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Adipocyte heterogeneity and tumor infiltration of adipose tissue in patients with metastatic breast cancer

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ABSTRACT

Background: The adipose tissue may serve as a source of energy supporting cancer growth and metastasis. Our understanding of the adipocytes which compose the adipose tissue in different anatomical locations of the body as well as potential microscopic tumor infiltration in patients with metastatic breast cancer remains limited. This study therefore investigates regional variations in adipocyte size and adipose tissue tumor infiltration in patients with metastatic breast cancer.

Methods: Within the UPTIDER rapid autopsy program, (NCT04531696), 94 adipose tissue samples from subcutaneous, visceral, retroperitoneal, and mammary depots of 22 patients with metastatic breast cancer were collected and analyzed. Distant adipocyte size was quantified using digital pathology, and tumor infiltration was assessed histologically. Linear mixed quantile regression analyzed the associations between adipocyte size, fat depot type and major histological subtypes.

Results: Distant adipocyte size did not significantly differ across fat depots. A trend towards smaller adipocytes in mammary fat at autopsy versus diagnosis was observed, suggesting potential age and/or treatment effects. Adipocyte size correlated positively with BMI at death, especially in subcutaneous and visceral fat. Visceral fat exhibited higher tumor infiltration, notably in patients with invasive lobular carcinoma (ILC).

Conclusion: This study highlights the relatively uniform adipocyte size across fat depots in patients with metastatic breast cancer, with potential changes in mammary adipocytes over the disease course. The microscopic tumor cell infiltration observed in the visceral fat, mainly for ILC, underscores the need to undertake additional research to understanding the contribution of the adipose tissue in breast cancer metastasis.

1. Introduction

Many tumors develop in close proximity to or in direct contact with adipose tissue. This is particularly true for breast cancer, where mammary glands are surrounded by adipose-rich tissue [1,2]. The interaction between tumor cells and their microenvironment is critical for tumor growth, tissue invasion and metastasis. Adipocytes are an often under-investigated component of the tumor microenvironment, despite the important, bi-directional interaction exists between tumors and adipose tissue, where both tumor and adipose tissue mutually respond to and influence each other [3,4].

Obesity is defined as abnormal or excessive fat accumulation and is usually reflected by a body mass index (BMI) of 30 kg/m^2 or more [5]. It

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Abbreviations

BMI	Body Mass Index
CAAs	Cancer-associated adipocytes
FABP4	Fatty Acid Binding Protein 4
FFPE	Formalin-Fixed Paraffin Embedded
H&E	Hematoxylin and Eosin
IBC-NST	Invasive Breast Cancer of No Special Type
ILC	Invasive Lobular Carcinoma
MAT	Mammary Adipose Tissue
SAT	Subcutaneous Adipose Tissue
RAT	Retroperitoneal Adipose Tissue
UPTIDER	UZ/KU Leuven Program for Tissue Donation to Enhance Research
VAT	Visceral Adipose Tissue
WAT	White Adipose Tissue

is associated with an increased risk of developing breast cancer, especially in postmenopausal women, and a worse prognosis after diagnosis of breast cancer. In individuals with obesity, adipocyte hypertrophy leads to metabolic dysregulation, immune cell recruitment, and inflamed white adipose tissue (WAT), contributing to an underlying chronic subclinical inflammation. WAT inflammation occurs across the spectrum of BMI and is likely contributing to the pathogenesis of breast cancer [6]. As BMI has limitations in correctly assessing adiposity, adipocyte size has been proposed as an alternative method to quantify adiposity at tissue level using a digital pathology tool that we previously described [7] and successfully applied [8]. A recent study also demonstrated a link between breast cancer and adipose tissue dysfunction by assessing adipocyte size and macrophage infiltration [9].

Adipose tissue is distributed throughout the human body, but the most prominent adipose tissue depots are grouped anatomically, including mammary (MAT), subcutaneous (SAT), retroperitoneal (RAT) and visceral adipose tissue (VAT). SAT lies beneath the skin and provides insulation, whereas VAT surrounds internal organs within the abdominal cavity [10]. It is generally known that SAT is more involved in lipolysis and considered healthier than VAT [11]. However, it is still unknown whether adipocyte size is similar in these different adipose tissue depots.

Tumor infiltration in adipose tissue has been recognized as a common feature of primary invasive breast carcinomas, especially of invasive lobular carcinoma (ILC) [12]. Comparative analysis has highlighted differences in lipid metabolism between primary ILC and invasive breast carcinoma of no special type (IBC-NST), with higher expression of fatty acid binding protein 4 (FABP4) in ILC [13,14]. The role of FABP4 is currently under thorough investigation, particularly in conjunction with its key partner fatty acid translocase/CD36, primarily in patients with ovarian cancer [15]. However, it is now also being studied in breast cancer as a potential therapeutic target to inhibit the fatty acid import into breast cancer cells [16].

In our post-mortem tissue donation program UPTIDER (NCT04531696) [17], we have been systematically collecting samples of different fat depots from patients with metastatic breast cancer. In the present study, we investigated the adipocytes to understand regional differences in adipocyte size and tumor infiltration of adipose tissue in the advanced stages of the disease.

2. Methods

2.1. Patients

Following written consent, patients with metastatic breast cancer were included in UPTIDER (NCT04531696) at UZ/KU Leuven. The set-up and conduct of our post-mortem tissue donation program have been published [17]. Before the start of an autopsy, a harvest planning document is created for every patient to list the metastatic lesions and non-pathological tissues to be sampled. A sample is encoded as malignant when tumor is visible during gross inspection, otherwise it is encoded as normal. For this study, we included all retroperitoneal, subcutaneous, visceral and (contralateral) mammary fat tissue samples encoded as normal. If available, historical samples from the primary breast tumor were obtained. The slides were reviewed and one representative non-tumor infiltrated slide with mammary fat was selected. We additionally collected data on patient and tumor characteristics from medical files (Supplementary Table 1).

Rapid autopsies of the patients included in the present study were performed between January 2021 and April 2023. During autopsy, tissue samples of the retroperitoneal, subcutaneous, visceral, and mammary fat depots were taken if possible and fixed into formalin-fixed paraffin embedded (FFPE) blocks.

2.2. Histopathological characteristics

Histopathological characterization was performed on the hematoxylin and eosin (H&E)-stained slides. The quality of the sample was assessed before digitalizing the slide. In case of tissue tearing or air bubbles present on the slide, another H&E-stained slide was obtained. In case tumor cells were detected in the autopsy samples, tumor cellularity and histological subtype were defined according to the WHO classification of breast tumors [18].

2.3. Digital pathology analysis

Based on microscopic evidence and literature [19], we defined adipocytes distant from the tumor as those being at least 2 mm away from cancer cells as well as 2 mm away from normal epithelial and fibrotic areas. Thin fibrous septa that carry blood supply for the adipocytes (corresponding to perilobular fibrosis score 1) were not excluded since these do not affect the adipocyte size [20]. Distant adipocyte size was measured using HALO version 2.3 (Vacuole module, Indica Labs, Corrales, CA) on digitally scanned H&E-stained slides [7]. Annotation, segmentation, count and measurements of area and diameter of adipocytes were performed on the scanned fat tissue slides per patient. We extracted the area, perimeter, and diameter of each single adipocyte for each annotated region. The areas to be analyzed were drawn by H.I. and checked by breast pathologist in training G.Z. to obtain approximately 500 adipocytes and calculate the median adipocyte area, perimeter, and diameter. Adipocytes with incomplete membranes or adipocytes that touched the border of the image were manually excluded.

2.4. Statistics

The violin plots describing the intra-patient inter-adipocyte region heterogeneity were created using the ggplot2 package (v3.4.3). Quantile regression analysis was performed to measure the association between median adipocyte area (as the outcome, in μm^2) and the groups of interest (as independent co-variates: Fat type of interest vs Reference fat type, ILC vs IBC-NST histological subtype) with an included random effect on patient ID. The analysis was performed using the lqmm package (v1.5.8) and the results were plotted using a forest plot with the ggplot2 package (v3.4.3) on R version 4.3. Spearman rank correlation was employed to study the strength of linear association between fat types of interest and independent variables (Age and BMI at death). A spearman correlation coefficient ($Rs \geq 0.6$) was considered as a strong correlation.

3. Results

3.1. Fat tissue sampling

To characterize the main histopathological features and the adipocytes of the primary breast tumors, resection specimens at time of surgery were retrieved, of which 13 out of 22 (59.1 %) had sufficient fat available for investigation (Fig. 1 and Supplementary Table 1). At diagnosis, 4 out of 22 patients (18.2 %) had triple-negative breast cancer, 1 patient had ER+/PR+/HER2+ breast cancer (4.5 %), 1 patient had ER-/PR+/HER2- breast cancer (4.5 %), 2 patients had ER+/PR-/HER2- breast cancer (9.1 %) and the remaining 14 patients had ER+/PR+/HER2- breast cancer (63.6 %). Histologically, 5 patients (22.7 %) presented with ILC, 3 patients (13.6 %) with mixed IBC-NST/ILC, 1 patient (4.5 %) with mixed IBC-NST/metaplastic squamous cell carcinoma and the remaining 13 patients (59.1 %) with IBC-NST. Of these patients, 6 patients received neo-adjuvant therapy followed by mastectomy (n = 5) or breast conserving surgery (n = 1), while 12 other patients had primary surgery (mastectomy n = 5, breast conserving surgery n = 7). The median number of treatment lines was 6 with a range from 2 to 11 treatment lines.

The patients had metastatic lesions present in different anatomical locations (overview from autopsy findings in Supplementary Fig. 1). Additionally, fat tissue samples were collected from subcutaneous (SAT), retroperitoneal (RAT), visceral (VAT), and (contralateral) mammary fat depots (MAT) from all patients during the post-mortem tissue donation (Fig. 2A). Only 1 visceral non-tumor infiltrated fat sample and 6 contralateral mammary fat samples were not taken (Fig. 2C).

3.2. Regional differences of fat samples

For each patient, adipocyte parameters including area, diameter, and perimeter were assessed using HALO® to compare size across different fat depots (Supplementary Table 2). As shown in Fig. 3A and B, median adipocyte area exhibited a heterogeneous distribution across patients and regions, with no statistically significant differences observed among the distinct fat regions (Fig. 3C and Supplementary Table 3). We compared distant adipocyte size between MAT from the resection specimen at time of surgery and MAT at autopsy. Only 7 samples of the 15 MAT samples at autopsy showed sufficient adipose tissue for HALO® analysis. We observed no statistically significant differences in distant adipocyte size between the mammary fat from the resection specimen and the (contralateral) MAT taken at autopsy, although there was a trend towards smaller adipocytes in the mammary fat samples obtained at autopsy.

To assess the correlation between adipocyte size and BMI or age at death, these parameters were visualized using scatter plots and correlations were investigated (Fig. 4). The strongest positive correlation was observed between BMI at death and adipocyte size in both the subcutaneous and visceral regions, with Spearman correlation coefficients of 0.55 and 0.54, respectively.

3.3. Infiltration of fat samples by the tumor

In addition to the assessment of adipocyte size on post-mortem fat tissue samples initially deemed macroscopically devoid of tumor, we further conducted assessments to discern microscopic tumor presence or infiltration (Fig. 5).

We observed a higher frequency of tumor infiltration in VAT (9 out of 21 or 42.9 %) compared to other regions (SAT 2 out of 22 or 9.1 %, RAT 2 out of 22 or 9.1 % and MAT 1 out of 16 or 6.3 %). Specifically, we found a higher incidence of tumor infiltration in post-mortem fat tissue samples in patients with primary breast cancer histologically classified as ILC. Among the patients with primary IBC-NST, 3 out of 13 (23.1 %) exhibited fat tissue infiltration, while 4 out of 5 patients (80 %) with primary ILC and all 3 patients with primary mixed IBC-NST/ILC showed infiltration in at least one of the fat tissue regions. Remarkably, patients with mixed IBC-NST/ILC histology exclusively demonstrated infiltration of the fat tissue by the ILC component of the tumor [21].

We also examined adipocyte size of the distant adipocytes by histological subtype and found a trend of larger adipocytes in IBC-NST lesions compared to ILC lesions across all regions (Fig. 5C).

4. Discussion

While the adipose tissue plays a crucial role in the tumor microenvironment and significantly influences tumor development and metastasis, our understanding of the contribution of adipocytes in patients with breast cancer remains limited [2]. This paper aimed to shed light on this topic by first exploring regional variations in adipocytes across different fat depots collected in the context of post-mortem tissue donation using digital pathology [7] and, second, by investigating fat tissue tumor infiltration, to gain valuable insights into the complexities of adipose tissue in breast cancer.

Understanding adipocyte size in the context of breast cancer is relevant for several reasons. First, since adipocyte size reflects of adiposity, accurate measurement is crucial. This is particularly important because BMI may not consistently and reliably reflect adipose tissue

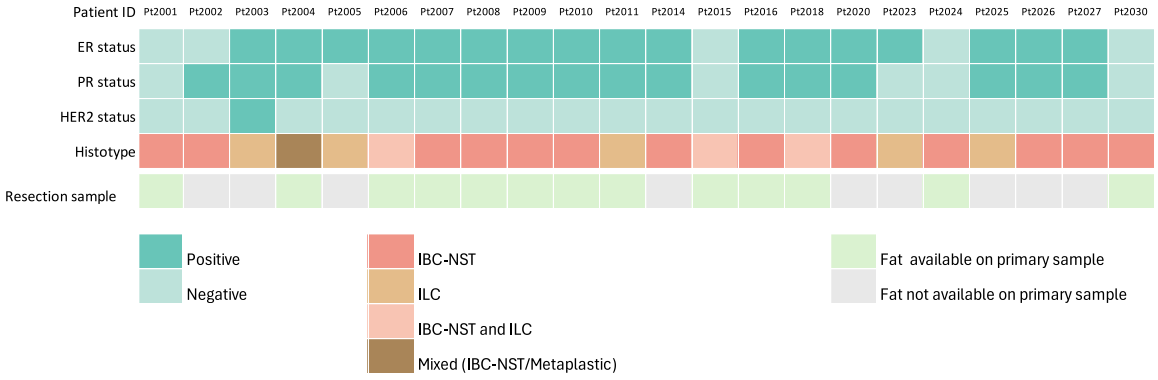


Fig. 1. Characteristics of primary tumor and availability of adipocytes from primary tumor resection specimen. General information on the primary breast tumor: estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) status, and histological subtype for the primary resection specimen (invasive breast carcinoma of non-special type (IBC-NST), invasive lobular carcinoma (ILC), IBC-NST and ILC (mixed), and mixed IBC-NST/metaplastic squamous cell carcinoma) (Mixed IBC-NST/Metaplastic)). Overview of the availability of the fat tissue samples containing distant adipocytes on the mammary fat samples at time of surgery.

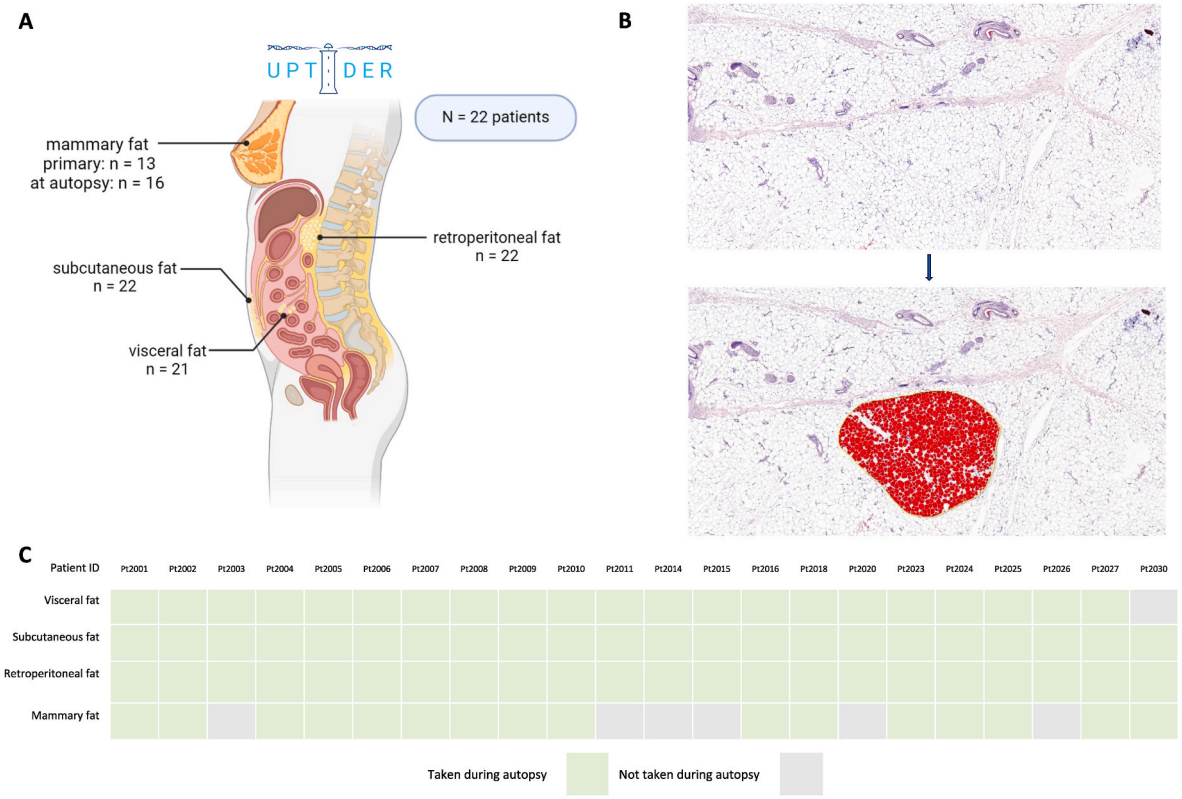


Fig. 2. Overview of the available fat samples from the regions of interest annotated with HALO®. (A) Fat tissue was sampled from various depots across the body during autopsy. These depots included mammary fat nestled in the female breast, retroperitoneal fat located behind the abdominal or peritoneal cavity, subcutaneous fat located underneath the abdominal skin, and visceral fat found between the organs of the abdomen or peritoneal cavity. (B) Example annotation with HALO® of the adipose tissue region on an H&E-stained slide, taken from the subcutaneous fat depot of Pt2017. Adipocytes distant from the tumor and fibrotic areas were annotated. (C) The availability of fat tissue samples from four different fat depots across 22 patients with metastatic breast cancer included in the UPTIDER post-mortem tissue donation program.

distribution throughout the body, adipose tissue inflammation or metabolic health [6]. Additionally, the extent to which adipocytes biopsied from distinct anatomic regions differ from each other has not been investigated previously in patients with breast cancer. Obtaining these different fat tissues from living patients is not feasible or ethical due to the invasive nature and practical challenges associated with acquiring multiple biopsies from various adipose depots.

Looking into previous studies, researchers have identified variations in fat metabolism among different fat regions in healthy women [10,22]. These studies often highlighted increased lipolysis, higher lipid content, larger adipocytes, and greater metabolic and inflammatory activity in SAT compared to VAT and have generally considered SAT as healthier than VAT [11]. Driven by our interest in assessing fat tissue variations in patients with breast cancer, we investigated regional disparities in distinct fat tissue depots, encompassing SAT, VAT, RAT, and MAT. Our analysis investigated characteristics of distant adipocytes—area, diameter, and perimeter—using specimens from resection surgeries or post-mortem samples.

We found no significant differences in adipocyte size when comparing fat regions to each other, which could indicate relatively uniform adipose tissue status across these different fat depots in patients with metastatic breast cancer. This uniformity may indicate similar metabolic activity across these fat depots.

Even though no significant differences could be demonstrated, there was a trend towards smaller adipocytes in the MAT obtained at autopsy compared to the matched mammary fat samples taken from the resection specimen at time of surgery. This could suggest a progressive change in the adipose tissue microenvironment in the breast during the course of metastatic breast cancer. This change might indicate a shift in the overall health of patients near the end of life, including factors like BMI, menopausal status, and effects of aging. Alternatively, it could also reflect the effects of treatment over time in this heavily treated patient cohort. Almost all patients were treated with multiple lines of endocrine therapy, however as previously reported we would expect to see a hypertrophic appearance of adipocytes in these samples which we did not observe here [23]. Next to endocrine therapy, all patients received chemo- and/or radiotherapy which has been associated with smaller adipocyte size [24,25]. Additionally, the results may also be influenced by the significant weight loss some patients experience at the end of their life, a factor that could be attributed to cancer-associated cachexia [26]. It is widely recognized that cachexia, a multifactorial condition, impacts the quality of life of patients and response to therapy, which leads to poorer outcomes for cancer patients [27,28]. Finally, the change in adipocyte size could signify a transition towards a more adverse adipose tissue phenotype, potentially influencing disease progression, treatment response, and patient outcomes [29]. Further exploration of the underlying mechanisms behind these changes is necessary to fully grasp their clinical implications and potential therapeutic strategies.

While a connection between BMI and adipocyte size is anticipated, the highest correlation between adipocyte size and BMI was found in VAT and SAT. This suggests that changes in BMI may be more reflective of changes in adipocyte size or adipose dysfunction in VAT and SAT compared to MAT and RAT.

In addition to examining adipocyte size differences across fat depots, we also investigated tumor infiltration of fat tissue samples initially presumed to be macroscopically tumor-free. Contrary to initial expectations, we found tumor infiltration of fat tissue regions in 10 out of 22 patients. These samples revealed a more extensive disease state at

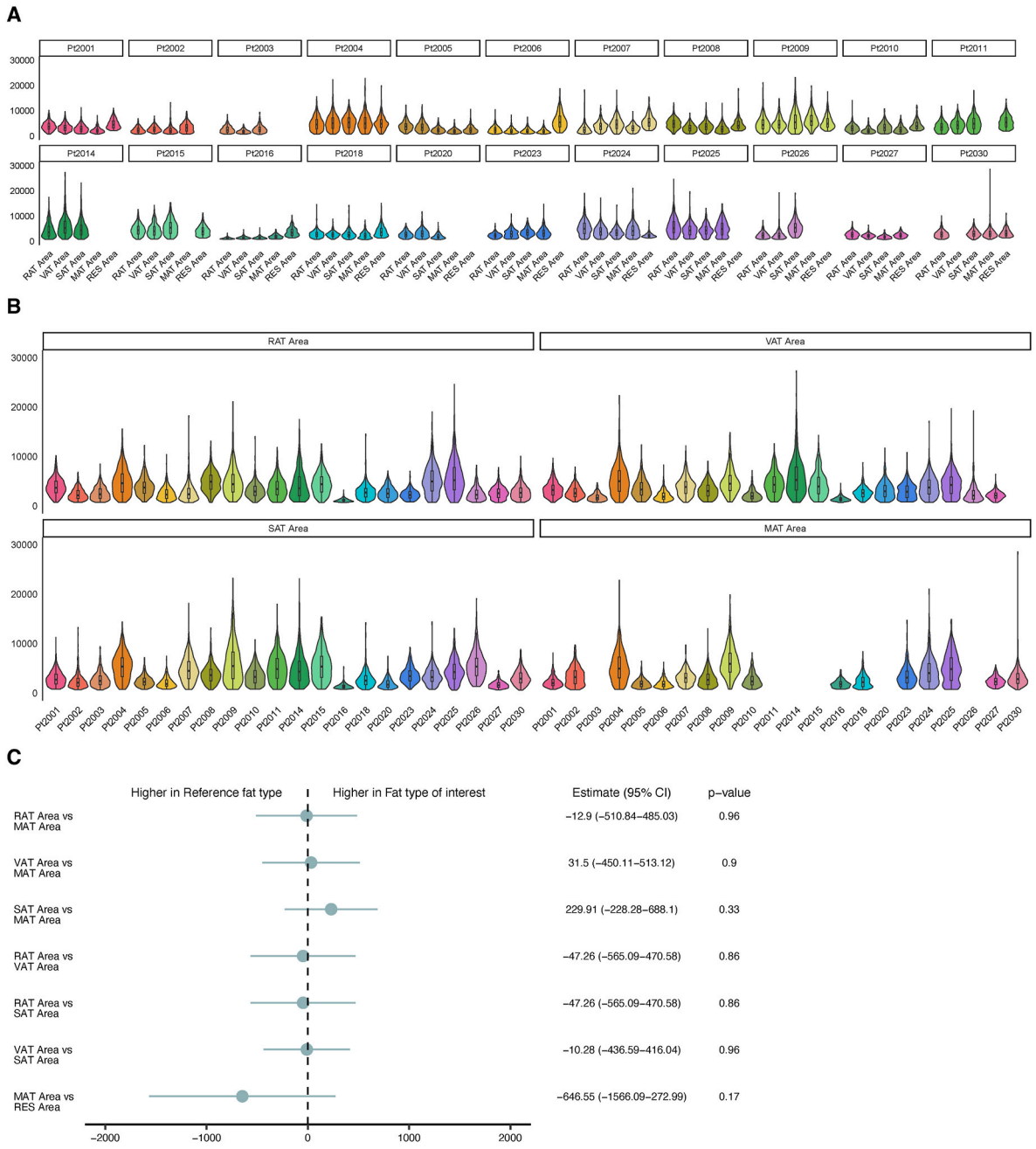


Fig. 3. Distribution of adipocyte area across patients and regions. (A and B) Heterogeneous distribution of the adipocyte area across patients (A) and fat regions (B). (C) Forest plot depicting median change in adipocyte area between regional fat types. We observed no statistically significant differences observed among the distinct fat regions. Mammary adipose tissue taken at autopsy was associated with smaller adipocyte area compared to the resection specimen taken at time of surgery. Note that 9 out of 22 (40.9 %) of mammary fat tissue samples taken at autopsy did not contain sufficient adipose tissue and 6 out of 22 (27.3 %) of mammary fat tissue samples were not taken during autopsy. RAT: Retroperitoneal adipose tissue, VAT: Visceral adipose tissue, SAT: Subcutaneous adipose tissue, RES: adipose tissue from resection specimen.

autopsy, underscoring the pervasive nature of breast cancer metastases.

One notable observation was the higher tumor infiltration in VAT as compared to other regions, irrespective of tumor histotype. When comparing different tumor histotypes, however, we found more fat tissue infiltration by ILC compared to IBC-NST. This is a common observation for primary ILC [12] but was previously unexplored in the metastatic setting, despite being acknowledged in clinical contexts [30]. It is worth noting that this observation might also be influenced by the greater number of visceral fat samples collected from patients with ILC, as more extensive random sampling of VAT was undertaken during autopsies for this specific cancer histotype.

Although the comparison was not statistically significant, we observed that adipocytes were larger in IBC-NST when compared to ILC in all regions, further underscoring the divergent characteristics between these two breast cancer histotypes. This finding complements our previous findings of smaller size of cancer-associated adipocytes in ILC [7].

Emerging evidence suggests that ILC exhibits a distinct lipid metabolism profile compared to IBC-NST [13,14,31]. Pre-clinical studies have highlighted key differences, notably the upregulation of hormone-sensitive lipase and FABP4, both key proteins involved in lipid metabolism, as well as lower expression of perilipin A, a regulator of

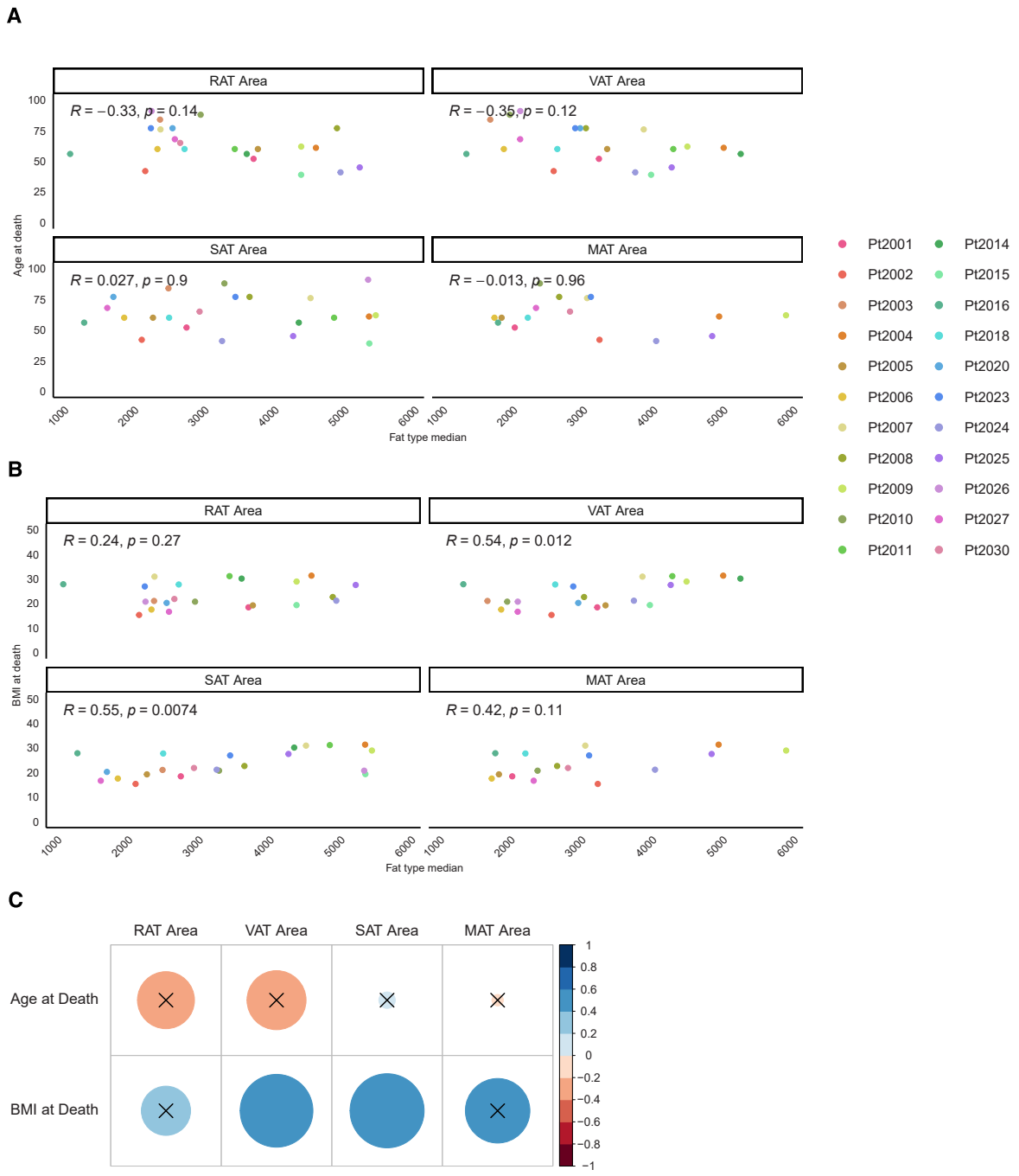
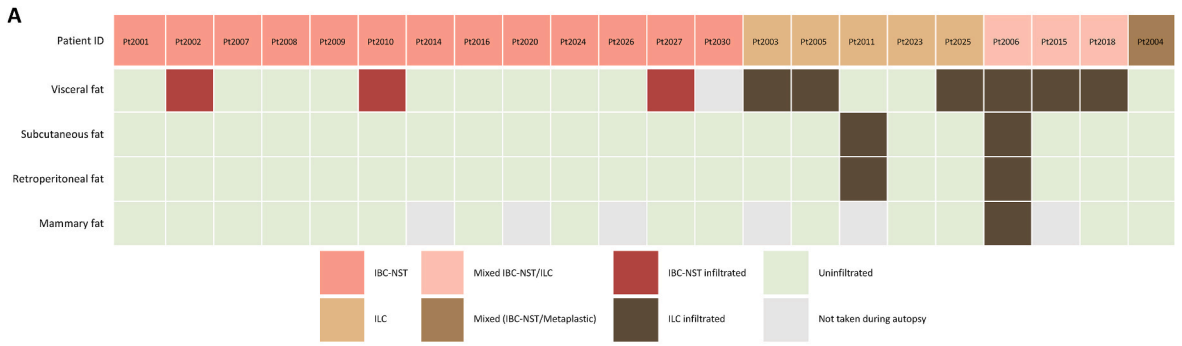


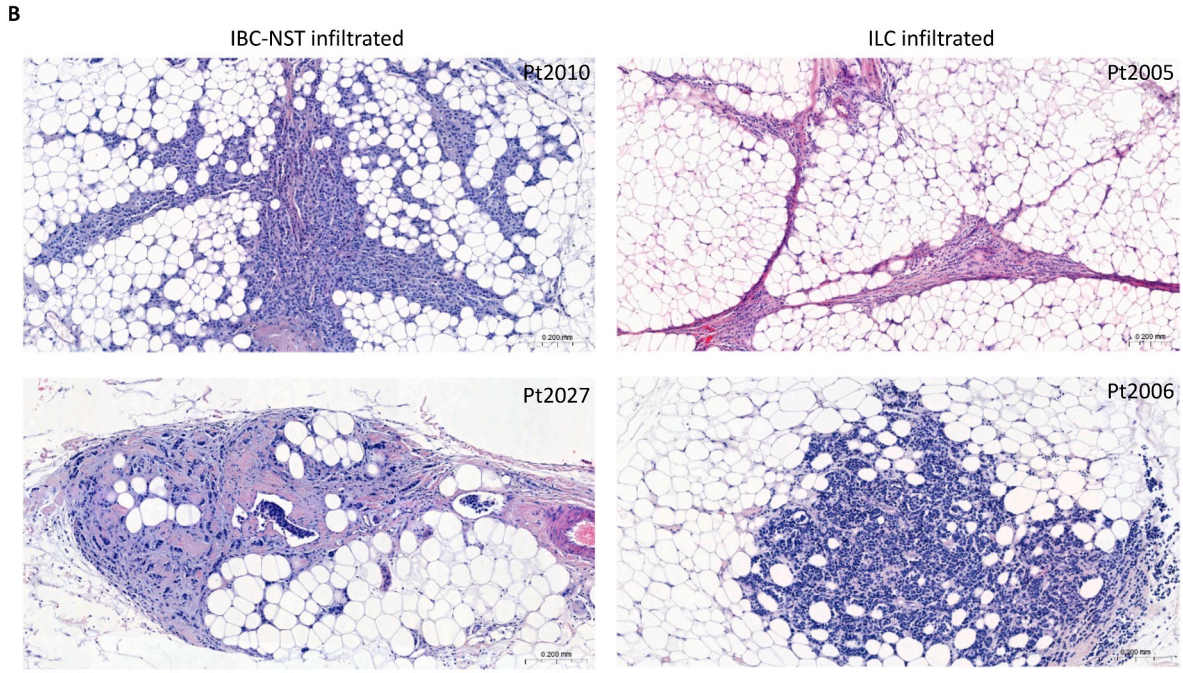
Fig. 4. The correlation between BMI and age at death with adipocyte size. Scatter plots illustrate the relationship between BMI or age at death and median adipocyte area per region. Spearman correlation coefficients (Rs) show the strongest positive relationship between BMI at death and adipocyte area in subcutaneous or visceral fat samples. RAT: Retroperitoneal adipose tissue, VAT: Visceral adipose tissue, SAT: Subcutaneous adipose tissue, MAT: mammary adipose tissue, RES: adipose tissue from resection specimen.

lipid droplet formation, in ILC as compared to IBC-NST [13,15]. Another report also highlighted the role of lipid metabolism in endocrine resistance in ILC cell lines, showing distinct expression patterns in ILC compared to IBC-NST [32]. The distinct lipid metabolism profile observed in ILC, characterized by alterations in key lipid metabolism-related proteins, could possibly be partially responsible for the different biological behaviour of ILC.

We faced certain challenges in this descriptive study, such as the paucity of fat tissue within the primary resection specimens and at autopsy. Nevertheless, the immense effort of the UPTIDER rapid autopsy program enabled us to investigate invaluable adipose tissue samples that might otherwise not be available in patients with metastatic breast cancer [17].

While the investigation primarily focused on distant adipocytes, it is crucial to note that cancer-associated adipocytes may hold greater clinical relevance for studies examining tumor-fat interactions or adipocyte size changes. These nuances underscore the intricacies involved in studying adipose tissue in the context of advanced breast cancer and emphasize the necessity for cautious interpretation and further research to elucidate the clinical implications of these findings.





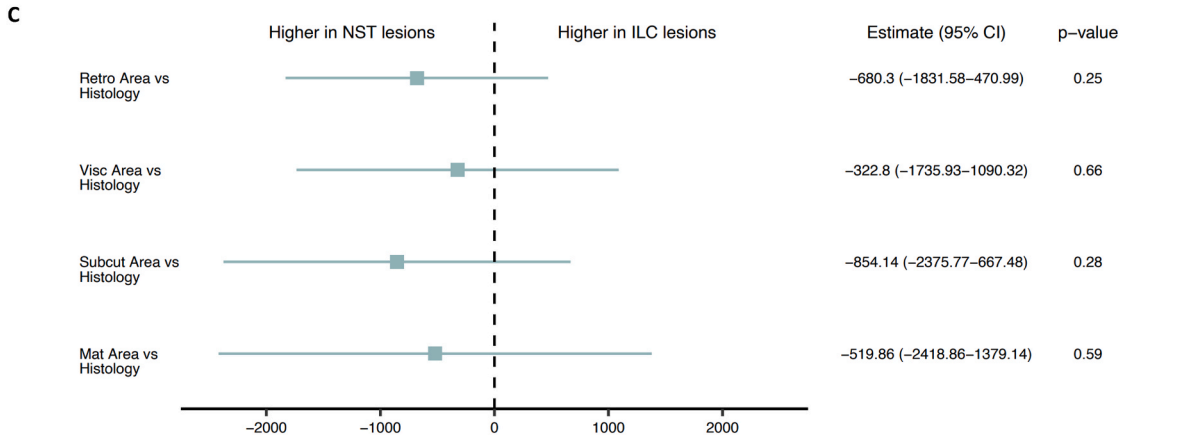


Fig. 5. Tumor infiltration of adipose tissue showing higher frequency in ILC patients. (A) Visceral fat tissue samples exhibited a higher frequency of tumor infiltrations (9 out of 21 or 42,9 %) compared to other regions (maximum of 2 out of 22 or 9.1 %). Patients diagnosed with primary breast cancer classified as invasive lobular carcinoma (ILC) demonstrated a notably higher incidence of tumor infiltration in post-mortem fat tissue samples compared to invasive breast cancer of no-special type (IBC-NST) (respectively, 4 out of 5 patients (80 %) and 3 out of 13 patients (23.1 %)). All patients with primary mixed IBC-NST/ILC histology displayed tumor infiltration of the adipose tissue and only the ILC component was present. (B) Depiction of tumor infiltration of fat tissue in patients with a primary diagnosis of IBC-NST (left side) and ILC or mixed IBC-NST/ILC (right side) breast cancer. (C) Forest plots depicting differences in adipocyte area between major histological subtypes, IBC-NST and ILC, showing a trend towards larger adipocyte area across all regions in the IBC-NST lesions, in comparison to ILC lesions.

5. Conclusion

Our study investigated adipose tissue samples, collected during rapid autopsy from patients with metastatic breast cancer. The results revealed that adipocyte characteristics are relatively uniform across different fat depots in patients with metastatic breast cancer. Yet, we observed a trend towards smaller adipocytes in mammary fat at autopsy compared to surgery after diagnosis, indicating potential metabolic shifts over time. As could be expected, we found a significant positive correlation between adipocyte size and BMI, which was most pronounced for the subcutaneous and visceral adipose regions. Additionally, when we explored tumor infiltration in fat tissue, we found the highest frequency in the visceral component, mainly in patients with ILC. We also observed a trend for smaller adipocytes in ILC, underscoring the distinct nature of ILC tumors. These findings emphasize the importance of adipose tissue dynamics in understanding breast cancer metastasis. Further research is essential to elucidate these mechanisms and the role of adipose tissue in disease progression and resistance to treatment of metastatic breast cancer.

CRediT authorship contribution statement

Hava Izci: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Gitte Zels:** Writing – review & editing, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Anirudh Pabba:** Writing – review & editing, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Marion Maetens:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **François Richard:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Maxim De Schepper:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Josephine Van Cauwenberge:** Writing – review & editing, Resources, Investigation, Data curation. **Ha-Linh Nguyen:** Writing – review & editing, Resources, Investigation, Data curation. **Kristien Borremans:** Writing – review & editing, Resources, Investigation, Data curation. **Sophia Leduc:** Writing – review & editing, Resources, Investigation, Data curation. **Karen Van Baelen:** Writing – review & editing, Resources, Investigation, Data curation. **Sigrid Hatse:** Writing – review & editing, Investigation. **Tatjana Geukens:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Amena Mahdami:** Writing – review & editing, Resources, Investigation, Data curation. **Hans Wildiers:** Writing – review & editing, Resources. **Patrick Neven:** Writing – review & editing, Resources. **Wouter Van Den Bogaert:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Giuseppe Floris:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Christine Desmedt:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Ethical approval

The project has been approved on November 30th, 2020, by the ethics committee of the University Hospitals Leuven (S64410).

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Declaration of competing interest

All authors have no competing financial or non-financial interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2024.103852.

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