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Comparison of Axillary Lymph Node Size in Breast Cancer and Non-Cancer Female Cadavers

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Abstract

Background

Axillary lymph node size is central to breast cancer staging and surgical decision-making. However, existing data are primarily derived from imaging or operative series, which are influenced by resolution limits, treatment effects, and selective nodal sampling. Direct anatomical comparisons of lymph node size in breast cancer and non-cancer populations remain limited. This study aimed to evaluate whether a history of breast cancer is associated with measurable differences in axillary lymph node area using systematic cadaveric dissection.

Methods

Twenty-nine female cadavers (8 with breast cancer, 21 controls) were dissected to collect axillary lymph nodes from six chains: central, humeral, infraclavicular, parasternal, pectoral, and subscapular. Lymph node area (mm^2) was measured, and outliers ($z \geq 3$) were excluded. Welch's t-test was used to compare overall node size between groups. A two-factor ANOVA assessed the effects of group, location, and their interaction on node size. Statistical significance was set at $\alpha = 0.05$.

Results

Lymph node area was significantly greater in the breast cancer group compared to controls (mean difference = 56.7 mm^2, $p < 0.001$). All node chains demonstrated significant group-level differences. The two-factor ANOVA revealed significant main effects of group ($p < 0.001$) and location ($p = 0.014$), while the group × location interaction was not significant ($p = 0.103$), suggesting generalized enlargement across chains.

Conclusions

Systematic anatomical assessment demonstrates generalized axillary lymph node enlargement in cadavers with a history of breast cancer. These findings provide direct structural data that complement imaging-based staging paradigms and establish baseline anatomical evidence for future investigations incorporating histologic correlation and larger, more diverse cohorts.

Introduction

The lymphatic system is a network of vessels, nodes, and organs that maintains homeostasis and fluid balance, supports immune function, and facilitates the absorption of dietary fats. It transports lymph, a fluid containing white blood cells, proteins, and waste, back into the bloodstream while filtering pathogens and abnormal cells through lymph nodes. In addition to their critical role in immune

surveillance, lymph nodes serve as primary routes for metastatic spread in various cancers, particularly breast and colon malignancies, and are primary sites for immune activation of these cancers (1, 2). Lymph nodes are an important part of this system and are regularly assessed when staging cancer and planning treatment. Node size has been shown to influence diagnostic accuracy and staging in breast cancer (3). Knowing the location of lymph nodes, their size, and number helps guide procedures such as sentinel lymph node biopsy (SLNB) and axillary lymph node dissection (ALND) (4, 5). ALND, which involves the removal of lymph nodes from specific levels in the axilla, has been a common method for staging and regional control in nodal clearance. However, it is associated with complications, the most notable of which is lymphedema, a condition characterized by impaired lymphatic drainage, leading to swelling, fibrosis, and functional impairment (2, 6). Since the risk of lymphedema increases when more lymph nodes are removed, many practitioners now prefer SLNB, which involves taking out fewer lymph nodes and leads to fewer complications (7).

Although preoperative imaging techniques such as ultrasound (8, 9), magnetic resonance imaging, and diffusion-weighted imaging (10) have improved the preoperative evaluation of lymph nodes, their diagnostic accuracy remains imperfect. These modalities provide indirect assessments that rely on size-based or morphologic criteria rather than direct anatomical measurement, and interpretation is influenced by factors such as resolution limits, operator dependence, and threshold selection. Substantial natural variation in lymph node size and configuration further complicates interpretation, as normal anatomical differences or reactive enlargement may mimic malignancy. Peters et al. (11) demonstrated considerable variation in axillary lymph node arrangement, which may contribute to inconsistencies in detection, staging, and clinical outcomes.

Similarly, data derived from surgical series are limited by selection bias, as lymph nodes are typically removed selectively rather than comprehensively, and are frequently altered by neoadjuvant chemotherapy, radiation, or surgical manipulation. These factors can obscure baseline nodal anatomy and size, making it difficult to distinguish disease-related changes from treatment effects. Together, these limitations highlight the need for alternative approaches that allow direct anatomical assessment of lymph node size and distribution.

The aim of this study is to compare lymph node sizes in measurements of area in the axillary regions of female cadavers with a known history of breast cancer to those without. By analyzing this relationship, we seek to provide anatomical evidence of enlarged lymph nodes potentially associated with cancer. If a consistent difference is observed, it may suggest that lymph node enlargement is linked to cancer spread, the body’s response to the tumor, or lymphatic changes associated with the cancer. We hypothesize that lymph node size in the axillary regions will be significantly larger in female cadavers with breast cancer compared to those without breast cancer. This difference may be attributed to metastatic involvement of the lymph nodes by the cancer or reactive hyperplasia. Clarifying this relationship may offer insight into cancer progression, improve preoperative planning, and refine criteria for surgical decision-making.

Methods

Embalming and dissection of cadavers

The cadavers used for this study were donated to the Georgia Campus of the Philadelphia College of Osteopathic Medicine (PCOM). Dissections were conducted by students from the Doctor of Osteopathic Medicine, Physician Assistant, and Doctorate of Physical Therapy programs across several academic terms between 2019 and 2025. Prior to participation, students received detailed instruction on the lymphatic system, lymph nodes, and study protocols from Dr. Shelley DiCecco, lead investigator and faculty member in the Physical Therapy Department. All cadavers were embalmed onsite at PCOM using standardized procedures.

The cadavers were preserved using the Maryland State Blend (MSB), a chemical embalming solution manufactured by Hydrol Chemical Company (Pennsylvania) and formulated for Georgia's climate. Prior to use, MSB was diluted at a ratio of one gallon of water per ten ounces of solution. Most cadavers received an initial infusion of 4 to 5 gallons of the diluted solution over 2 to 3 days, with an additional 3 to 4 gallons administered as needed until full vascular saturation was achieved, as assessed by PCOM's Director of Anatomical Donor Services. Following arterial infusion, the bodies were wrapped in cotton saturated with the diluted solution, sealed in plastic, placed in zipper pouches, and refrigerated for approximately 4 to 8 months prior to dissection.

Beginning in 2022, a revised embalming protocol was adopted that included a B-4 preinjection, followed sequentially by Metaflow, Rectifiant, Introfiant DC, and Introfiant OTC, before final MSB administration. Although embalming protocols used between 2019 and 2021 are not fully documented, MSB was consistently used as the primary preservative for all cadavers included in this study.

The cadavers were thoroughly dissected to examine the lymphatic system. Lymph nodes from the axillary region were the target group. Lymph nodes from the inguinal and cervical regions were also excised as a control group, providing an additional reference for lymph node size comparison. Once excised, each lymph node was measured using a soft tape measure to determine its longest diameter, notated as length, as well as the longest perpendicular diameter, notated as width. The surface area of each node was calculated in millimeters.

Cadavers

All 29 cadavers were female, ranging in age from 39 to 103 years (mean ± SD: 72.8 ± 16.2). The cohort included 24 Caucasian, 3 African American, and 2 South Asian individuals. Eight cadavers with a documented history of breast cancer were assigned to the cancer group, while 21 cadavers with no known cancer and whose cause of death was unrelated to malignancy were assigned to the control group. Each cadaver was dissected to identify lymph nodes from the following axillary regions: central, humeral, infraclavicular, parasternal, pectoral, and subscapular. Nodes were identified and classified using consistent anatomical landmarks for each region.

Anatomical landmarks for identifying lymph nodes per region

Standard anatomical landmarks were used to identify and distinguish the different lymph node regions. PCOM students conducting the dissections received a lecture on the research being conducted and the location of the nodes, accompanied by illustrations of anatomical landmarks. The following are the descriptions given to students, as well as the anatomical landmarks that were used to define the axillary lymph node groups in this study:

Central Nodes

Located in the fatty tissue at the base of the axilla, and adjacent to the intercostobrachial nerves and the second portion of the axillary artery, usually beneath the fascia and the pectoralis minor. In this study, 24 central nodes were identified from the cancer group, and 31 from the control group.

Humeral Nodes

Extend from the tendon of the latissimus dorsi muscle to the junction of the subscapular vein with the axillary vein, near the medial cutaneous brachial nerve. In this study, 11 humeral nodes were identified from the cancer group, and 22 from the control group.

Infraclavicular Nodes

Located in the adipose tissue at the clavipectoral triangle above the 1st intercostal space or the 1st indentation of the anterior serratus muscle. They are typically covered by the pectoral muscles and clavipectoral fascia. They can also be found in the apex of the axilla along the axillary vein and the first portion of the axillary artery. In this study, 10 infraclavicular nodes were identified from the cancer group, and 26 from the control group.

Parasternal Nodes

Along the inner surface of the thoracic wall, near the internal thoracic vein (usually laterally, but can be medial or both), are covered by the transverse thoracic muscle and the endothoracic fascia. Typically in the 1–6 intercostal spaces about 3 cm from the margin of the sternum. In this study, 12 parasternal nodes were identified from the cancer group, and 46 from the control group.

Pectoral Nodes

Located along the medial wall of the axilla on the anterior serratus muscle, following the lateral thoracic artery and vein between the 2nd and 7th ribs. They are usually embedded in a thin layer of adipose tissue and covered by fascia, and can also be found along the inferior border of the pectoralis minor and/or behind the pectoralis minor. Interpectoral nodes, which are present 50% of the time, are located between the pectoral muscles at the bifurcation angle of the thoracoacromial artery’s pectoral branch. In this study, 24 pectoral nodes were identified from the cancer group, and 73 from the control group.

Subscapular Nodes

Located along the subscapular and thoracodorsal vein on the posterior wall/fold of the axilla, on the inferior margin of the subscapularis muscle, and between the teres minor and latissimus dorsi muscles. They do not have much adipose coverage and are crossed over by the intercostobrachial, subscapular, and thoracodorsal nerves. In this study, 38 subscapular nodes were identified from the cancer group, and 38 from the control group.

Statistical analysis

Outliers were defined as lymph nodes with z-scores ≥ 3 and were excluded from analysis (5 cancer nodes and 22 control nodes). All analyses were conducted using IBM SPSS Statistics, with statistical significance defined as $\alpha = 0.05$ for all tests.

Normality of lymph node area distributions was assessed visually (using histograms and Q-Q plots) and through descriptive statistics. Due to non-normal distributions and unequal variances between groups, an independent samples t-test with equal variances not assumed was used to compare overall lymph node size between the cancer and control groups. A one-sided alpha was used for this test based on the a priori hypothesis that lymph nodes would be larger in the cancer group. A two-factor ANOVA was used to evaluate the effects of group (cancer vs. control) and lymph node location, as well as their interaction, on lymph node area. When a significant interaction was detected, estimated marginal means were used to interpret differences between regions.

Results

Comparison of Axillary Lymph Node Area Between Groups

Axillary lymph nodes were significantly larger in cadavers with a history of breast cancer compared to non-cancer controls. An independent-samples t-test using Welch's correction (equal variances not assumed) demonstrated a significant difference in mean lymph node area between groups ($t(140.38) = -7.64$, $p < 0.001$), with a mean difference of 56.73 mm² (Table 1).

Table 1
Mean axillary lymph node area by group

Group	N	Mean area (mm²)	SD
Breast Cancer	119	94.11	77.53
Control	236	37.38	33.31

Values are presented as mean ± standard deviation. Group comparisons were performed using Welch's independent-samples t-test due to unequal variances. n = number of lymph nodes measured; SD = standard deviation.

Effects of Group and Lymph Node Location

A two-factor analysis of variance (ANOVA) was conducted to examine the effects of group (breast cancer vs. control) and lymph node location, as well as their interaction, on lymph node area. There was a significant main effect of group, $F(1, 343) = 72.58$, $p < 0.001$, partial $\eta^2 = 0.175$, indicating that lymph nodes were larger overall in breast cancer cadavers. A significant main effect of lymph node location was also observed, $F(5, 343) = 2.90$, $p = 0.014$, partial $\eta^2 = 0.041$, reflecting variation in lymph node size across anatomical regions. The group × location interaction did not reach statistical significance, $F(5, 343) = 1.85$, $p = 0.103$, suggesting that the effect of breast cancer on lymph node size was consistent across nodal chains (Table 2).

Table 2
Two-factor ANOVA examining effects of group and lymph node location on lymph node area

Source	SS	df	MS	*F*	*p*	Partial η²
Group	193,877.91	1	193,877.91	72.58	< 0.001	0.175
Location	38,732.32	5	7,746.46	2.90	0.014	0.041
Group × Location	24,655.54	5	4,931.11	1.85	0.103	0.026
Error	916,275.67	343	2,671.36			

Two-way ANOVA was conducted to evaluate the main effects of group (breast cancer vs. control), lymph node location, and their interaction on lymph node area. SS = sum of squares; df = degrees of freedom; MS = mean square; F = F statistic; p = significance value; partial η² = measure of effect size.

Table 3
Lymph Node Area by Anatomical Location and Group

Chain	Breast Cancer n	Mean ± SD (mm²)	Control n	Mean ± SD (mm²)
Central	24	86.50 ± 75.81	31	38.97 ± 35.22
Humeral	11	86.00 ± 86.71	22	27.77 ± 22.46
Infraclavicular	10	45.70 ± 39.07	26	26.42 ± 26.93
Parasternal	12	122.25 ± 59.29	46	28.50 ± 29.17
Pectoral	24	98.33 ± 87.13	73	45.77 ± 37.44
Subscapular	38	102.45 ± 79.99	38	43.76 ± 32.87

Values are presented as mean lymph node area (mm²) ± standard deviation. n indicates the number of lymph nodes measured per anatomical location and group.

Across all examined lymph node chains, breast cancer cadavers exhibited significantly larger lymph node areas compared to controls (Fig. 1). The estimated group difference was largest for the parasternal chain, based on the marginal means plot; however, the overall group × location interaction did not reach statistical significance. These findings suggest that breast cancer is associated with generalized enlargement of axillary lymph nodes, affecting all major nodal chains. Descriptive statistics for each lymph node chain, including sample size and mean area by group, are summarized in Table 3.

Estimated marginal means of lymph node area (mm²) across anatomical locations for breast cancer and control cadavers.

Discussion

This study demonstrates a statistically significant increase in axillary lymph node size in female cadavers with a history of breast cancer compared to controls. These findings support our hypothesis and align with the existing literature, which suggests that lymph node enlargement may be related to metastatic involvement or reactive hyperplasia associated with malignancy (1, 7). The findings indicate that all examined nodal chains exhibit significantly greater mean area in the breast cancer cadavers. The estimated group difference was largest for the parasternal chain, though the overall group × location interaction did not reach statistical significance. These findings suggest that breast cancer is associated with generalized enlargement of axillary lymph nodes, affecting all major nodal chains.

While preoperative imaging techniques, such as ultrasound, magnetic resonance imaging, and diffusion-weighted imaging, are routinely used to evaluate lymph nodes, these methods are not entirely reliable, and diagnostic accuracy is influenced by factors including nodal size (3). Unlike imaging techniques, our cadaveric approach allows for direct measurement of lymph nodes without the confounding effects of clinical variables such as edema, fibrosis, or treatment effects or imaging limitations.

Additionally, our findings contribute to existing evidence connecting tumor progression with regional lymph node spread. Sopik and Narod (12) described a continuum connecting tumor size, lymph node status, and distant metastasis in patients with invasive breast cancer. The consistent lymph node enlargement observed in breast cancer cadavers further demonstrates the change in the lymphatic system due to the presence of malignancy. These findings are important for both surgical practice and anatomical education. Accurate knowledge of which lymph node regions are most affected by a specific cancer may aid in surgical planning. Given that these findings suggest that breast cancer is associated with generalized enlargement of axillary lymph nodes, affecting all major nodal chains, clinicians may be prompted to closely evaluate all major chains during sentinel lymph node biopsy and axillary dissection.

Limitations

While our results provide valuable insight into the difference between axillary lymph node sizes of breast cancer and non-cancer cadavers, there are several limitations that must be acknowledged. A relatively small sample size, particularly the 8 cadavers used in the breast cancer group, limits statistical inference

when comparing regional lymph nodes. Additionally, the cadaver population was primarily caucasian and spanned a wide age range (39–103 years old), introducing a higher potential for variability in lymph node size that is independent of breast cancer status.

Lack of complete medical records not only prevents confirmation of malignancy in lymph nodes, but also prevents assessing the impact of tumor stage, origin, and prior treatments on regional lymph node enlargement. Only area measurements were collected, rather than volume, due to limited collection capabilities, which could potentially underestimate the extent of enlargement.

It is also possible that embalming and post-mortem changes could alter lymph node dimensions. While cadavers remove the confounding variables in clinical and imaging analysis, they also lack the in vivo tissue behavior of lymph nodes, such as the dynamic flow of fluid and immune activity. It is important to remember that lymph nodes represent a static state at the time of death, and different nodes may have been in varying stages of reactivity.

Furthermore, the study lacks histological confirmation of metastasis or reactive hyperplasia, making it unclear whether enlargement was due to cancer or benign changes. Exclusion of statistical outliers, while appropriate for our analysis, reduced the sample size even further and could potentially include clinically relevant variation.

Future Directions

The limitations discussed highlight the need for future research that integrates histological analysis and more detailed inclusion of clinical data to better interpret the relationship between regional lymph node enlargement and the progression of breast cancer.

The greatest difference was seen between the pectoral and parasternal lymph node chains. This could be due to both sites being primary drainage sites for lateral and medial breast tumors, respectively. It has been found in previous research that sternal or parasternal swelling is a symptom that reveals “involvement of internal mammary nodes secondary to breast cancer” (13). Further investigation into a correlation between lymph node enlargement and a tumor’s site of origin may offer valuable insight into metastatic pathways. This knowledge could lead to earlier detection of breast cancer and guide more effective, targeted treatment strategies.

As data continues to compile, expansion of sample size and increased diversity of cadavers will strengthen the generalizability of our results if they hold true with the improved data. Future studies should include histological analysis to distinguish between metastatic involvement and benign causes of enlargement, as nodal size has been shown to influence diagnostic accuracy and staging in breast cancer (3). Additional cadaver data could be used to evaluate whether patterns of lymph node enlargement seen in the axilla also occur in other nodal regions, such as the pelvic lymph nodes, or whether they extend to cadavers with other types of cancer. Lastly, a comparison between cadaver node

sizes and pre-mortem imaging data could further evaluate the accuracy of clinical imaging in determining node morphology.

Conclusion

This study demonstrated a statistically significant increase in axillary lymph node size in female cadavers with a history of breast cancer compared to controls. The most pronounced differences were observed in the pectoral and parasternal chains, which correspond to the primary lymphatic drainage pathways of the breast. These findings support the association between breast cancer and axillary lymph node enlargement, possibly due to metastatic involvement or reactive hyperplasia.

Cadaveric dissection allowed for direct anatomical assessment of lymph nodes without the confounding effects of treatment or imaging limitations. These insights may inform surgical decision-making and staging by identifying which nodal chains are most commonly affected, potentially helping to reduce unnecessary tissue removal during axillary procedures.

However, results should be interpreted with caution given the small sample size, especially for the infraclavicular region, the lack of histopathologic confirmation of nodal involvement, and the limited demographic diversity of the cadavers. Future cadaver-based studies incorporating histological analysis and broader clinical data are warranted to further clarify the relationship between nodal size and breast cancer progression.

Declarations

Ethics approval and consent to participate

Human cadaveric specimens were obtained through the Philadelphia College of Osteopathic Medicine (PCOM) body donation program, with documented donor consent for educational and research use. According to institutional policy and applicable federal regulations, research involving cadaveric specimens does not constitute human subjects research and therefore did not require approval by the PCOM Institutional Review Board (IRB). All procedures were conducted in accordance with PCOM policies for the ethical procurement, handling, and use of donated human remains.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Shelley DiCecco: Conceptualization, methodology, supervision, manuscript review
Adam Davis: Data collection, analysis, manuscript drafting and editing
Harshita Yepuri, Julia Girgis, Sara Kurtovic, Celeste Pak, Erek Arsiniega, Jasdeep Singh: Data collection, literature review, manuscript drafting
Michael Roberts: Statistical analysis, manuscript editing
All authors read and approved the final manuscript.

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Figures

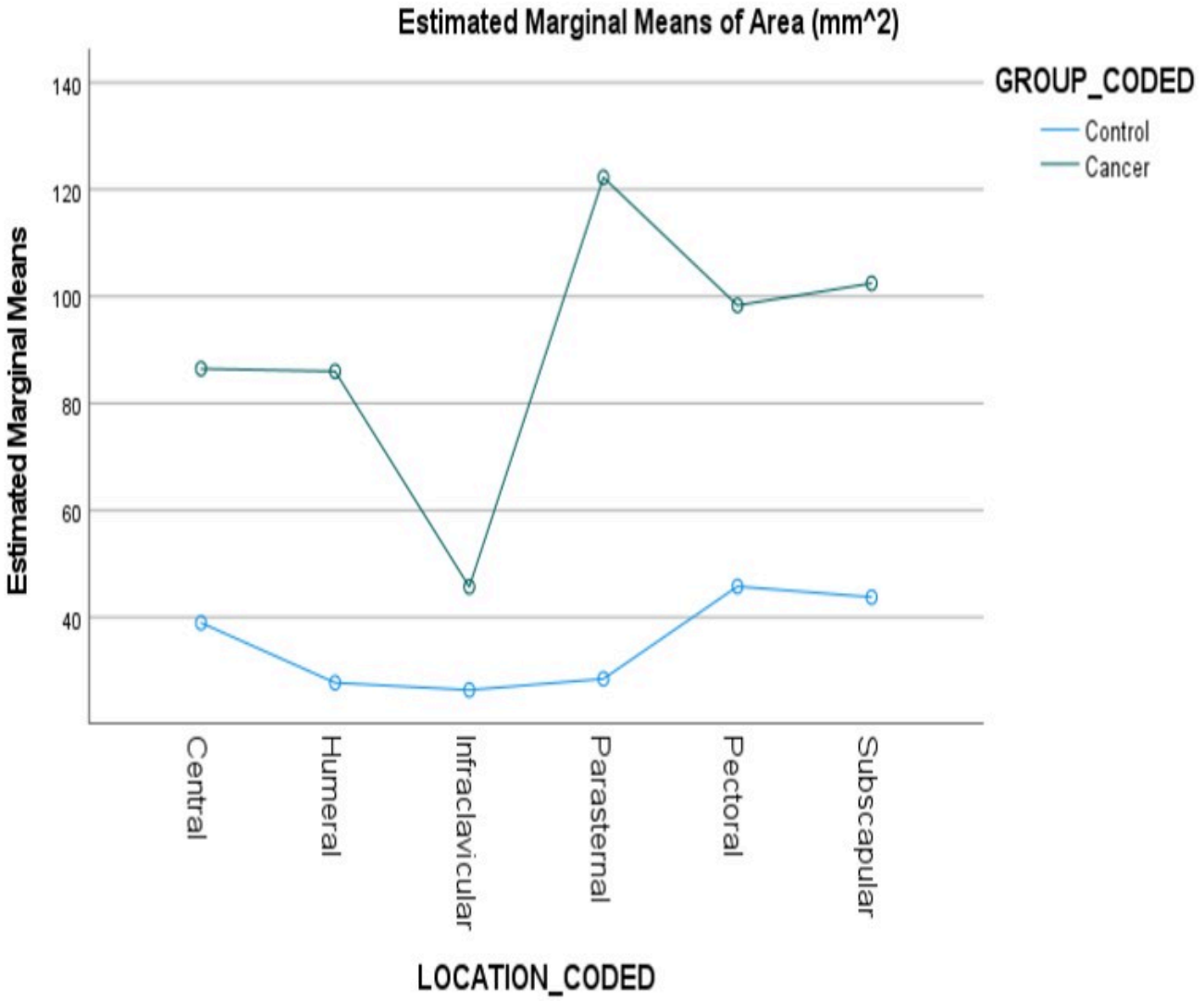


Figure 1

Estimated Marginal Means of Lymph Node Area by Group and Anatomical Location