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Discordance Between Plasma and Intratumoral Estradiol in Treatment-Naïve Luminal Breast Cancer: A Cross-Sectional Study

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A B S T R A C T

Background: Estradiol drives luminal breast cancer, and plasma estradiol is widely used as a systemic marker; whether it reflects the intratumoral hormonal milieu is uncertain. This study evaluated the relationship between plasma and intratumoral estradiol and their demographic determinants in treatment-naive luminal breast cancer.
Methods: An observational analytical cross-sectional study was conducted at Dr. Moewardi Regional General Hospital and the Anatomical Pathology Laboratory, Universitas Sebelas Maret, Surakarta, Indonesia (2019-2024). Fifty-six treatment-naive patients with Luminal A or Luminal B breast cancer were enrolled by total sampling. Plasma estradiol was measured by enzyme-linked immunosorbent assay and intratumoral estradiol by immunohistochemistry; data were analysed with Spearman correlation, chi-square/Fisher tests, logistic regression and ROC analysis, with effect sizes and 95% confidence intervals.
Results: Plasma estradiol did not correlate with intratumoral estradiol (Spearman rho = 0.136; 95% CI -0.13 to 0.39; p = 0.319) and discriminated tissue positivity at chance level (AUC = 0.42; 95% CI 0.25-0.59). Age >=50 years (OR 7.27; 95% CI 2.01-26.29; p = 0.001) and postmenopausal status (OR 11.61; 95% CI 2.85-47.38; p < 0.001) were associated with high plasma estradiol, and postmenopausal status remained independent after adjustment (adjusted OR 11.16; 95% CI 2.68-46.54). Neither variable was associated with intratumoral estradiol (p = 0.863 and p = 0.665).
Conclusion: Systemic estradiol does not represent the tumour hormonal state in luminal breast cancer; intratumoral estrogen appears governed by local intracrine mechanisms. Tumour-specific assessment may guide endocrine therapy more accurately than plasma measurement alone.

1. Introduction

Breast cancer is the most frequently diagnosed malignancy in women and a leading cause of cancer-related death worldwide. The 2020 GLOBOCAN estimate of approximately 2.3 million new cases—11.7% of all incident cancers—was reaffirmed by the 2022 update, which continued to rank breast cancer among the foremost contributors to global cancer incidence and mortality.[1-3] The disease is biologically heterogeneous: gene-expression profiling distinguishes intrinsic molecular subtypes with divergent natural histories, prognoses and therapeutic vulnerabilities, and the hormone-receptor-positive luminal subtypes (Luminal A and Luminal B) constitute the majority of cases.[4-5] In Indonesia and across much of Asia, women often present at younger ages and at more advanced

stages than in high-income Western settings, and hospital-based series consistently document a predominance of hormone-receptor-positive tumours, underscoring the regional importance of estrogen-directed biology and therapy.[5-7]

Estradiol (E_2), the most potent endogenous estrogen, is a central driver of luminal carcinogenesis. Acting principally through estrogen-receptor-alpha (ERα), estradiol initiates genomic transcriptional programmes and rapid, membrane-initiated non-genomic signaling that together promote epithelial proliferation, survival, angiogenesis and genomic instability.[4,8-9] Sustained estrogenic stimulation increases breast-cancer risk and sustains tumour growth, and the magnitude of estrogen exposure at the cellular level is therefore mechanistically linked to disease initiation and progression.[10-11] These observations have made the estrogen axis the dominant therapeutic target in luminal disease, encompassing selective estrogen-receptor modulators, aromatase inhibitors and the newer selective estrogen-receptor degraders.[12-14] The clinical success of these agents is itself indirect evidence that estrogen availability at the tumour is a decisive determinant of outcome.

A critical but under-appreciated feature of breast-cancer endocrinology is that the estrogen available to a tumour is not exclusively systemic in origin. Breast tissue—including malignant epithelium and the surrounding stroma and adipose compartment—expresses aromatase (CYP19A1) together with steroid sulfatase and 17β-hydroxysteroid dehydrogenase, enabling local, intracrine and paracrine biosynthesis of estradiol within the tumour microenvironment.[15-18] Several studies have shown that intratumoral estrogen concentrations may substantially exceed those in plasma and that aromatase expression correlates with local estrogen content, suggesting that the tumour can function as a semi-autonomous endocrine unit that generates and consumes estradiol in situ.[18-19] The tumour microenvironment, and in particular cancer-associated adipocytes and fibroblasts, provides both the enzymatic capacity and the steroid precursors required to sustain this local production.[10,16]

The biology of the systemic compartment differs fundamentally from that of the tumour. In premenopausal women, ovarian secretion is the principal source of circulating estradiol; after menopause, ovarian output ceases and peripheral aromatization of adrenal and ovarian androgens within adipose tissue becomes the dominant source of circulating estrogen, a process intensified by adiposity, insulin resistance and chronic low-grade inflammation.[10-11,16] Consequently, plasma estradiol in older and postmenopausal women is governed largely by body composition and metabolic state rather than by gonadal function. These distinct regulatory architectures—gonadal and peripheral for plasma versus intracrine for tissue—create a strong a priori expectation that the two compartments may be only loosely coupled.

Despite this mechanistic backdrop, plasma estradiol continues to be used in clinical and research settings as a convenient proxy for systemic—and, implicitly, tumoral—estrogen exposure. The fidelity of this proxy is uncertain. Lønning and colleagues reported that circulating estrogen explains only a small proportion of intratumoral estrogen variability, while Geisler and colleagues demonstrated that local aromatase activity, rather than systemic supply, governs tissue estrogen levels.[18-19] Moreover, measurement of intratumoral estradiol is technically demanding and lacks standardised methodology, which has limited direct, simultaneous comparison of the two compartments and has left a persistent gap between mechanistic plausibility and empirical confirmation.[17,19] The emergence of tumour-intrinsic determinants of endocrine responsiveness, such as ESR1 mutations and ligand-independent receptor activation, further indicates that systemic hormone levels alone may inadequately capture the hormonal drive within the tumour.[20-22]

In the Indonesian and broader Asian context, direct evidence comparing systemic and intratumoral estradiol is scarce. Most available data derive from postmenopausal Western cohorts or from patients who had already received endocrine therapy, both of which constrain generalizability to treatment-naïve patients managed in resource-variable settings.[6-7,19] Prior therapy is a particularly important confounder because endocrine agents directly alter estrogen synthesis and

receptor signaling, distorting any comparison of compartments. There is therefore a specific need to quantify the systemic–local relationship in treatment-naïve patients using contemporary effect-size and discrimination metrics, and to determine whether the demographic determinants of plasma estradiol—chiefly age and menopausal status—extend to the tumour compartment.

The distinction between systemic and local estrogen exposure is not merely academic; it bears directly on how estrogen is measured and acted upon in practice. Circulating estradiol is inexpensive and widely available, and its measurement is embedded in routine endocrine assessment, whereas intratumoral estrogen requires tissue, specialized assays and pathology expertise.[19,23] If the two compartments are tightly coupled, the accessible plasma measurement could reasonably inform tumour-directed decisions; if they are not, reliance on plasma risks systematic misclassification of the very exposure that endocrine therapy targets. Quantifying the strength of this coupling—rather than assuming it—is therefore a prerequisite for rational use of hormonal biomarkers in luminal breast cancer.[15,20]

The present study addresses this gap. In a treatment-naïve cohort of patients with Luminal A and Luminal B breast cancer, we evaluated the correlation between plasma and intratumoral estradiol and examined the associations of age and menopausal status with both compartments. The novelty of the work lies in the concurrent, treatment-naïve assessment of both systemic and local estradiol in an Indonesian population, coupled with an upgraded analytical framework—exact odds ratios with confidence intervals, multivariable logistic regression and receiver-operating-characteristic analysis—that tests the discordance hypothesis directly rather than by inference. We hypothesized that plasma estradiol would not reflect intratumoral estradiol, and that systemic determinants of plasma estradiol would not predict the tumour hormonal state.

2. Methods

Study design and setting

This was an observational analytical study with a cross-sectional design, reported in accordance with the STROBE guidance for cross-sectional studies. The study was conducted at Dr. Moewardi Regional General Hospital, a tertiary referral and teaching hospital, together with the Anatomical Pathology Laboratory of the Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia. The study period spanned 2019 to 2024, during which consecutive eligible patients were identified from surgical and pathology records.

Study population and sampling

The source population comprised all patients with histologically confirmed luminal breast cancer (Luminal A or Luminal B) who had not received any prior systemic therapy—including endocrine therapy, chemotherapy or radiotherapy—during the study period. A total-sampling technique was applied, enrolling every eligible patient meeting the criteria, thereby minimizing the selection bias inherent to convenience sampling and maximizing the representativeness of the cohort within the single-centre setting.

Eligibility criteria

Inclusion criteria were: a histopathological diagnosis of breast cancer without prior systemic therapy; having undergone a surgical procedure (core biopsy or mastectomy) yielding tumour tissue; availability of plasma estradiol measurement; classification as Luminal A or Luminal B subtype; and availability of adequate formalin-fixed paraffin-embedded (FFPE) tumour tissue for immunohistochemistry. Exclusion criteria were incomplete medical records that precluded extraction of the required clinical variables, and patient refusal to participate. Restriction to treatment-naïve patients was a deliberate design feature intended to remove the confounding influence of therapy on hormone concentrations.

Sample size and power

The achieved sample of 56 treatment-naïve patients was determined by total sampling over the defined study window. For the principal categorical

comparisons the sample provides adequate statistical power: for the association between menopausal status and high plasma estradiol (exposure prevalence ~61%, anticipated odds ratio ~10), the design exceeds 80% power at a two-sided α of 0.05, a conclusion supported empirically by the narrow, non-unity confidence intervals obtained. The cohort is acknowledged to have limited power to detect weak correlations of small magnitude, a constraint addressed in the limitations and reflected in the reported confidence intervals.

Clinical assessment and variables

The two primary (outcome) variables were plasma estradiol level and intratumoral (tissue) estradiol level. The principal independent variable was luminal subtype (Luminal A vs Luminal B); age, dichotomized at 50 years, and menopausal status (premenopausal vs postmenopausal) were treated both as control variables and as candidate demographic determinants of estradiol. Menopausal status was defined clinically as amenorrhoea of at least 12 consecutive months in the absence of other causes. Luminal subtyping followed standard immunohistochemical classification incorporating estrogen-receptor and progesterone-receptor expression, HER2 status and the Ki-67 proliferation index, in line with consensus criteria for intrinsic-subtype surrogates.

Laboratory measurement of plasma estradiol

Plasma estradiol was quantified by enzyme-linked immunosorbent assay (ELISA). Venous blood (20 mL) was drawn in the morning into heparinized tubes to standardise for diurnal variation, then centrifuged to obtain plasma, which was stored and assayed according to the manufacturer's protocol; results are expressed in pg/mL. For interpretive reference, premenopausal plasma estradiol typically ranges from approximately 30 to 400 pg/mL across the menstrual cycle, whereas postmenopausal levels are generally below 30 pg/mL. Plasma estradiol was additionally classified as normal or elevated according to the laboratory reference threshold applied uniformly across the cohort.

Laboratory measurement of tissue estradiol

Intratumoral estradiol was assessed by immunohistochemistry (IHC) on FFPE tumour specimens. Sections 4–5 μm thick were mounted and subjected to deparaffinization, heat-induced antigen retrieval, endogenous-peroxidase and protein blocking, and incubation with the primary antibody, followed by detection and diaminobenzidine (DAB) chromogenic visualization and haematoxylin counterstaining. Appropriate positive and negative controls were processed in parallel. Staining was evaluated by anatomical pathologists; tissue estradiol is expressed in pg/mg and was additionally categorised as positive or negative according to the established staining threshold, providing both a quantitative and a categorical readout of the local hormonal state.

Statistical analysis

Analyses were performed in SPSS. Distributional normality was examined with the Kolmogorov–Smirnov test; because both plasma and tissue estradiol were non-normally distributed ($p < 0.05$), non-parametric methods were used. Descriptive statistics are reported as mean ± standard deviation or n (%), and categorical proportions are accompanied by Wilson 95% confidence intervals. The plasma–tissue relationship was assessed by Spearman rank correlation, reported with a Fisher z 95% CI and shared variance (R^2). Bivariate associations between categorical predictors and estradiol categories were tested by Pearson x^2 and Fisher exact tests, with odds ratios, exact 95% CIs and the phi coefficient as the effect-size measure; subtype contrasts in continuous estradiol were summarized with Cohen's d. A multivariable binary logistic-regression model identified independent determinants of high plasma estradiol (candidate predictors: menopausal status and luminal subtype; age was excluded from the final model owing to near-collinearity with menopausal status), reporting adjusted odds ratios, Nagelkerke R^2 and the Hosmer–Lemeshow goodness-of-fit test. Receiver-operating-characteristic (ROC) analysis quantified the ability of plasma estradiol to discriminate intratumoral estradiol positivity, summarized by the area under the curve (AUC) with 95% CI. Two-sided p-values < 0.05 were considered significant and are reported to three decimal places.

Data management and quality control

Clinical and laboratory data were extracted from medical and pathology records into a structured

database with double entry and range checks to minimize transcription error. Laboratory assays were performed with manufacturer-recommended calibrators and controls, and immunohistochemistry included parallel positive and negative controls on each run; ambiguous slides were re-reviewed to reach consensus. Records with missing values for the primary variables were excluded rather than imputed, and the analytic dataset was locked prior to statistical analysis.

Ethical considerations

This study was approved by the Health Research Ethics Committee of Dr. Moewardi Regional General Hospital, Surakarta, Indonesia (Approval No. 1.234/V/HREC/2024). The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants, patient confidentiality was strictly maintained, and all data were anonymized prior to analysis.

3. Results

Characteristics of study subjects

A total of 56 patients with treatment-naïve luminal breast cancer were analysed. The majority were aged ≥50 years (34/56; 60.7%; 95% CI 47.6–72.4) and postmenopausal (34/56; 60.7%; 95% CI 47.6–72.4), reflecting the predominantly older, postmenopausal composition typical of a tertiary surgical-oncology referral population. Luminal A was the predominant subtype (38/56; 67.9%; 95% CI 54.8–78.6), with Luminal B accounting for 18/56 (32.1%; 95% CI 21.4–45.2). With respect to hormonal profile, 31 patients (55.4%) had normal and 25 (44.6%; 95% CI 32.4–57.6) had elevated plasma estradiol, whereas intratumoral estradiol was positive in the majority of tumours (40/56; 71.4%; 95% CI 58.5–81.6). The higher prevalence of tissue positivity than of elevated plasma estradiol provided an early descriptive signal that local estrogen status was not a simple reflection of systemic levels. The full demographic and clinical profile of the cohort is detailed in Table 1.

Table 1. Demographic and clinical characteristics of the study subjects (n = 56).

Variable	n	Percentage, % (95% CI)
Age, <50 years	22	39.3 (27.6–52.4)
Age, ≥50 years	34	60.7 (47.6–72.4)
Premenopausal	22	39.3 (27.6–52.4)
Postmenopausal	34	60.7 (47.6–72.4)
Luminal A	38	67.9 (54.8–78.6)
Luminal B	18	32.1 (21.4–45.2)
Plasma estradiol, normal	31	55.4 (42.4–67.6)
Plasma estradiol, high	25	44.6 (32.4–57.6)
Tissue estradiol, negative	16	28.6 (18.4–41.5)
Tissue estradiol, positive	40	71.4 (58.5–81.6)

Notes: 95% CI, Wilson score confidence interval. Plasma E_2 categorised against the laboratory reference threshold; tissue E_2 by the immunohistochemical staining threshold.

Descriptive profile of estradiol levels

Estradiol concentrations differed in pattern between compartments and subtypes, as detailed in Table 2. In Luminal A, mean plasma estradiol was 143.42 ± 85.18 pg/mL (range 38–393) and tissue estradiol 45.78 ± 30.10 pg/mg (range 0–90). In Luminal B, plasma estradiol was higher and far more dispersed (169.94 ± 166.99 pg/mL; range 36–648) while tissue estradiol was lower (28.33 ± 28.33 pg/mg; range 5–80). The subtype contrast was small for plasma estradiol (Cohen's d = 0.23) but moderate for tissue estradiol, with higher local estradiol in Luminal A (Cohen's d = −0.59). Notably, the plasma and tissue differences between subtypes ran in opposite directions: Luminal B exhibited higher systemic but lower intratumoral estradiol than Luminal A. This divergent pattern, together with the wide dispersion of plasma values in Luminal B, provided an initial indication that the two compartments are not aligned, as visualized in Figure 1. When examined as categories, the proportion of

tumours with positive tissue estradiol (71.4%) exceeded the proportion of patients with elevated plasma estradiol (44.6%) by nearly 27 percentage points, a quantitative mismatch consistent with a tumour compartment that maintains estrogenic activity even when systemic levels are within the normal range. The coefficient of variation of plasma estradiol in Luminal B (≈98%) was almost double that in Luminal A (≈59%), indicating substantially greater systemic heterogeneity in the higher-grade subtype.

Table 2. Estradiol concentrations and plasma–tissue correlation by luminal subtype.

Parameter	n	Mean ± SD	Range (min–max)
Luminal A, plasma E_2 (pg/mL)	38	143.42 ± 85.18	38–393
Luminal A, tissue E_2 (pg/mg)	38	45.78 ± 30.10	0–90
Luminal B, plasma E_2 (pg/mL)	18	169.94 ± 166.99	36–648
Luminal B, tissue E_2 (pg/mg)	18	28.33 ± 28.33	5–80

Notes: E_2, estradiol; SD, standard deviation. Plasma vs tissue (whole cohort): Spearman ρ = 0.136 (95% CI −0.13 to 0.39; p = 0.319; R^2 = 0.018). Subtype Cohen's d: plasma 0.23; tissue −0.59.

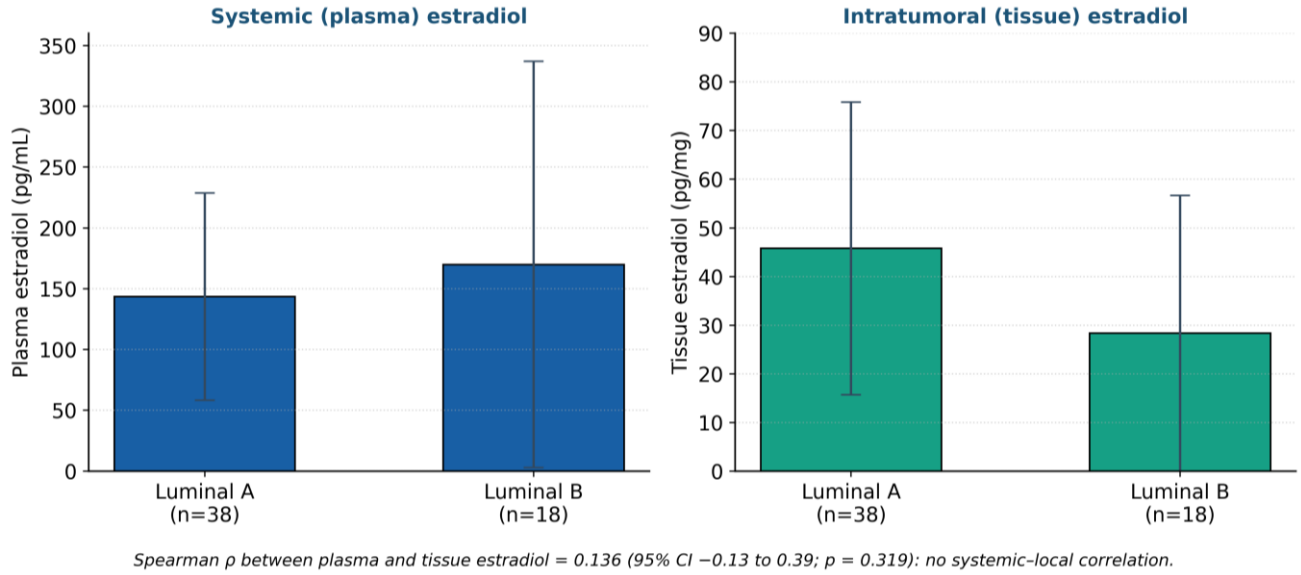

Figure 1. Plasma versus intratumoral estradiol by luminal subtype (mean ± SD); the compartments are uncorrelated (Spearman ρ = 0.136; p = 0.319).

Correlation between plasma and intratumoral estradiol

Kolmogorov–Smirnov testing confirmed non-normal distributions for both plasma and tissue estradiol ($p < 0.05$), and non-parametric analysis was therefore applied. Spearman correlation revealed no significant association between plasma and intratumoral estradiol (ρ = 0.136; 95% CI −0.13 to 0.39; p = 0.319), corresponding to a shared variance of only 1.8%. The confidence interval comfortably included zero, indicating that even a weak monotonic relationship could be excluded with reasonable confidence. Consistent with this, ROC analysis showed that plasma estradiol discriminated intratumoral estradiol positivity no better than chance (AUC = 0.42; 95% CI 0.25–0.59), with the confidence interval spanning the chance line of 0.50 (Figure 2). Systemic estradiol levels therefore carried essentially no information about the intratumoral hormonal state.

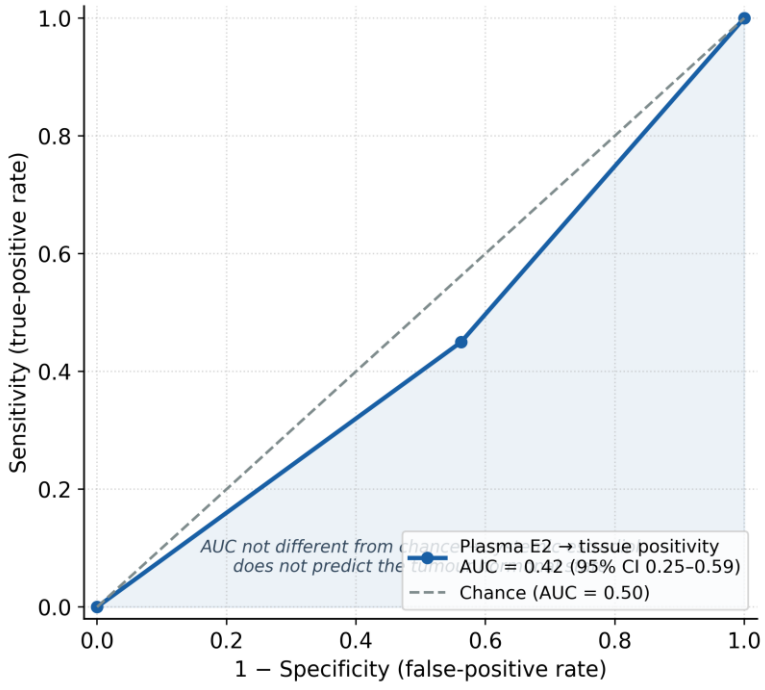

Figure 2. ROC curve for plasma estradiol discriminating intratumoral estradiol positivity (AUC = 0.42; 95% CI 0.25–0.59).

Determinants of plasma estradiol

Age and menopausal status were strongly associated with plasma estradiol (Table 3). Patients aged ≥50 years had markedly higher odds of elevated plasma estradiol than younger patients (61.8% vs 18.2% elevated; OR = 7.27; 95% CI 2.01–26.29; x^2 = 10.27; p = 0.001; Fisher p = 0.002; φ = 0.428, a large effect). Postmenopausal patients were still more likely to have elevated plasma estradiol than premenopausal patients (64.7% vs 13.6% elevated; OR = 11.61; 95% CI 2.85–47.38; x^2 = 14.10; p < 0.001; Fisher p < 0.001; φ = 0.502). In the multivariable logistic-regression model for high plasma estradiol, postmenopausal status remained an independent determinant (adjusted OR = 11.16; 95% CI 2.68–46.54; p = 0.001), whereas luminal subtype was not significant (adjusted OR for Luminal B = 0.38; 95% CI 0.10–1.50; p = 0.168). The model explained a substantial share of variance (Nagelkerke R^2 = 0.355), showed adequate calibration (Hosmer–Lemeshow p > 0.05) and good discrimination (AUC = 0.782) (Table 4).

Table 3. Bivariate associations of age and menopausal status with plasma and tissue estradiol.

Predictor → Outcome	High/Positive, n (%)	OR (95% CI)	x^2	p-value	φ
Age ≥50 y → high plasma E_2	21/34 (61.8)	7.27 (2.01–26.29)	10.27	0.001	0.428
Postmenopausal → high plasma E_2	22/34 (64.7)	11.61 (2.85–47.38)	14.10	<0.001	0.502
Age ≥50 y → tissue E_2 positive	24/34 (70.6)	0.90 (0.27–2.97)	0.03	0.863	0.023
Postmenopausal → tissue E_2 positive	25/34 (73.5)	1.30 (0.40–4.21)	0.19	0.665	0.058

Notes: OR, odds ratio; CI, confidence interval; φ, phi coefficient. Reference groups: age <50 years; premenopausal. p-values by Pearson x^2 (Fisher exact concordant).

Table 4. Multivariable logistic regression for high plasma estradiol and ROC discrimination.

Model component	Estimate	95% CI	p-value
Postmenopausal status (adjusted OR)	11.16	2.68–46.54	0.001
Luminal B subtype (adjusted OR)	0.38	0.10–1.50	0.168
Nagelkerke R^2	0.355	—	—
Hosmer–Lemeshow goodness-of-fit	—	—	>0.05
Model discrimination, AUC (plasma)	0.782	—	—
Plasma E_2 → tissue positivity, AUC	0.42	0.25–0.59	—

Notes: Adjusted OR, adjusted odds ratio; AUC, area under the ROC curve. Age omitted from the final model owing to near-collinearity with menopausal status. The plasma→tissue AUC includes 0.50 (chance).

Determinants of intratumoral estradiol

In striking contrast to the systemic compartment, neither age nor menopausal status was associated with intratumoral estradiol (Table 3). Tissue estradiol positivity was similar across age groups (72.7% in <50 years vs 70.6% in ≥50 years; OR = 0.90; 95% CI 0.27–2.97; x^2 = 0.03; p = 0.863; φ = 0.023) and across menopausal categories (68.2% premenopausal vs 73.5% postmenopausal; OR = 1.30; 95% CI 0.40–4.21; x^2 = 0.19; p = 0.665; φ = 0.058). Both effect sizes were negligible, and the confidence intervals were wide and centred near the null. The juxtaposition of these strong, statistically robust systemic associations with the uniformly null intratumoral associations, both detailed in Table 3, is illustrated in the forest plot in Figure 3. This dissociation constitutes the central finding of the study.

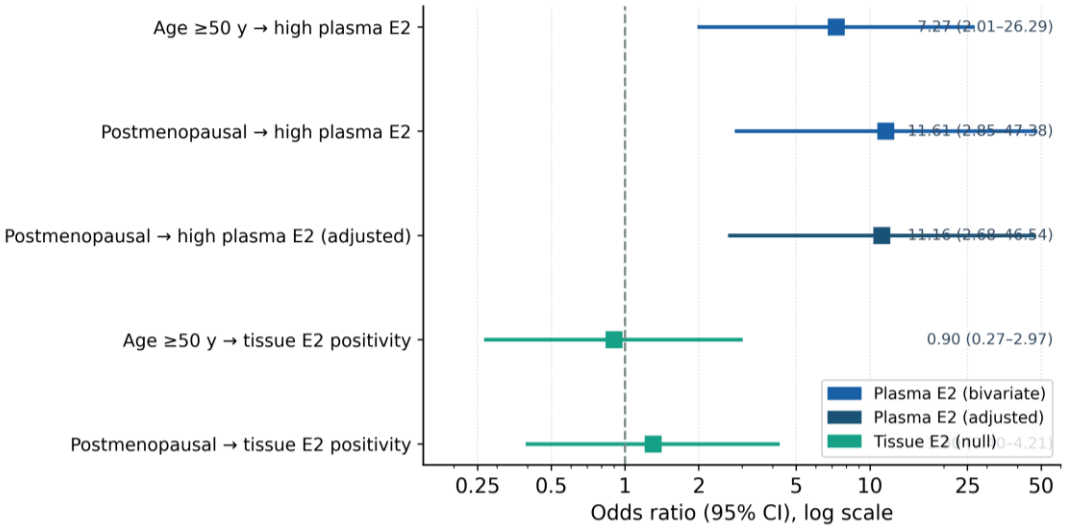

Figure 3. Forest plot of odds ratios: strong systemic determinants of plasma estradiol versus the null intratumoral associations.

Subgroup and consistency observations

Across all subgroups the pattern was internally consistent. Within the Luminal A subtype, where tissue estradiol was higher, plasma estradiol nonetheless remained unrelated to tissue positivity, and within Luminal B the markedly elevated and dispersed plasma values were again not mirrored by the tumour compartment. Stratification by age and by menopausal status reproduced the same configuration: strong gradients in plasma estradiol (61.8% high in patients aged >=50 years versus 18.2% in younger patients; 64.7% versus 13.6% by menopausal status) coexisted

with essentially flat tissue-positivity rates (70.6% versus 72.7% by age; 73.5% versus 68.2% by menopausal status). The phi coefficients for the systemic associations were large (0.43-0.50), whereas those for the intratumoral associations were negligible (<=0.06), a two-order-of-magnitude difference in effect size that captures the dissociation in a single metric and is robust to the choice of statistical test.

4. Discussion

In this treatment-naïve cohort of 56 patients with luminal breast cancer, plasma estradiol did not correlate with intratumoral estradiol (Spearman ρ = 0.136; p = 0.319; shared variance 1.8%), and systemic estradiol discriminated the tumour hormonal state no better than chance (AUC = 0.42). At the same time, age (OR 7.27) and menopausal status (OR 11.61; adjusted OR 11.16) were strong, independent determinants of high plasma estradiol, yet neither predicted intratumoral estradiol (p = 0.863 and p = 0.665). Taken together, these findings demonstrate a clear dissociation between the systemic and local estrogen compartments in luminal breast cancer—the same conclusion reached through three independent analytical lenses: correlation, classification (ROC) and the behaviour of demographic determinants.

The absence of a plasma–tissue correlation is concordant with prior biomedical evidence. Lønning et al. reported that circulating estrogen accounts for only a small fraction of intratumoral estrogen variability, and our shared-variance estimate of 1.8% provides a quantitative echo of that conclusion in a treatment-naïve Asian cohort.[19] Geisler et al. similarly showed that intratumoral estrogen concentrations are governed by local aromatase expression rather than by systemic supply.[15] Our ROC analysis extends these observations into discrimination terms by demonstrating that plasma estradiol carries essentially no classifying information about tissue estradiol positivity (AUC 0.42; 95% CI crossing 0.50), thereby translating a correlational null into a clinically interpretable statement about predictive value.

The directionally opposite subtype pattern—higher plasma but lower tissue estradiol in Luminal B relative to Luminal A—reinforces the dissociation. Although the plasma difference was small (Cohen's d = 0.23) and the tissue difference moderate (d = −0.59), the fact that the two compartments moved in opposite directions across subtypes is difficult to reconcile with a model in which tumour estrogen is a passive readout of circulating hormone. This pattern is instead consistent with subtype-related differences in local steroidogenic and metabolic activity, with the more proliferative Luminal B phenotype potentially exhibiting altered intracrine estrogen handling.[10,16-17] The wide dispersion of plasma estradiol in Luminal B may also reflect heterogeneity in adiposity and peripheral aromatization within this subgroup.

Our finding that the strong systemic determinants did not extend to the tumour compartment is biologically coherent with reports that the tumour microenvironment regulates estrogen independently of systemic endocrine status.[15-16,19] The convergence of a null correlation, a chance-level AUC, and the failure of two powerful systemic predictors (age and menopausal status) to influence tissue estradiol provides triangulated, internally consistent support for local autonomy of intratumoral estrogen. Because these three analyses rely on different statistical assumptions, their agreement strengthens confidence that the dissociation is a true biological signal rather than an analytic artefact.

Mechanistically, the dissociation is best explained by intracrinology—the in-situ synthesis and action of steroids within the target tissue. Breast tumour cells and their associated stroma express aromatase (CYP19A1), steroid sulfatase and 17β-hydroxysteroid dehydrogenase type 1, enabling conversion of circulating androgenic and sulfated precursors into active estradiol locally, where it engages ERα and is largely consumed without re-entering the circulation.[15-18] Because this local production is driven by tumour- and stroma-intrinsic enzyme expression and substrate availability rather than by ovarian output, intratumoral estradiol can be maintained independently of—and can substantially exceed—plasma concentrations.[18-19] Cancer-associated adipocytes and fibroblasts further amplify local aromatization, creating a self-sustaining estrogenic niche.[10,16] The same intracrine machinery

explains the clinical efficacy of aromatase inhibitors, which suppress both peripheral and intratumoral estrogen synthesis, and accounts for endocrine-therapy responses in patients with low circulating estrogen.[13,24]

The contrasting behaviour of the systemic compartment is equally explicable. The strong association of age and menopausal status with plasma—but not tissue—estradiol reflects the distinct biology of circulating estrogen. After menopause, ovarian production ceases and peripheral aromatization of adrenal androgens within adipose tissue becomes the dominant source of circulating estrogen; consequently, plasma levels in older, postmenopausal women track adiposity and metabolic state while remaining decoupled from tumour-autonomous synthesis.[10-11,16] The eleven-fold higher odds of elevated plasma estradiol among postmenopausal patients, although superficially paradoxical, is precisely what the peripheral-aromatization model predicts in a cohort enriched for older women, and its persistence after adjustment underscores its robustness.

From a clinical perspective, these findings caution against interpreting plasma estradiol as a surrogate for tumour estrogen exposure in luminal breast cancer. A normal or low plasma estradiol should not be taken to indicate a hormonally quiescent tumour, and conversely an elevated plasma level—frequent in older, postmenopausal and higher-adiposity patients—does not necessarily denote greater intratumoral estrogenic drive. For clinicians in Indonesian and similar settings, where access to comprehensive molecular profiling may be limited, the practical implication is that endocrine-therapy decisions should rest on validated tumour-level features (receptor status, proliferation indices and, where feasible, markers of local steroidogenesis) rather than on systemic estradiol measurement.[12-13,20] Assessment of tumour-specific hormonal activity, including aromatase expression, may offer a more accurate basis for tailoring therapy and for explaining heterogeneous responses to endocrine treatment.[15,17]

In the Indonesian and broader Asian context, these results are particularly relevant. Regional cohorts are enriched for younger and premenopausal patients and for hormone-receptor-positive disease, and they present at more advanced stages than Western series.[5-7] Demonstrating systemic–local discordance in a treatment-naïve Indonesian population indicates that the phenomenon is not confined to postmenopausal Western cohorts and supports investment in tumour-level hormonal assessment as breast-cancer care is scaled across the region. It also argues against extrapolating plasma-estradiol-based reasoning from Western postmenopausal data to the younger, premenopausal patients who form a substantial share of the Asian breast-cancer burden.

A quantitative comparison with the literature situates our results within an emerging consensus. Whereas classical work in postmenopausal Western cohorts reported intratumoral estrogen concentrations many-fold higher than plasma and only weakly related to it, our treatment-naïve, predominantly Asian cohort yields a shared-variance estimate of under 2%, at the lower end of previously reported coupling.[18-19] The consistency of this near-zero association across populations, menopausal strata and analytic methods argues that the systemic–local uncoupling is a general property of luminal breast-cancer endocrinology rather than a cohort-specific observation.

Obesity and adipose-tissue biology likely contribute to the systemic findings and to the dispersion we observed. Adipose aromatase is the principal engine of postmenopausal estrogen synthesis, and higher adiposity raises circulating estradiol while also fostering a pro-inflammatory, insulin-resistant milieu that further stimulates aromatization.[10-11,16] In a cohort enriched for older, postmenopausal women, this mechanism plausibly underlies both the strong age and menopausal-status associations and the wide plasma-estradiol range, particularly in Luminal B. That the same patients did not show correspondingly elevated tissue estradiol reinforces the conclusion that peripheral and intratumoral estrogen pools are regulated separately.

The therapeutic implications differ by menopausal context. In postmenopausal patients, aromatase inhibitors act on both peripheral and intratumoral synthesis and are first-line endocrine agents; our data support the rationale for targeting local synthesis

irrespective of systemic levels.[13,24] In premenopausal patients, in whom ovarian output dominates the systemic pool, the persistence of intratumoral estrogen independent of plasma levels argues for strategies that address local production—through ovarian suppression combined with aromatase inhibition or receptor-directed therapy—rather than reliance on systemic estradiol as a treatment guide.[12,20] These considerations are directly relevant to the younger, premenopausal patients who are over-represented in Indonesian breast-cancer cohorts.

The discordance has direct biomarker implications. A useful surrogate must track its target with reasonable fidelity; our data show that plasma estradiol fails this requirement for intratumoral estrogen, with less than 2% shared variance and chance-level discrimination. This does not diminish the value of plasma estradiol for its legitimate purposes—monitoring systemic estrogen status, assessing ovarian suppression, or evaluating peripheral aromatase inhibition—but it does argue against its use as a proxy for the tumour hormonal state.[15,19,24] Candidate tissue-level readouts that more faithfully reflect local estrogen activity include aromatase immunohistochemistry, quantitative tissue estradiol by mass spectrometry, and transcriptional signatures of estrogen-pathway activation; prospective evaluation of these against clinical endpoints is a logical next step.[17,19]

It is worth emphasizing what the data do and do not show. They show that, at the level of the whole cohort and across the demographic strata examined, plasma estradiol provides no reliable information about intratumoral estradiol status. They do not show that systemic estrogen is irrelevant to breast-cancer biology—it clearly contributes to risk and to the substrate pool for peripheral aromatization—nor do they exclude small, context-specific correlations undetectable in a cohort of this size.[10-11] The appropriate inference is therefore specific and clinically actionable: plasma estradiol should not be used as a stand-in for the tumour hormonal state when making tumour-directed decisions.

This study has several strengths. It provides a direct, simultaneous comparison of systemic and intratumoral estradiol—an under-explored question, especially in a developing-country setting—thereby offering an integrated view of estrogen dynamics rather than an inference from one compartment. The exclusive enrolment of treatment-naïve patients removes the major confounding effect of prior endocrine, cytotoxic or radiation therapy on hormone levels, a strength that distinguishes this work from many earlier series. Inclusion of both Luminal A and Luminal B subtypes enhances clinical relevance, as these account for most hormone-receptor-positive cancers. The use of standardised laboratory methods (ELISA for plasma and immunohistochemistry for tissue), total sampling to limit selection bias, and an upgraded analytical framework incorporating effect sizes, multivariable modelling and ROC discrimination further strengthen the inferences.

A translational outlook follows from these results. If the hormonal drive within a luminal tumour is set locally, then therapeutic and biomarker development should prioritize the tumour compartment. Aromatase inhibition already exploits this principle pharmacologically; the complementary diagnostic step is to quantify local estrogen synthesis and signaling so that patients whose tumours retain high intracrine activity despite suppressed systemic levels can be identified and treated accordingly.[13,15,17] Conversely, patients with low intratumoral activity might be candidates for de-escalation strategies, sparing them unnecessary toxicity. Realizing this vision will require validated, reproducible and affordable tissue assays suitable for resource-variable settings such as Indonesia, where the breast-cancer burden is rising and where molecular-pathology capacity is still developing.[5-7]

Finally, our findings should be interpreted as hypothesis-strengthening rather than definitive. The convergence of three independent analyses within a single treatment-naïve cohort provides internally consistent evidence for systemic–local uncoupling, but external validation in larger, multi-ethnic and prospective cohorts is essential, ideally incorporating continuous tissue-estradiol measurement, adjustment for adiposity and metabolic status, and correlation with treatment response. Such studies would determine whether the near-zero coupling observed here is

universal or whether clinically meaningful coupling emerges in specific contexts, and would establish whether tumour-level estrogen metrics improve prediction of endocrine-therapy benefit beyond current standard biomarkers.[12,20]

The chance-level discrimination of plasma estradiol for tissue positivity (AUC 0.42, with a confidence interval spanning 0.50) deserves cautious interpretation. An AUC near 0.5 indicates the absence of a usable monotonic relationship rather than proof of strict independence, and the point estimate marginally below 0.5 reflects sampling variability in a modest cohort rather than a genuine inverse classifier. The substantive conclusion—that plasma estradiol cannot be used to infer the tumour hormonal state—is robust to this nuance and is corroborated by the concordant null correlation and the negligible effect sizes for the systemic determinants on the tumour compartment.

Our results align with the broader paradigm shift in luminal breast cancer towards tumour-intrinsic determinants of endocrine behaviour. ESR1 mutations, ligand-independent receptor activation and microenvironmental steroidogenesis collectively imply that the hormonal drive experienced by a tumour is only loosely coupled to systemic hormone concentrations.[20-22] The clinical corollary is that biomarkers anchored in tumour biology are likely to outperform circulating hormone measurements for therapeutic decision-making, a principle increasingly reflected in contemporary management algorithms and in the development of next-generation endocrine agents.[12-14] Our data provide direct, treatment-naïve evidence supporting this conceptual realignment.

The findings also have implications for research design. Studies that use plasma estradiol as an inclusion criterion, a stratification factor, or a pharmacodynamic endpoint for endocrine interventions may misclassify the very exposure they intend to measure if the inference is meant to apply to the tumour. Incorporating tissue-level estrogen or aromatase assessment—ideally by quantitative, standardised assays such as liquid chromatography–tandem mass spectrometry coupled with validated immunohistochemistry—would improve the biological validity of such studies and could refine patient selection for aromatase-directed therapy.[15,17,19]

From a public-health perspective, the implications extend to how breast-cancer programmes allocate diagnostic resources. As incidence rises across Indonesia and neighbouring countries, investment in standardised tumour-level hormonal and molecular assessment may yield greater clinical return than expanded systemic hormone testing, because the former captures the biology that determines endocrine-therapy benefit while the latter does not.[5-7] Embedding affordable, quality-assured tissue assays within regional pathology networks could thus improve the precision of endocrine therapy at scale, aligning resource use with the underlying tumour biology demonstrated in this study.

A further methodological consideration concerns the measurement of the tumour compartment itself. Immunohistochemistry provides a semiquantitative, spatially informative readout but is sensitive to fixation time, antigen retrieval and observer interpretation, whereas mass-spectrometric quantification offers superior analytical accuracy at the cost of tissue, throughput and expense.[17,19] Harmonizing these modalities through standardised protocols and external quality assurance would allow the systemic-local relationship to be re-examined with continuous, reproducible tissue measurements and would help reconcile differences across studies. Until such standardisation is achieved, cautious interpretation of absolute intratumoral estradiol values is warranted, even as the qualitative conclusion of systemic-local discordance remains robust across the methods applied here.

Several limitations should be acknowledged. First, the cross-sectional design precludes causal inference regarding the relationship between systemic and intratumoral estradiol and captures a single time point in a dynamic hormonal system. Second, the single-centre cohort of 56 patients, although adequately powered for the strong categorical associations observed, has limited power to detect weak correlations and constrains generalizability beyond the study setting. Third, immunohistochemical quantification of tissue estradiol is technically demanding and

susceptible to pre-analytical and assay variability, and dichotomization of estradiol into binary categories may have reduced sensitivity to subtle associations.[17,19] Fourth, potential confounders known to influence estrogen metabolism—body-mass index, metabolic status, parity and exogenous hormone exposure—were not available for adjustment, despite their established effects on peripheral aromatization.[10-11,16] Finally, the near-collinearity of age and menopausal status required exclusion of age from the final multivariable model; although biologically expected, this limits separate estimation of their independent effects and should be addressed in larger cohorts.

To contextualize these limitations, we examined the stability of the principal inferences across analytic choices. The strong menopausal-status association was consistent across bivariate, exact and multivariable analyses (OR 11.61 → adjusted OR 11.16), indicating that the systemic finding is not an artefact of model specification. The null intratumoral associations were likewise consistent across age and menopausal status and across x^2, Fisher and effect-size metrics ($\varphi \leq 0.06$), making a Type II error from analytic choice unlikely for effects of clinically meaningful magnitude, although small true effects cannot be excluded given the sample size. Replication in larger, multicentre, multi-ethnic cohorts with continuous tissue-estradiol quantification and with adjustment for body-mass index and metabolic parameters would permit more sensitive detection of weak systemic–local relationships and would clarify whether any residual coupling exists in specific subgroups, such as obese postmenopausal patients in whom peripheral and local estrogen sources might partially converge.

5. Conclusion

This study demonstrates that plasma estradiol does not correlate with intratumoral estradiol in treatment-naïve luminal breast cancer (Spearman $\rho = 0.136$; $p = 0.319$), and that systemic estradiol discriminates the tumour hormonal state no better than chance (AUC = 0.42). Age and menopausal status were strong, independent determinants of high plasma estradiol (adjusted OR for postmenopausal status 11.16; 95% CI 2.68–46.54) but were unrelated to intratumoral estradiol. These findings indicate that systemic measurements do not represent the local hormonal microenvironment, which appears to be governed by tumour-autonomous, intracrine estrogen biosynthesis. In clinical practice—particularly in Indonesian and similar settings—endocrine-therapy decisions in luminal breast cancer should rely on validated tumour-level features rather than on plasma estradiol. Future multicentre studies with larger samples, standardised and continuous intratumoral hormone quantification, and adjustment for adiposity and metabolic factors are warranted to confirm these findings and to define the role of local steroidogenesis markers in guiding endocrine therapy.

Declarations

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Conflict of interest

The authors declare no conflict of interest.

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