Evaluation of stable calcium isotope ratios in women undergoing neoadjuvant chemotherapy (NACT) with and without denosumab (GeparX)

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Background

Calcium (Ca) is an essential mineral that plays a fundamental role in skeletal integrity, neuromuscular function, and intracellular signaling, with its homeostasis tightly regulated through hormonal feedback mechanisms involving parathyroid hormone, calcitonin, and vitamin D. Disorders of bone metabolism such as osteoporosis can disrupt this delicate balance, leading to altered calcium fluxes and reduced bone mineral density. The standard diagnostic tool for assessing BMD remains dual-energy X-ray absorptiometry (DXA).

Recently, the calcium isotope method (CIM) has emerged as an innovative biomarker approach that measures natural calcium isotope ratios ($\delta^{44/42}$Ca) in blood or urine to evaluate bone metabolism, particularly in postmenopausal women at risk for osteoporosis. This technique is based on the principle that calcium isotopes in blood and bone exist in isotopic equilibrium, differing only by a constant value:

($\Delta^{44/42}$CaBone-Blood = $\delta^{44/42}$CaBone - $\delta^{44/42}$CaBlood = −0.3‰)

Under conditions of metabolic equilibrium, where bone formation and resorption are balanced, the calcium isotope ratio in blood remains stable. However, during chronic bone resorption—such as in osteoporosis—lighter calcium isotopes are preferentially released from bone, leading to a measurable decrease in the blood $\delta^{44}/^{42}$Ca value.

Studies have demonstrated that shifts in calcium isotope composition correlate with bone turnover markers and bone density loss, suggesting that CIM may serve as a noninvasive, early indicator of bone metabolic imbalance before structural changes become detectable by DXA (Heuser et al., 2016). As such, calcium isotopic analysis holds promise as a dynamic biomarker for monitoring treatment efficacy and metabolic bone health across various populations.

This is the first study to evaluate the effects of neoadjuvant chemotherapy (NACT), with and without denosumab, on bone calcium balance in postmenopausal women with early breast cancer enrolled in the GeparX trial (NCT02682693).

Patients and Methods

This analysis was conducted on a predefined cohort of patients (pts) enrolled in the GeparX trial (Blohmer et al. 2022), specifically including postmenopausal pts with hormone receptor-positive (HR+), HER2-negative (HER2-) breast cancer who were treated with NACT.

Patients included in this cohort were randomized to receive denosumab (Dmab) or not in addition to NACT (weekly nab-paclitaxel [w-NabPac] followed by epirubicin/ cyclophosphamide).

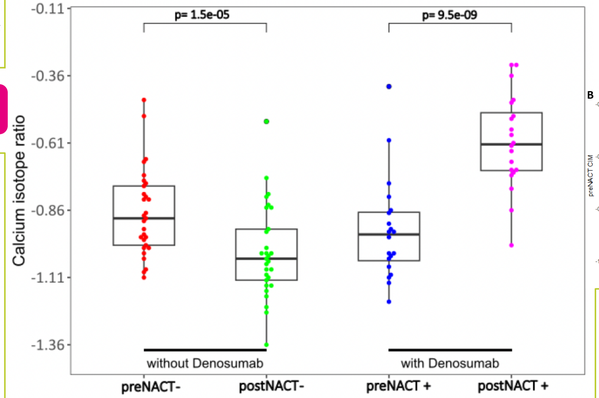
Eligible pts had two serum samples available: one prior to treatment and one after completion of NACT. In total, 20 pts included in this study were treated in the w-NabPac + Dmab arm and 29 received w-NabPac alone. Paired serum samples were analyzed for CIM ratios. Patient characteristics are summarized in **Table 1**.

Results

Table 1: Patient characteristics

Clinical parameter		Without Denosumab N (%)	With Denosumab N (%)	Overall N (%)	p-value
Age	≤59y	14 (48.3)	15 (75.0)	29 (59.2)	0.061
	>59y	15 (51.7)	5 (25.0)	20 (40.8)	
BMI	BMI ≤25	11 (37.9)	8 (40.0)	19 (38.8)	0.884
	BMI >25	18 (62.1)	12 (60.0)	30 (61.2)	
Tumor stage	cT1/2	26 (89.7)	18 (90.0)	44 (89.8)	0.969
	cT3	3 (10.3)	2 (10.0)	5 (10.2)	
Grade	G1/2	13 (44.8)	12 (60.0)	25 (51.0)	0.296
	G3	16 (55.2)	8 (40.0)	24 (49.0)	
Nodal status	cN-	20 (69.0)	11 (55.0)	31 (63.3)	0.319
	cN+	9 (31.0)	9 (45.0)	18 (36.7)	
ypT0_ypN0	no pCR	26 (89.7)	17 (85.0)	43 (87.8)	0.625
	ypT0_ypN0	3 (10.3)	3 (15.0)	6 (12.2)	
ypT0is_ypN0	no pCR	25 (86.2)	16 (80.0)	41 (83.7)	0.563
	ypT0is_ypN0	4 (13.8)	4 (20.0)	8 (16.3)	

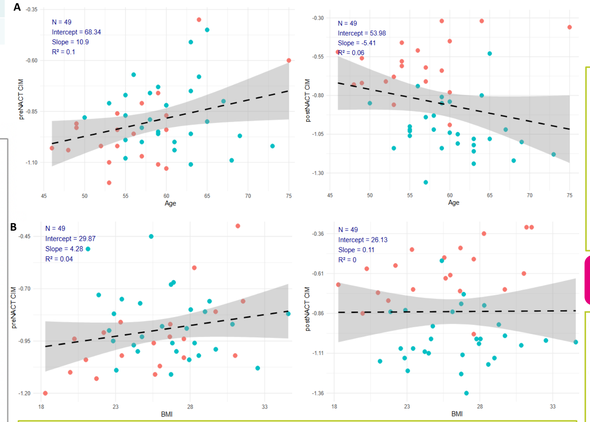
Figure 1: Changes in bone calcium balance pre- and post-neoadjuvant chemotherapy with and without Denosumab



A total of 98 serum samples were tested for CIM serum levels and valid measurements were obtained for all. Pre-treatment mean CIM values were comparable in both cohorts (mean±SD -0.87±0.16 ‰ vs -0.94±0.19 ‰; p=0.115; without vs with Dmab, respectively), indicating a net loss of calcium and the difference is not statistically significant. In pts treated with NACT alone, a significant decrease in CIM-serum levels to -1.02±0.17 ‰ could be detected signifying a bone calcium loss (p<0.001) and osteoporotic situation. In contrast, pts treated in the Dmab arm had a significant increase in CIM-serum levels reaching a mean of -0.61±0.18 ‰ (p<0.001), indicating a net accumulation of calcium and new bone formation (**Figure 1**).

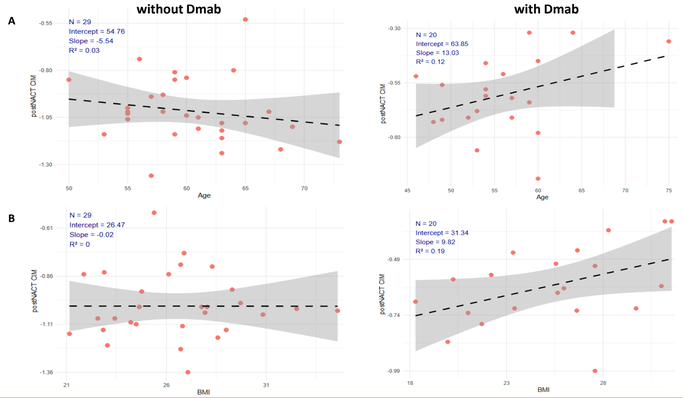
No relationship between CIM levels and pathologic complete response was observed.

Figure 2: Correlations of bone calcium balance pre and post neoadjuvant chemotherapy treated with and without Denosumab with (A) age and (B) BMI



Pre-treatment levels of CIM showed a mild positive correlation with age (slope = 10.9; R^2 = 0.10) and body mass index (BMI) (slope = 4.3; R^2 = 0.04). Post-treatment, the CIM levels showed a reverse trend with a mild negative correlation for age (slope = -5.4; R^2 = 0.06) and no correlation with BMI (slope = 0.11; R^2 = 0). Orange dots correspond to pts treated with Dmab, while cyan dots correspond to pts without Dmab (**Figure 2**).

Figure 3: Correlations of bone calcium balance post neoadjuvant chemotherapy treated without and with Denosumab with (A) age and (B) BMI



Within the post-treatment cohort, however, age and BMI showed contrasting, weak correlations with CIM levels in relation to treatment with Dmab: there was a negative correlation with age for pts who did not receive Dmab (slope = -5.54; R^2 = 0.03) and a positive correlation with age for pts who did (slope = 13.03; R^2 = 0.12); there was no correlation with BMI for pts who did not receive Dmab (slope = -0.02; R^2 = 0) and a positive correlation with BMI for pts who did (slope =9.82; R^2 = 0.19) (**Figure 3**). Hence, Dmab was associated with improved bone mineralization post-treatment, particularly in older pts and those with higher BMI.

Conclusions

Our analysis of CIM serum levels in postmenopausal pts undergoing NACT for HR+/HER2− breast cancer provide critical insights into the extent of bone mineral loss. Concurrent administration of denosumab appears to mitigate this effect by promoting bone mineralization. CIM serum levels may serve as a valuable, easily measurable biomarker for monitoring bone mineral balance in pts undergoing systemic treatments for breast cancer, enabling early intervention in case of bone mass loss and preventing osteoporosis.

References

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