PS4-03-29

N-nose as a non-invasive biomarker for predicting neoadjuvant chemotherapy response in breast cancer patients

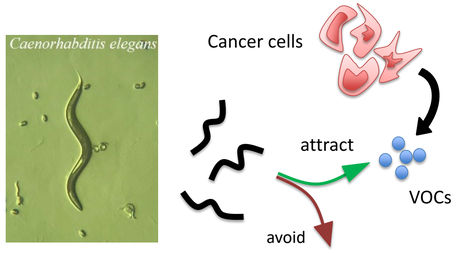
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Backgroud

- Breast cancer (BC) remains a leading cause of cancer-related mortality despite advances in diagnosis and treatment, and accurate assessment of response to neoadjuvant chemotherapy (NAC) is particularly important for HER2-positive and triple-negative subtypes.
- Liquid biopsy–based approaches have recently gained attention as non-invasive tools for cancer detection and treatment monitoring.
- **Nematodes-NOSE (N-NOSE)** is a novel assay that measures the chemotactic behavior of ***Caenorhabditis elegans* (C. elegans),** an organism with an exceptionally sensitive olfactory system in which one-third of its cells are neurons. (1)(2)
- Cancer alters metabolic pathways and releases characteristic metabolites, including volatile organic compounds (VOCs), into the urine; C. elegans moves toward attractive odors and away from repulsive ones, enabling it to detect even trace levels of cancer-associated metabolites. (3)

Features of *C. elegans*



Objective

In the current study, we aimed to clarify the ability of the N-NOSE method to predict the response to NAC in BC patients.

Materials and Methods

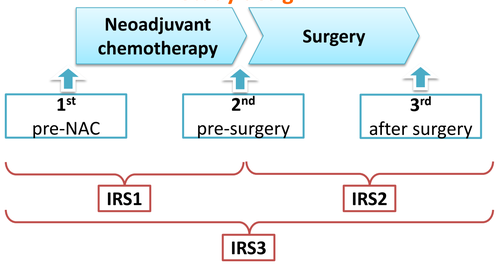
Study Population

- We prospectively enrolled 36 breast cancer patients scheduled for NAC followed by surgery between August 2020 and May 2023.

Urine Sample Collection

1. Within 1 week before NAC
2. the day before surgery
3. 4 weeks after surgery

Study design



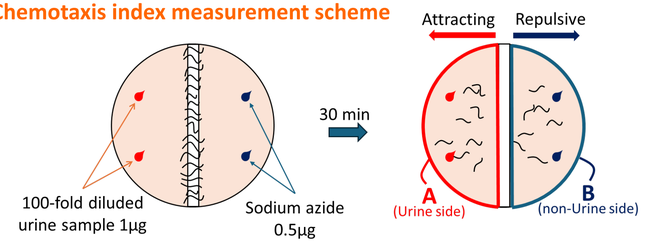
Outcome Measures

- Index reduction score (IRS)
  - ◆IRS1 : 1st -2nd
  - ◆IRS2 : 2nd -3rd
  - ◆IRS3 : 1st- 3rd
- The association between IRS and treatment efficacy was statistically evaluated using receiver operating characteristic (ROC) curve analysis.

Chemotaxis Assay Procedure

- Chemotaxis was assessed following established protocols.(1–3)
- On agar plates, 0.5 µL of 1 M sodium azide was applied to four locations. Urine diluted 1:100 was added to two sites (urine side). Approximately 100 adult nematodes were placed at the center, and after 30 minutes, the numbers in area A (urine side) and area B (non-urine side) were counted.

Chemotaxis index measurement scheme



$$\text{Chemotaxis Index(CI)} = \frac{\text{Number of nematodes in area A} - \text{Number of nematodes in area B}}{\text{Total number of nematodes}}$$

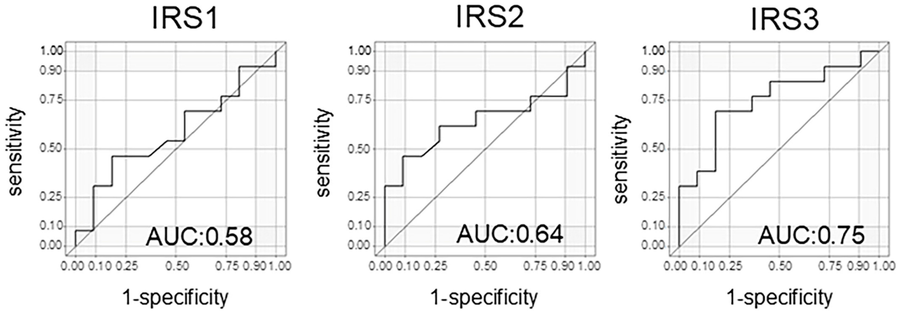
Results

- The median age was 51 years (range, 35–77), with 13 patients (36.1%) achieving pathological complete response (pCR) and 16 patients (44.4%) achieving pathological partial response (pPR).
- When the analysis was limited to patients achieving a complete response, **the area under the curve for IRS3 was the highest among all indices**, indicating that IRS3 provided the strongest predictive value for identifying pathological complete responders.

Patient characteristics (N=36)

Clinical Stage	
I	1 (2.8%)
IIA	10 (27.8%)
IIB	11 (30.6%)
IIIA	5 (13.9%)
IIIB	4 (11.1%)
IIIC	5 (13.9%)
Subtype	
Luminal	11 (30.6%)
Luminal HER2	4 (11.1%)
HER2	10 (27.8%)
Triple Negative	11 (30.6%)
Treatment response	
CR	13 (36.1%)
PR	16 (44.4%)
SD	6 (16.7%)
PD	1 (2.8%)

The association between IRS and CR



Discussion

- N-NOSE offers a non-invasive, repeatable way to monitor tumor dynamics during NAC, and we previously showed in esophageal cancer that IRS changes reflect treatment response. (4)
- By capturing patient-specific directional changes, IRS effectively minimizes the impact of variability in N-NOSE cut-off values.
- The stronger association of IRS3 (after surgery) with pCR compared with IRS1 (post-NAC) may suggest that **N-NOSE detected microscopic residual disease—such as intraductal components—more sensitively. This implies that NAC alone may leave behind extremely small residual lesions that cannot be detected by routine pathological examination.**
- N-NOSE has also been reported to outperform conventional tumor markers even in stage 0–1 gastrointestinal cancers. (5)
- This study is limited by its single-center design, small sample size, uncertain generalizability to luminal-type breast cancer, and the unproven mechanism underlying the differences between IRS1 and IRS3.

Conclusion

- IRS measured by N-NOSE may accurately reflect the efficacy of NAC in breast cancer.
- N-NOSE may also detect minimal or subclinical residual disease after NAC, which is clinically grouped but biologically heterogeneous.
- Large, multicenter prospective studies are needed to confirm these findings.

References

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All authors declare no conflicts of interest in this study.

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