer-induced sarcopenia. Psoas analysis should therefore not be conducted on patients with a medical history of spine surgery, lower back pain, degenerative lumbar instability, vertebral fracture, and deformity. This has a massive impact when studying cancer populations. First, medical records of all patients need to be evaluated to confirm which individuals are affected by the above conditions and, subsequently, will have to be excluded from the analysis. Second, degenerative diseases of the lumbar spine are rather prevalent in older patients, and in cancer populations (the mean age in our study population was >65 years). This would result in the psoas method being inapplicable in large numbers of individuals and especially elderly. Although the psoas muscle is a hip flexor muscle which could be expected to give a representation of physical fitness, the PA only represents 10% of the SMA measurable at L3. By using the PA alone you ignore vital information about the remaining skeletal muscles. Because the PA is so much smaller in comparison to the SMA, it is also much less sensitive to depict change. In our opinion, measuring change over time is the most accurate way to portray sarcopenia in ovarian cancer, and therefore we believe that PA should not be used to substitute SMA to predict survival. The rates of muscle loss

<table>
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<th>Table 2 Contingency tables</th>
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<tr>
<td>SMA vs. PA ($\kappa = 0.182$)</td>
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<tr>
<td>PA</td>
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<tr>
<td>SMA</td>
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<td>Loss</td>
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<td>Gain</td>
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SMA, skeletal muscle area; PA, psoas area; PLW, psoas length*width.
and gain we found seem to be consistent throughout the 15-year period of time that we studied because we have not noticed specific outliers within a certain time period.

Our results have shown an exceedingly well interobserver agreement with correlation coefficients between 0.96 and 0.99 for assessment of SMA and PA with SliceOmatic software. When comparing the results for Pearson’s correlation and intraclass correlation, we find almost identical results, which is suggestive for both a high consistency and high agreement between observers. A similar interobserver agreement \((r = 0.97)\) was found by Jones et al. for the evaluation of PA.\(^{11}\) Agreement between observers was less strong for the manual technique measuring PLW \((r = 0.80–0.85)\). This may be explained by the fact that the length and width of the psoas muscle are difficult to measure due to the great variation in shape of the psoas muscle. The psoas muscle is only a small muscle; therefore, any small errors in measurement may extrapolate to larger errors in rate of change when multiplying length and width in PLW measurements, contributing to a lower interobserver agreement. Additionally, when assessing psoas muscle with PLW, the intramuscular adipose tissue is included in the measurement which gives an overestimation of the actual muscle tissue. In the pathophysiology of cancer cachexia, skeletal muscle may be replaced by intramuscular adipose tissue. In this way, both the quantity and the quality of muscle are being influenced. Linear methods of assessing skeletal muscle such as PLW measurements cannot depict these important changes. Software delineation using predefined HU boundaries can take the intramuscular adipose tissue into account and therefore reflects a more accurate quantity of skeletal muscle. The overestimation of muscle surface area with a linear method is reflected in Table 1 where we find a higher mean PLW in comparison to mean PA.

**Limitations**

Due to the retrospective nature of this research, our analyses were to some extent restricted by missing data. Due to several irretrievable CT scans, a number of patients were excluded from the analysis. Also, by limiting our study population to patients who were treated with induction chemotherapy and interval debulking and excluding patients treated with primary debulking, we have created a selection bias. Effects on muscle mass might become more apparent in the population treated with induction chemotherapy which is prone to have more advanced tumour spread and/or a worse performance status. Whether evaluation of PA or muscle area estimation in general has any importance in ovarian cancer patients who receive primary debulking surgery without induction chemotherapy is unclear. Previous studies in other cancer populations have mainly focused on the relationship between PA and surgical complications and used only one CT measurement. Ovarian cancer patients selected for primary surgical treatment are also subjected to only one clinical CT scan, and it would be interesting to see if an association can be found between PA and complications in this group.

Part of our study population was used in previous investigations from which the results have been published recently.\(^4\) In this manuscript, we concluded that SMA loss was predictive of OS, which was confirmed in the current study. However, the additional value of this finding may be limited due to the overlap in patients studied (82% overlap). External validation of these findings in patients with other gynaecological malignancies and ovarian cancer specifically is imperative. Comparable results have been reported for patients with lung cancer, pancreatic cancer, and colorectal cancer, but these populations were primarily comprised of male patients and translation of these results to female patients—with lower muscle mass in general—should be applied carefully.\(^6–8\)

### Conclusion and implications for practice and research

Change in psoas muscle area is not representative of total muscle area change and should not be used to substitute total skeletal muscle to predict survival in patients with ovarian cancer undergoing induction chemotherapy and interval debulking. Assessment of psoas muscle area may be easier and quicker.
but is less sensitive to muscle change than standard assessment of total skeletal muscle. Measuring cross-sectional total SMA at L3 showed strong interobserver agreement and has proven to be a significant predictor for OS and should therefore not be substituted by psoas area evaluation alone.

This study does not answer the important question why some patients with ovarian cancer lose while others gain muscle mass. The present study underpins the observation that sarcopenia is a problem in patients with ovarian cancer and that it has a substantial effect on survival. External validation of our findings is crucial and may lead to prospective intervention trials investigating how prevention of muscle loss can improve prognosis of patients with ovarian cancer.

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The authors certify that they comply with the ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle: update 2015. \(^{32}\)

Conflict of interest

Iris Rutten, Jorne Ubachs, Roy Kruitwagen, Regina Beets-Tan, Steven Olde Damink, and Toon van Gorp declare that they have no conflicts of interest.

References


