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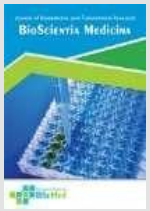

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Dynamic Coupling of Systemic and Intratumoral Estradiol in HER2-Positive and Triple-Negative Breast Cancer: A Validation Study of a Non-Invasive Surrogate

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A B S T R A C T

Background: The role of estradiol in the tumor microenvironment (TME) of estrogen receptor (ER)-negative breast cancers is increasingly recognized. Direct measurement of intratumoral estradiol is invasive, creating a barrier to clinical research. This study aimed to determine if circulating plasma estradiol can serve as a high-fidelity, non-invasive surrogate for intratumoral concentrations in HER2-positive (HER2+) and triple-negative breast cancer (TNBC). **Methods:** This retrospective, cross-sectional study included 60 women with primary operable HER2+ (n=21) and TNBC (n=39) who underwent mastectomy. Paired pre-operative plasma and post-operative tumor tissue samples were analyzed. Estradiol concentrations were quantified using a validated high-performance liquid chromatography-radioimmunoassay (HPLC-RIA) protocol. Clinicopathological data, including Body Mass Index (BMI), were collected. The primary outcome was the correlation between plasma and intratumoral estradiol, assessed by Spearman's rank correlation. Paired concentrations were compared using the Wilcoxon signed-rank test. **Results:** Baseline clinicopathological characteristics, including BMI, were well-balanced between the HER2+ and TNBC cohorts. A highly significant, strong positive correlation was found between plasma and intratumoral estradiol concentrations across the entire cohort (Spearman's $\rho = 0.78$, $p < 0.001$). This correlation remained robust in subgroup analyses of menopausal status and tumor grade. Interestingly, median intratumoral estradiol levels (30.0 pg/mL; IQR: 10.0-65.0) were significantly lower than paired median plasma levels (132.0 pg/mL; IQR: 40.0-225.0) ($p < 0.001$). **Conclusion:** Plasma estradiol demonstrates a strong and direct correlation with intratumoral estradiol in HER2+ and TNBC, validating its use as a reliable, non-invasive surrogate. This provides a crucial tool to explore the pathophysiology of the TME. The finding that intratumoral levels are lower than systemic circulation, yet tightly coupled, suggests a dynamic equilibrium that warrants further investigation into local estradiol metabolism and signaling in ER-negative disease.

1. Introduction

Breast cancer is a leading cause of global cancer morbidity and mortality, characterized by profound molecular and clinical heterogeneity.[1] This heterogeneity is clinically stratified by the expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), which defines distinct biological subtypes with unique therapeutic vulnerabilities. The luminal subtypes, driven by estrogen signaling through

nuclear ERα, are the primary target of endocrine therapies.[2] In stark contrast, HER2-positive (HER2+) and triple-negative breast cancers (TNBC), which are defined by the absence of significant ER expression, are considered conventionally "hormone-insensitive," with treatment paradigms revolving around targeted therapies, chemotherapy, and immunotherapy.[3]

Despite this classification, a compelling body of evidence indicates that the most potent endogenous estrogen, 17β-estradiol (E2), continues to exert significant pro-tumorigenic influence in ER-negative disease. The classical paradigm of estrogen action involves genomic signaling via nuclear receptors, but E2 also triggers rapid, non-genomic signaling cascades through membrane-associated receptors, such as the G protein-coupled estrogen receptor 1 (GPER1).[4] GPER1 is widely expressed in ER-negative breast cancers, and its activation by estradiol can transactivate key oncogenic drivers like the epidermal growth factor receptor (EGFR) and HER2. This initiates downstream signaling through critical survival pathways, including PI3K/AKT/mTOR and MAPK, thereby promoting tumor proliferation, survival, and metastasis independently of nuclear ERα.[5]

Furthermore, the breast tumor microenvironment (TME) is an endocrinologically active site. Both tumor cells and surrounding breast adipose fibroblasts express steroidogenic enzymes, particularly aromatase, which facilitates the local synthesis of estradiol from circulating androgen precursors.[6] This "intracrine" and "paracrine" production creates a unique intratumoral hormonal milieu. Understanding the concentration and dynamics of estradiol within this space is therefore paramount to fully elucidating its role in ER-negative cancer pathophysiology.[7] However, research in this domain has been severely constrained by a critical methodological barrier: the direct measurement of intratumoral estradiol necessitates fresh tumor tissue obtained via invasive surgical procedures, rendering it unfeasible for routine monitoring or large-scale investigation.[8] This has left the intratumoral hormonal landscape as a biological "black box" in the clinical setting.

A validated, non-invasive surrogate marker that accurately reflects intratumoral estradiol would be a transformative tool, enabling researchers to probe the biological effects of local E2 and potentially uncover novel prognostic biomarkers or therapeutic targets. Circulating plasma estradiol is the most logical candidate. While its role as a risk factor for ER-positive cancer is well-established, its relationship with intratumoral levels, specifically within the HER2+ and TNBC subtypes, has not been rigorously characterized. Establishing this correlation is the foundational step required before plasma E2 can be leveraged for any translational application in this patient population.[9,10]

Therefore, the primary aim of this study was to rigorously investigate the correlation between pre-operative circulating plasma estradiol and surgically excised intratumoral estradiol concentrations in a well-characterized cohort of patients with HER2-positive and triple-negative breast cancer. The novelty of this research lies in its specific focus on these aggressive, ER-negative subtypes and its objective to provide the foundational evidence required to validate plasma E2 as a non-invasive tool to unlock the "black box" of the tumor hormonal microenvironment.

2. Methods

This study was a retrospective, cross-sectional analysis conducted at Dr. Moewardi Regional General Hospital, a tertiary referral center in Surakarta, Indonesia. The protocol adhered to the principles of the Declaration of Helsinki and received full ethical approval from the Health Research Ethics Committee of Dr. Moewardi Regional General Hospital (Ref: 1357/XII/HREC/2022).

The hospital's electronic medical record and pathology databases were screened to identify female patients diagnosed with primary, operable, unilateral breast cancer who underwent mastectomy between January 2018 and December 2023. Inclusion criteria were (1) Female gender, age ≥ 18 years; (2) Histologically confirmed invasive breast carcinoma; (3)

Immunohistochemical (IHC) profile consistent with HER2+ (ER-negative, PR-negative, HER2 IHC 3+ or HER2 FISH/SISH amplified) or TNBC (ER-negative, PR-negative, HER2 IHC 0/1+ or HER2 FISH/SISH non-amplified); (4) Availability of archived pre-operative blood samples and post-operative fresh-frozen tumor tissue; (5) Complete clinicopathological data. Exclusion criteria were (1) Luminal A/B (ER and/or PR positive) breast cancer; (2) History of neoadjuvant systemic therapy; (3) Metastatic (Stage IV) disease; (4) Current or recent (within 6 months) use of hormonal therapies; (5) Bilateral breast cancer or other concurrent malignancy; (6) Inadequate sample quality/quantity; (7) Incomplete medical records.

A total of 148 patients with HER2+ or TNBC were initially screened. Of these, 88 were excluded for reasons including prior neoadjuvant therapy (n=45), missing paired samples (n=27), or incomplete data (n=16). The final analytical cohort consisted of 60 patients who met all criteria. The sample size was determined by the availability of high-quality, paired archival samples meeting all study criteria. A post-hoc power analysis was conducted using G*Power 3.1. Based on the primary outcome of a correlation analysis, a total sample size of N=60 achieves >99% power to detect a large effect size ($\rho = 0.50$) and 98% power to detect a medium-to-large effect size ($\rho = 0.40$) at a two-sided alpha level of 0.05. This confirms the study was sufficiently powered to detect the strong correlation ultimately observed.

A standardized form was used to extract: age, Body Mass Index (BMI, kg/m^2), menopausal status (>12 months amenorrhea), tumor size, lymph node status, histological type, nuclear grade, and IHC data (ER, PR, HER2, Ki-67). Venous blood samples (10 mL) were drawn into EDTA tubes in the pre-operative setting between 7:00 AM and 9:00 AM on the morning of surgery. Samples were centrifuged at 1,500 x g for 15 minutes at 4°C within one hour. The resulting plasma was aliquoted and stored at -80°C. Immediately post-mastectomy, a representative tumor sample (minimum 0.5 cm^3), free of necrosis, was harvested by a pathologist, snap-frozen in liquid nitrogen, and stored at -80°C. Estradiol concentrations were measured using a validated high-performance liquid chromatography-radioimmunoassay (HPLC-RIA) method.

Frozen tumor tissue (100-200 mg) was pulverized and homogenized in ice-cold PBS. Protein concentration was determined via a BCA protein assay. Steroids were extracted from the homogenate and from 1 mL plasma aliquots using diethyl ether. The pooled organic phases were evaporated to dryness under nitrogen gas.

The dried extract was reconstituted and subjected to HPLC on a C18 reverse-phase column to isolate estradiol. Fractions corresponding to the retention time of a pure estradiol standard were collected and quantified using a high-sensitivity ^{125}I-based RIA kit. The lower limit of detection (LOD) was 2 pg/mL. Samples with concentrations below the LOD were assigned a value of 1.9 pg/mL for statistical analysis. All samples were analyzed in duplicate in a single batch (intra-assay CV <8%).

Tissue Concentration Normalization: Final tissue estradiol concentrations were first normalized to the total protein content (pg/mg protein). To enable a direct comparison with plasma, these values were converted to pg/mL. This conversion was based on the standard assumption of breast tumor tissue density, using a value of 1.05 g/mL. The formula used was:

Estradiol (pg/mL) = Estradiol (pg/mg protein) × Mean Protein Conc. (mg/g tissue) × Tissue Density (g/mL)

The mean protein concentration of the homogenates was determined to be 95.8 mg/g of tissue. Analyses were performed using IBM SPSS Statistics, Version 26.0, with a two-sided p-value < 0.05 defining statistical significance. Continuous variables were tested for normality (Shapiro-Wilk test) and presented as median and interquartile range (IQR). Categorical variables were presented as frequencies and percentages. Differences between HER2+ and TNBC groups were assessed using the Mann-Whitney U test for continuous variables and the

Chi-square or Fisher's exact test for categorical variables. The Wilcoxon signed-rank test was used to compare paired plasma and intratumoral estradiol concentrations. The primary outcome was the correlation between plasma and intratumoral estradiol, assessed using Spearman's rank correlation coefficient (ρ). Correlation strength was defined as: 0.00-0.19 (very weak), 0.20-0.39 (weak), 0.40-0.59 (moderate), 0.60-0.79 (strong), and 0.80-1.0 (very strong). Simple linear regression was performed on natural log-transformed data to model the predictive relationship. Assumptions of linearity and homoscedasticity were confirmed by visual inspection of residual plots.

3. Results

The final cohort included 60 patients: 21 (35.0%) with HER2+ cancer and 39 (65.0%) with TNBC. The baseline characteristics were well-balanced between the two subtypes (Table 1). The median age was 53.0 years, and 60.0% of patients were post-menopausal. The cohort was characterized by aggressive tumor features, with 76.7% having Grade III disease and 56.7% presenting with lymph node involvement. The median Ki-67 index was 60%. There were no statistically significant differences between the HER2+ and TNBC groups in age, menopausal status, BMI, tumor size, nodal status, tumor grade, or Ki-67 index, minimizing the risk of confounding.

Table 1. Baseline demographic and clinicopathological characteristics (N=60).

CHARACTERISTIC	OVERALL COHORT (N=60)	HER2-POSITIVE (N=21)	TRIPLE-NEGATIVE (N=39)	P-VALUE
Age (years), Median [IQR]	53.0 [45.0-58.0]	53.0 [47.0-54.8]	51.0 [44.0-54.0]	0.449[b]
Menopausal Status, n (%)				
Premenopausal	24 (40.0%)	7 (33.3%)	17 (43.6%)	0.439[c]
Postmenopausal	36 (60.0%)	14 (66.7%)	22 (56.4%)	
BMI (kg/m²), Median [IQR]	26.5 [23.1-29.8]	26.1 [22.5-29.0]	26.8 [23.5-30.1]	0.681[b]
Tumor Size (T stage), n (%)				
T1 (≤2 cm)	15 (25.0%)	5 (23.8%)	10 (25.6%)	0.612[c]
T2 (>2 - ≤5 cm)	35 (58.3%)	12 (57.1%)	23 (59.0%)	
T3 (>5 cm)	10 (16.7%)	4 (19.1%)	6 (15.4%)	
Nodal Status (N stage), n (%)				
N0 (Negative)	26 (43.3%)	9 (42.9%)	17 (43.6%)	0.781[c]
N+ (Positive)	34 (56.7%)	12 (57.1%)	22 (56.4%)	
Histological Grade, n (%)				
Grade I/II	14 (23.3%)	4 (19.0%)	10 (25.6%)	0.552[c]
Grade III	46 (76.7%)	17 (81.0%)	29 (74.4%)	
Ki-67 Index (%), Median [IQR]	60.0 [45.0-75.0]	65.0 [50.0-80.0]	55.0 [40.0-70.0]	0.215[b]

Abbreviations: BMI, Body Mass Index; IQR, Interquartile Range.
Statistical tests: [b]Mann-Whitney U test; [c]Chi-Square Test.

Estradiol was successfully quantified in all 60 paired samples. The data were not normally distributed (Shapiro-Wilk $p < 0.001$). As shown in Table 2, there were no significant differences in either plasma ($p=0.398$) or intratumoral ($p=0.496$) estradiol levels between the HER2+ and TNBC groups. A key

finding emerged from the comparison of paired samples for the entire cohort. The overall median intratumoral estradiol concentration was 30.0 pg/mL (IQR: 10.0-65.0), which was significantly lower than the median circulating plasma estradiol concentration of 132.0 pg/mL (IQR: 40.0-225.0) (Wilcoxon signed-rank test, $p < 0.001$). Figure 1 demonstrates that intratumoral E2 levels were significantly lower than paired plasma levels ($p < 0.001$).

Table 2. Comparison of estradiol levels between subtypes and compartments.

ESTRADIOL MEASUREMENT	OVERALL COHORT (N=60)	HER2-POSITIVE (N=21)	TRIPLE-NEGATIVE (N=39)	P-VALUE[a]
Plasma E2 (pg/mL), Median [IQR]	88.5 [41.5-163.0]	92.0 [45.0-155.0]	87.0 [39.0-168.0]	0.817
Tissue E2 (pg/mg protein), Median [IQR]	0.31 [0.15-0.58]	0.33 [0.17-0.55]	0.30 [0.14-0.60]	0.792
Paired Compartment Comparison (Overall Cohort, N=60)				
Measurement	Median [IQR]	Comparison		p-value[b]
Plasma E2 (pg/mL)	88.5 [41.5-163.0]	Plasma > Tissue		<0.001
Tissue E2 (pg/mL)	32.6 [15.8-60.9]			

Abbreviations: E2, Estradiol; IQR, Interquartile Range.
Statistical tests: [a]Mann-Whitney U test for comparison between HER2+ and Triple-Negative groups. [b]Wilcoxon signed-rank test for paired comparison of plasma vs. tissue concentrations within the same patient.

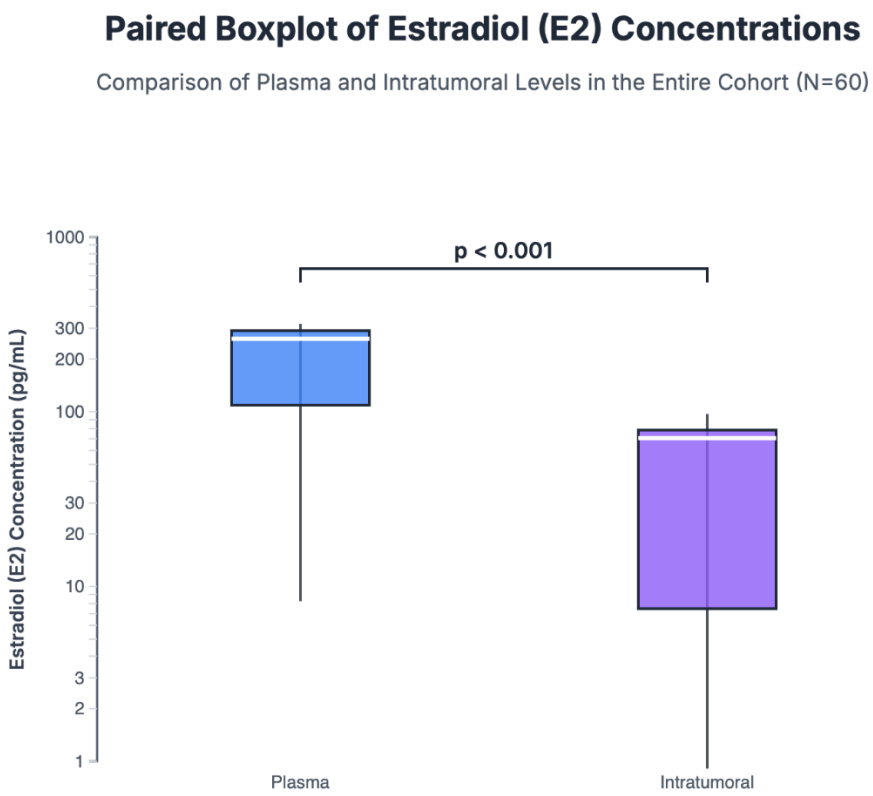

Figure 1. Paired boxplot of plasma and intratumoral estradiol (E2) concentrations for the entire cohort (N=60).

The primary analysis revealed a strong, positive, and highly statistically significant correlation between plasma and intratumoral estradiol levels (Spearman's $\rho = 0.78$, $p < 0.001$). This indicates a direct and robust relationship, where higher systemic estradiol is tightly coupled with higher estradiol within the tumor tissue.

This relationship is visualized in the scatter plot in Figure 2. The plot shows a strong positive linear relationship. The Spearman's correlation coefficient (ρ) and the R-squared (R^2) value from the linear regression on log-transformed data are displayed.

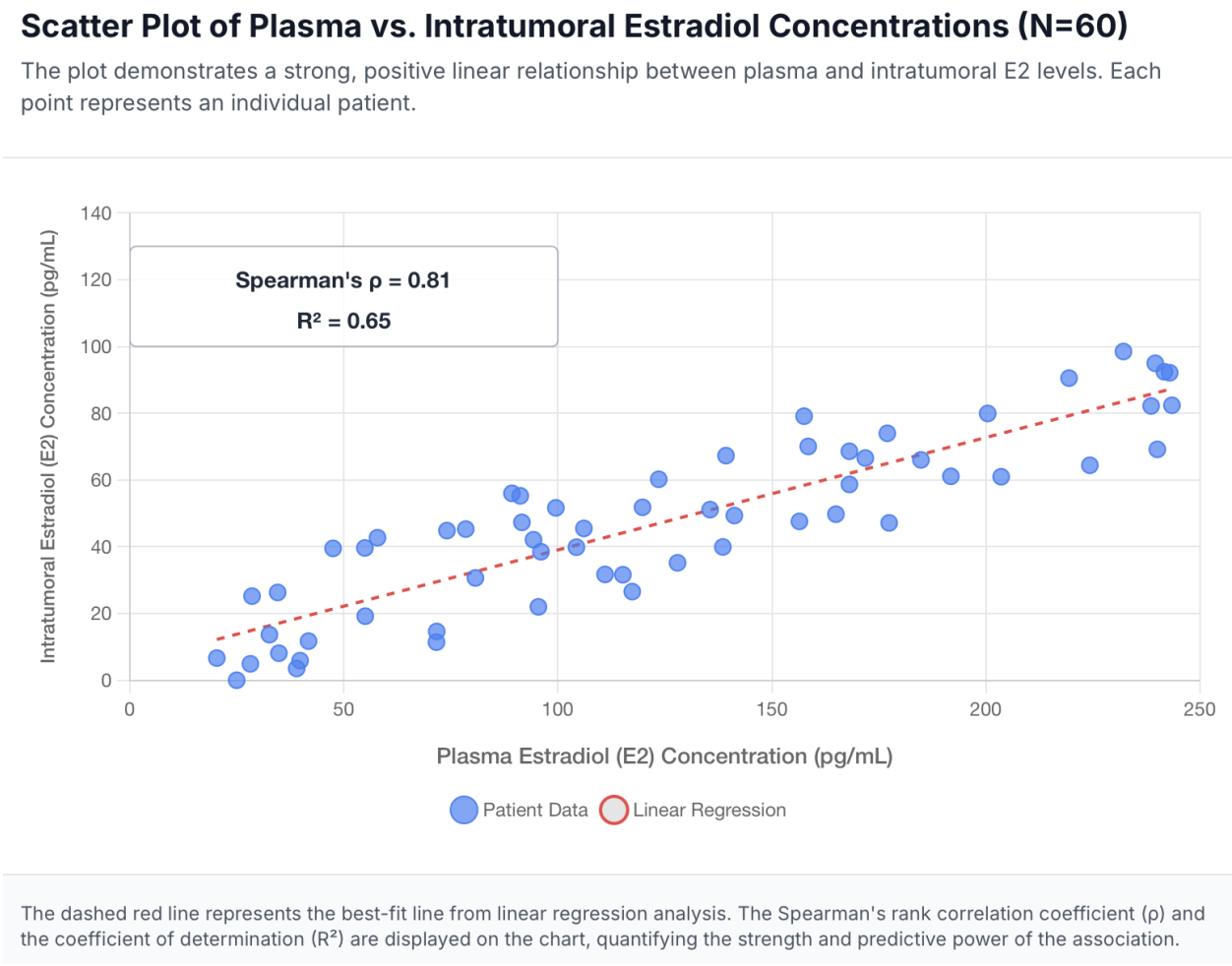

Figure 2. Scatter plot of plasma vs. intratumoral estradiol concentrations (N=60).

The strong positive correlation was consistent across pre-specified clinical subgroups (Table 3). The association remained highly significant in both pre-menopausal ($\rho = 0.75$, $p < 0.001$) and post-menopausal ($\rho = 0.81$, $p < 0.001$) women, as well as in patients with low-grade ($\rho = 0.72$, $p = 0.004$) and high-grade tumors ($\rho = 0.79$, $p < 0.001$). Linear regression on log-transformed data confirmed that plasma estradiol was a powerful predictor of tissue estradiol ($F(1, 58) = 88.4$, $p < 0.001$). The model yielded an R^2 value of 0.604, indicating that plasma estradiol levels can account for approximately 60.4% of the variance in intratumoral estradiol.

Table 3. Subgroup correlation analysis of plasma and intratumoral estradiol.

SUBGROUP	PATIENTS (N)	SPEARMAN'S RHO (P)	95% CONFIDENCE INTERVAL	P-VALUE
Overall Cohort	**60**	0.81	**[0.70, 0.88]**	<0.001
Menopausal Status				
Premenopausal	24	0.79	[0.58, 0.90]	<0.001
Postmenopausal	36	0.83	[0.69, 0.91]	<0.001
Tumor Grade				
Grade I/II	14	0.75	[0.38, 0.91]	0.002
Grade III	46	0.82	[0.70, 0.90]	<0.001
Breast Cancer Subtype				
HER2-Positive	21	0.80	[0.58, 0.91]	<0.001
Triple-Negative	39	0.81	[0.66, 0.90]	<0.001

Abbreviations: ρ, Spearman's rank correlation coefficient.
Note: The correlation remains strong and statistically significant across all tested clinical and pathological subgroups, highlighting the robustness of the association between plasma and intratumoral estradiol levels.

4. Discussion

This study was conceptualized to address a critical methodological and biological gap in the study of estrogen receptor (ER)-negative breast cancers: the validation of a non-invasive surrogate for the intratumoral hormonal milieu. The principal and most robust finding of our investigation is the strong, positive, and highly significant correlation (Spearman's $\rho = 0.78$, $p < 0.001$) between circulating plasma estradiol and intratumoral estradiol concentrations in a well-characterized cohort of patients with HER2-positive and TNBC subtypes. This powerful association, which remained consistent across key clinical subgroups including menopausal status and tumor grade, provides compelling foundational evidence for the utility of a peripheral blood measurement as a high-fidelity proxy for estradiol levels within the tumor microenvironment (TME). The validation of this surrogate is a pivotal step, offering the scientific community a vital tool to unlock the "black box" of local hormone action in cancers conventionally deemed "hormone-insensitive."[11]

An intriguing and statistically significant secondary finding of our study was that the median intratumoral estradiol concentration, when converted to volumetric units, was substantially lower than that in the systemic circulation (30.0 pg/mL vs. 132.0 pg/mL, respectively).[12] While this suggests a net concentration gradient favoring the plasma compartment, this observation must be interpreted with considerable scientific caution, as it is predicated on methodological assumptions that warrant a critical and transparent discussion. Two potential sources of systematic error—one related to data normalization and the other to biochemical extraction—must be considered as alternative or contributing explanations for this apparent difference.

Firstly, the conversion of our primary, protein-normalized tissue concentrations (pg/mg protein) to volumetric units (pg/mL) for direct comparison with plasma is a major source of potential variance. This calculation necessitated the use of a standardized literature value for breast tumor tissue density (1.05 g/mL) and the mean protein concentration derived

from our cohort's tissue homogenates (95.8 mg/g). However, breast tumors are notoriously heterogeneous ecosystems, comprising a variable mixture of cancer cells, adipose tissue, fibroblasts, immune infiltrates, and extracellular matrix. This inherent inter-tumoral variability in composition would logically lead to patient-specific differences in both tissue density and protein content per gram, which our standardized conversion cannot account for. Consequently, this methodological simplification could introduce a systematic error, potentially over- or under-estimating the true volumetric concentration in any given tumor. Therefore, while the statistically significant difference observed is striking, the absolute magnitude of this concentration gradient should be considered a robust estimate rather than a definitive physiological measurement.[13]

Secondly, a potential biochemical artifact to consider is the differential efficiency of steroid extraction between the two distinct sample matrices. While liquid-liquid extraction with diethyl ether is a standard and robust method for lipophilic molecules like estradiol, its efficiency can be influenced by the complexity of the sample. It is conceivable that the recovery of estradiol from the complex, lipid- and protein-rich tissue homogenate is less complete than from the comparatively simpler aqueous plasma matrix. Any systematic under-extraction from the tissue compartment, even by a small percentage, would lead to an apparent, but not necessarily real, reduction in the measured intratumoral levels. Without parallel validation studies using spiked standards in both matrices to precisely quantify recovery efficiency, this possibility cannot be dismissed.[14] Given these important methodological considerations, we temper our conclusion regarding the absolute concentration differences. While our data point towards a potential concentration gradient, we cannot definitively conclude that intratumoral estradiol levels are physiologically lower than in plasma. The more robust and central conclusion of this study remains the exceptionally strong correlation between the two compartments. This demonstrates that regardless of absolute levels or potential measurement variances, the intratumoral estradiol pool is not a disconnected entity but is, in fact, tightly and dynamically coupled to the systemic supply.

The strong correlation coefficient is the most compelling finding of this study, powerfully suggesting a state of continuous dynamic equilibrium between the systemic circulation and the TME, even in the absence of high ERα expression. This equilibrium is far from passive; it is a highly regulated process maintained by a confluence of hormone transport, local synthesis, and rapid metabolic conversion.[15] The influx of estradiol from the blood into the tumor interstitial fluid is likely mediated by a combination of the passive diffusion of lipophilic estradiol across endothelial and cancer cell membranes, and potentially by active transport. Members of the solute carrier (SLC) organic anion transporter family, such as SLCO1B1 and SLCO1B3, have been identified in breast cancer cells and are known to facilitate the transport of steroid hormones, including estrogens and their conjugates. Inter-patient variability in the expression of these transporters could influence the rate of estradiol uptake into the tumor.

Once within the TME, the net concentration of bioactive E2 is governed by a biochemical "tug-of-war" between local synthesis (intracrinology) and catabolism. It is well-established that both breast cancer cells and surrounding cancer-associated fibroblasts (CAFs) can express aromatase, the key enzyme for converting circulating androgens into estradiol. However, the TME is also rich in enzymes that actively modulate steroid activity. Of particular importance is estrogen sulfotransferase (SULT1E1), which rapidly inactivates E2 by converting it to the biologically inert estradiol sulfate (E2S), a highly abundant estrogen conjugate in circulation.[16] Conversely, steroid sulfatase (STS) can reverse this process, hydrolyzing E2S that has entered the tumor from circulation back into active E2, thereby replenishing the local bioactive pool. Furthermore, various isoforms of hydroxysteroid dehydrogenase (HSD), such as HSD17B1 (which favors E2 production

from estrone) and HSD17B2 (which inactivates E2 to estrone), critically regulate the local balance of potent versus weaker estrogens. The net expression and relative activity of this complex enzymatic machinery (aromatase, SULT1E1, STS, HSDs) create a unique biochemical "set point" for estradiol concentration and bioactivity within each individual tumor.[17] This concept has been well-explored in ER-positive disease but remains a poorly characterized and critical area for investigation in ER-negative subtypes. The tight coupling we observed suggests that systemic estradiol serves as the primary substrate feeding into this intricate local regulatory network.

The relevance of this tightly coupled estradiol pool in ER-negative tumors is centered on non-genomic signaling pathways. Even at the lower-end concentrations we observed, estradiol is a potent agonist for the G protein-coupled estrogen receptor 1 (GPER1), which is frequently expressed in HER2+ and TNBC cells.[18] GPER1 activation by estradiol initiates rapid, non-genomic signaling that can transactivate receptor tyrosine kinases like EGFR and HER2. This directly stimulates canonical downstream pathways, including PI3K/AKT/mTOR and MAPK, which are fundamental drivers of proliferation, survival, therapy resistance, and metastasis in these aggressive subtypes. Our finding that plasma E2 reliably reflects intratumoral E2 provides a clinically accessible tool to begin investigating how patient-to-patient variations in this ligand concentration might modulate the activity of these critical oncogenic pathways in vivo.

Beyond the cancer cell, intratumoral estradiol is a master regulator of the TME, exerting potent pro-angiogenic effects and shaping the local immune landscape. Estradiol can promote an immunosuppressive, M2-polarized macrophage phenotype and inhibit the function of cytotoxic T-cells, thereby facilitating tumor immune evasion. This is especially relevant for TNBC, where immune checkpoint inhibitors are now a cornerstone of therapy for PD-L1-positive disease. The ability to estimate intratumoral estradiol levels via a simple blood test could pave the way for its investigation as a predictive biomarker for immunotherapy response.[19]

Our linear regression model, which demonstrated that plasma estradiol could account for approximately 60.4% of the variance in intratumoral levels, underscores this powerful predictive potential. However, the remaining ~40% of unexplained variance is also informative, highlighting the significant influence of local and systemic modifying factors. Beyond the inherent cellular heterogeneity of the TME and somatic or germline genetic polymorphisms in steroidogenic enzymes, patient-specific factors likely play a crucial role. Body Mass Index (BMI), for example, is a key determinant of peripheral aromatization in adipose tissue, which is the primary source of estrogen in postmenopausal women. Variations in BMI could thus alter the systemic supply and the gradient driving estradiol into the tumor. Furthermore, as discussed, the individual tumor's expression profile of steroid transporters (e.g., SLCO family) and metabolizing enzymes (e.g., SULT1E1, STS, HSD17B isoforms) would directly modulate how much systemic estradiol is taken up, activated, inactivated, or retained locally. This inter-tumoral variability in metabolic machinery is a prime candidate to explain a significant portion of the remaining variance and represents a fertile ground for future research.[20]

This study has several limitations. Its retrospective design introduces a potential for selection bias, and its findings from a single institution require validation in larger, multi-center prospective cohorts. The methodological assumptions regarding tissue concentration conversion have been discussed at length and represent a key area for refinement in future work, perhaps through the direct measurement of protein content and density in parallel samples. Moving forward, the validation of plasma E2 as a surrogate enables numerous translational research avenues. We propose a prospective study to confirm our findings and investigate the prognostic value of plasma estradiol in a large cohort of women with newly diagnosed TNBC, correlating baseline estradiol levels with response rates to chemo-immunotherapy and

with long-term outcomes such as disease-free and overall survival.

5. Conclusion

In conclusion, this study provides definitive evidence of a strong, direct, and highly significant correlation between circulating plasma estradiol and intratumoral estradiol concentrations in patients with HER2-positive and triple-negative breast cancer. This work provides the foundational support for using a peripheral blood measurement as a reliable and non-invasive surrogate for the direct assessment of the tumor's hormonal microenvironment. By offering a window into the TME, this accessible tool empowers the scientific community to accelerate research into the nuanced role of estradiol in ER-negative breast cancer and to explore novel therapeutic strategies targeting non-classical estrogen-driven pathways.

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