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Detection of Hematological Malignancies Using N-NOSE (Nematode-NOSE)

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ABSTRACT

Hematological malignancies often lack defined risk factors and present with non-specific symptoms, underscoring the urgent need for simple and reliable detection methods. To address this challenge, Hirotsu et al. innovated N-NOSE, a novel, non-invasive cancer screening test that utilizes the chemotaxis response of the nematode *Caenorhabditis elegans* to detect tumor-related odors in urine. In this clinical study, we assessed the performance of N-NOSE in patients with various hematological malignancies at diagnosis and during treatment. Urine samples were collected from 30 healthy individuals and 89 patients, including those with leukemia ($n = 13$), malignant lymphoma ($n = 53$), multiple myeloma ($n = 15$), primary AL amyloidosis ($n = 3$), Waldenström's macroglobulinemia ($n = 2$), myelodysplastic syndrome ($n = 2$), and blastic plasmacytoid dendritic cell neoplasm ($n = 1$). Based on the optimal cut-off values in detecting hematological malignancies, N-NOSE demonstrated high positivity rates in treatment-naïve patients: leukemia and multiple myeloma were very high (over 90%), whereas malignant lymphoma was slightly lower than 80%. In the small subset of malignant lymphoma patients who tested N-NOSE-negative, confounding factors included steroid administration and hemodialysis. Importantly, no significant correlation emerged between N-NOSE index values and baseline characteristics or comorbidities other than the presence of cancer. Moreover, in all 32 patients who achieved clinical response following chemotherapy, the N-NOSE index declined, reflecting disease status. These findings highlight N-NOSE's strong potential as a sensitive, non-invasive screening tool for hematological malignancies—particularly multiple myeloma—and support its use in initial detection and monitoring of therapeutic response.

1 | Introduction

Blood cancer, also known as hematological malignancy, is mainly categorized into three major types: acute/chronic myelogenous leukemia (AML/CML), malignant lymphoma, and multiple myeloma [1]. AML is induced by the abnormality of myeloid stem/progenitor cells, whereas CML is affected by the abnormality of hematopoietic stem cells and is frequently triggered by chromosomal aberration like *BCR-ABL1* fusion [2]. In general, AML is more serious because AML has strong symptoms and shows more rapid disease progression than CML. The pathogenesis of multiple myeloma is the propagation of cancerized plasmacytes, and malignant lymphoma mainly consists of three subtypes: B-cell lymphoma, T-cell lymphoma, and

Hodgkin lymphoma. The risk factors inducing these hematological malignancies are increasing age, high doses of radiation and chemicals for therapeutics of other diseases, and infection of pathogens and viruses, and the initial symptoms of these hematological malignancies are often non-specific and heterogenetic.

In comparison with the most common six malignant solid tumors like gastric, lung, colorectal, breast, cervical, and prostate cancers, the new cases and the mortality of hematological malignancies are relatively low in Japanese population: leukemia (incidence rate, 11.3 per 100,000; mortality rate, 8.0 per 100,000), multiple myeloma (incidence rate, 5.8 per 100,000; mortality rate, 3.5 per 100,000), and malignant lymphoma (incidence rate, 28.5 per 100,000; mortality rate, 11.5 per 100,000) [3]. In addition to a routine blood test, a highly invasive needle biopsy of the patients' bone marrow is also performed to diagnose hematological malignancies. Therefore, minimally invasive, cost-effective, affordable, and highly accurate cancer screening methods are urgently required for detecting hematological malignancies.

In recent years, there's been a surge in interest in biological diagnostics, particularly cancer detection, using living organisms as biosensors. One such innovative method is N-NOSE, a cancer screening technique that employs *Caenorhabditis elegans* (*C. elegans*) as an olfactory biosensor. This method has gained significant attention, with over 700,000 individuals having undergone the cancer test in Japan at the time of writing [4].

C. elegans, with approximately 1200 candidates for G protein-coupled olfactory receptor genes [5–7] and only five types of olfactory neurons (AWA and AWC for excitatory responses to pleasant odors, and AWB, ASH, and ADL for excitatory responses to unpleasant odors), possesses a sophisticated sense of smell that can detect specific odors in the urine of cancer patients. A previous study revealed that *C. elegans* can recognize cancer-associated volatile organic compounds (VOCs) and exhibit specific chemotactic behavior, moving toward cancer patients' urine and away from healthy individuals' urine [8].

We recently reported a prospective clinical study involving a cohort of over 1600 patients demonstrated the success of N-NOSE in detecting over 20 types of malignancies, including the most common six types of cancer worldwide (lung, stomach, colorectal, breast, cervical, and prostate) and malignant lymphoma, with high sensitivities ranging from 60% to 90% [9]. However, the efficacy of N-NOSE in detecting various hematological malignancies remains to be fully proven.

In this study, we sought to assess the efficacy of N-NOSE in detecting hematological malignancies. N-NOSE exhibited remarkable discrimination between 30 healthy individuals and 89 patients with hematological malignancies. Our findings contribute to the accumulating evidence supporting nematode-based cancer screening methods. They elucidate the potential of N-NOSE in developing detection strategies for various types of hematological malignancies and numerous types of malignant solid tumors. This could revolutionize whole-body cancer screening.

2 | Materials and Methods

2.1 | Study Population

This study was approved by the institutional review board of the Tokushima University Ethics Committee (Approval number 3045-1, 3045-4). Urine samples were collected from 30 healthy individuals (mean age, 40.6 ± 10.0 years; male: female, 17:13) and 89 patients (mean age, 64.3 ± 14.3 years; male: female, 41:48) diagnosed with hematological malignancies at the Tokushima University Hospital (Tokushima, Japan) between June 2018 and July 2024. The characteristics of these 89 patients are listed in Table 1. To minimize potential confounding factors, strict eligibility criteria for patients recruitment were applied. Patients with comorbid malignancies or active systemic infectious diseases, including urinary tract infections, were excluded in this study. Urine samples were further collected from 32 patients who received cytotoxic chemotherapy by June 2021. The complete/partial response criteria were used as previously reported for lymphoma [10], myeloma [11], and leukemia [12]. The characteristics of these 32 patients who received chemotherapy are also listed in Supporting Information Table S1. We obtained written informed consent from all participants. Furthermore, the key exclusion criteria for the recruitment of healthy individuals in this study were as follows: (i) individuals

TABLE 1 | The characteristics of patients with hematological malignancies enrolled in the study.

	ML	MM	Leukemia	AL	Macro	MDS	BPDCN	Total	Healthy
Number of cases	53	15	13	3	2	2	1	89	30
Male	22	6	6	2	2	2	1	41	17
Female	31	9	7	1	—	—	—	48	13
Mean age (years)	67.1	66.7	50.1	62.3	68.5	55.5	80	64.3 ± 14.3	40.6 ± 10.0
Stage I	6	5	—	—	—	—	—	11	—
Stage II	12	—	—	—	—	—	—	12	—
Stage III	10	10	—	—	—	—	—	20	—
Stage IV	25	—	—	—	—	—	—	25	—
Undefined	—	—	13	3	2	2	1	21	—

Abbreviations: AL: AL amyloidosis, BPDCN: blastic plasmacytoid dendritic cell neoplasm, Macro: Waldenström's macroglobulinemia, MDS: myelodysplastic syndrome, ML: malignant lymphoma, MM: multiple myeloma.

without a history of cancer, (ii) individuals not suffering from any (systemic or urinary tract) infectious diseases, (iii) no pregnant, no lactating, or no possible pregnant. All participants in the study were Asian people (Mongoloid).

2.2 | Collection of Urine Samples

Urine samples were collected from the patients in the morning before breakfast. The collected urine samples were frozen (−20°C) within 2 h of collection until analysis by N-NOSE.

2.3 | Measurement by N-NOSE

Based on a seminal study by Bargmann et al. [5], the N-NOSE chemotaxis method was developed to analyze the behavior of *C. elegans* toward urine samples from cancer patients [8]. To perform the chemotaxis assay, we cultured *C. elegans* (wild-type N2) at 20°C under well-fed and uncrowded conditions with the *Escherichia coli* strain NA22. Chemotaxis assay plates were prepared as previously described (10 mL of media composed of 2% agar, 5 mM KPO_4, 1 mM $CaCl_2$, and 1 mM $MgSO_4$ in 9-cm diameter dishes) [5, 8, 9, 13]. Chemotaxis assays were performed as described previously [8, 9]. Briefly, 0.5 μL of 1 M sodium azide was spotted at two points at both ends of the plates, and 1 μL of urine sample diluted 10-fold and 100-fold with ultrapure water was added to two points. Sodium azide, a chemical agent used to immobilize nematodes, facilitating quantification of their number, was added before introducing the nematodes to the plate. Sodium azide was added to four points on the plate (urine side and non-urine side), with urine added to two points (urine side and sodium azide location). We confirmed that nematodes showed no chemotaxis behavior to 1 μL of water (data not shown).

Approximately 100 adult nematodes were collected, washed three times with chemotaxis buffer (0.05% gelatin, 5 mM KPO_4, 1 mM $CaCl_2$, and 1 mM $MgSO_4$), and placed in the center of the plate. After removing the excess buffer, the nematodes were allowed to roam for 30 min. After 30 min, the number of nematodes that moved toward the urine samples [N(A)] and that moved away from the samples [N(B)] were counted. The chemotaxis index was calculated using the following equation (Figure 1): Chemotaxis index = [N(A) − N(B)]/[N(A) + N(B)]; Where [N(A)] is the number of nematodes near the urine samples, and [N(B)] is the number of nematodes in the region without the samples. For each sample, chemotaxis assays were performed at both 10-fold and 100-fold urine dilutions ($N = 10$ for each dilution), and obtained data were averaged. A negative index (− 1 to 0) indicates the trend of "repulsion" to the sample, and a positive index (0–1) means the trend of "attraction" to the sample, respectively. In this study, we performed 3020 N-NOSE assays in total.

2.4 | Statistical Analyses

One-way analysis of variance (ANOVA) and Wilcoxon signed-rank tests were performed using JMP version 14 (SAS Institute Inc., Cary, NC, USA). Statistical significance was set at $p < 0.05$. ROC, AUC calculation, graphs, and plots were performed using GraphPad Prism version 10.0.0 (GraphPad Software, Boston, MA, USA).

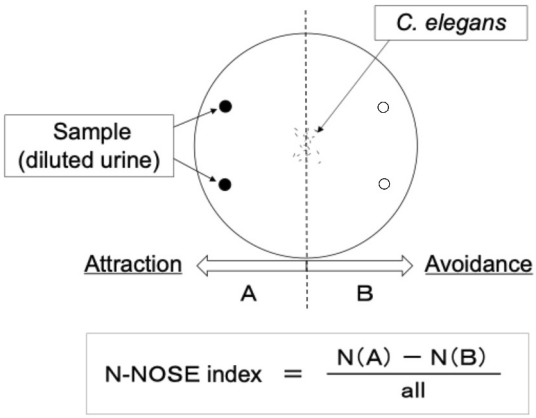

FIGURE 1 | Measurement of nematode chemotactic behavior and N-NOSE index.

3 | Results

3.1 | Patients

The characteristics of the 89 patients are summarized in Table 1. There were 48 females and 41 males enrolled in this study. The mean age was 64.3 ± 14.3 years. Most of the participants had malignant lymphoma (ML, $N = 53$), symptomatic multiple myeloma (MM, $N = 15$), or leukemia ($N = 13$). Among ML, there were 19 cases of diffuse large B-cell lymphoma (DLBCL), 9 cases of follicular lymphoma (FL), 8 cases of T-cell lymphoma, 4 cases of Hodgkin lymphoma (HL), 4 cases of mucosa-associated lymphoid tissue lymphoma (MALT), and other subtypes. We also analyzed 32 patients to investigate the influence of pre-/post-treatment on the changes in the chemotactic behavior of nematodes (Supporting Information Table S1). This study also included 30 healthy individuals to newly set the appropriate N-NOSE cut-off values in detecting hematological malignancies.

3.2 | Sensitivity of N-NOSE in Detecting Hematological Malignancies

First, we addressed whether N-NOSE can discriminate between healthy individuals and patients with hematological malignancies. The N-NOSE chemotaxis assays were performed using two different conditions of 10-fold and 100-fold urine dilution. In contrast to healthy individuals, most parts of hematological malignancy patients before chemotherapy showed positive for N-NOSE chemotaxis assay (Figure 2A). Area under the curve (AUC), calculated from the receiver operating characteristics (ROC) analysis of each urine dilution indicates that N-NOSE has good performance to discriminate between healthy individuals and patients with hematological malignancies (AUC = 0.7753 at 10-fold dilution; AUC = 0.8260 at 100-fold dilution, respectively). Notably, the optimal cut-off values for both conditions estimated in this study were slightly lower than zero (−0.020 at 10-fold dilution; −0.024 at 100-fold dilution,

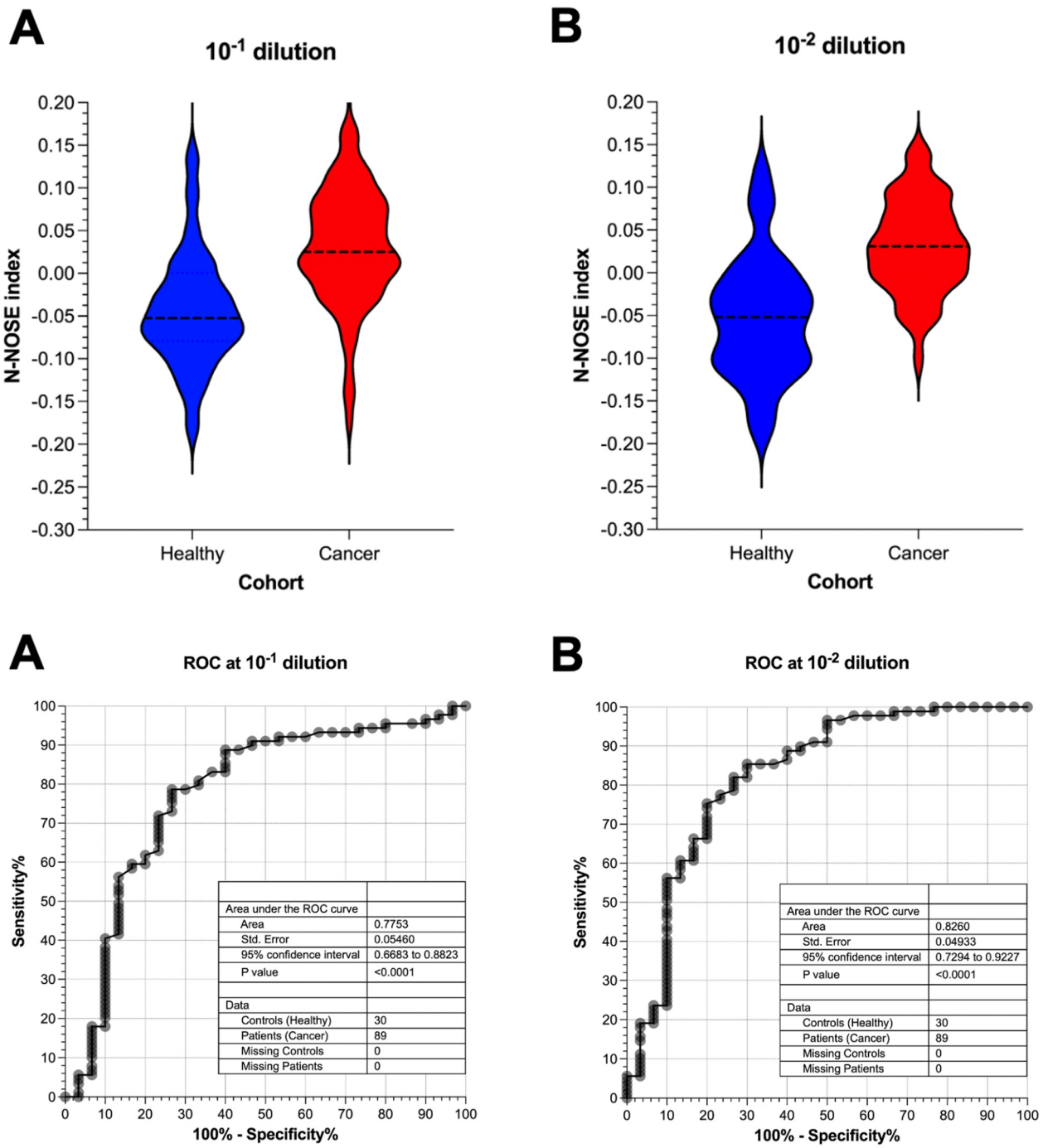

FIGURE 2 | (A) Comparison of N-NOSE index distributions between healthy and cancer cohorts at two sample dilutions (10^{-1} and 10^{-2}). (B) ROC curve analysis at two dilution levels (10^{-1} and 10^{-2}). Sensitivity (%) = True Positive Rate; 100%—Specificity (%) = False Positive Rate.

respectively) (Figure 2B), and the calculated sensitivities [78.7% (70/89) at 10-fold dilution; 85.4% (76/89) at 100-fold dilution, respectively] and specificities [73.3% (22/30) at 10-fold dilution; 70.0% (21/30) at 100-fold dilution, respectively] using optimal cut-off values at each condition were also high.

Among patients with hematological malignancies, some cases were negative for N-NOSE, especially the patients with ML (Figure 3). Similar trends for N-NOSE chemotaxis assay were observed between the two urine dilutions (10-fold and 100-fold). We performed regression analysis between the N-NOSE index and the three major types of hematological malignancy (ML, MM, and leukemia). The N-NOSE index was not correlated with any of the three hematological malignancies by one-way ANOVA ($p = 0.0537$ at 10-fold dilution; $p = 0.0861$ at 100-fold dilution). The cancer-type-specific sensitivities and stage-specific sensitivities of N-NOSE for hematological malignancies were calculated and summarized in Tables 2 and 3. The N-NOSE tests had overall sensitivity for each condition (78.7% at 10-fold dilution; 85.4% at 100-fold dilution). Among ML patients with a negative for N-

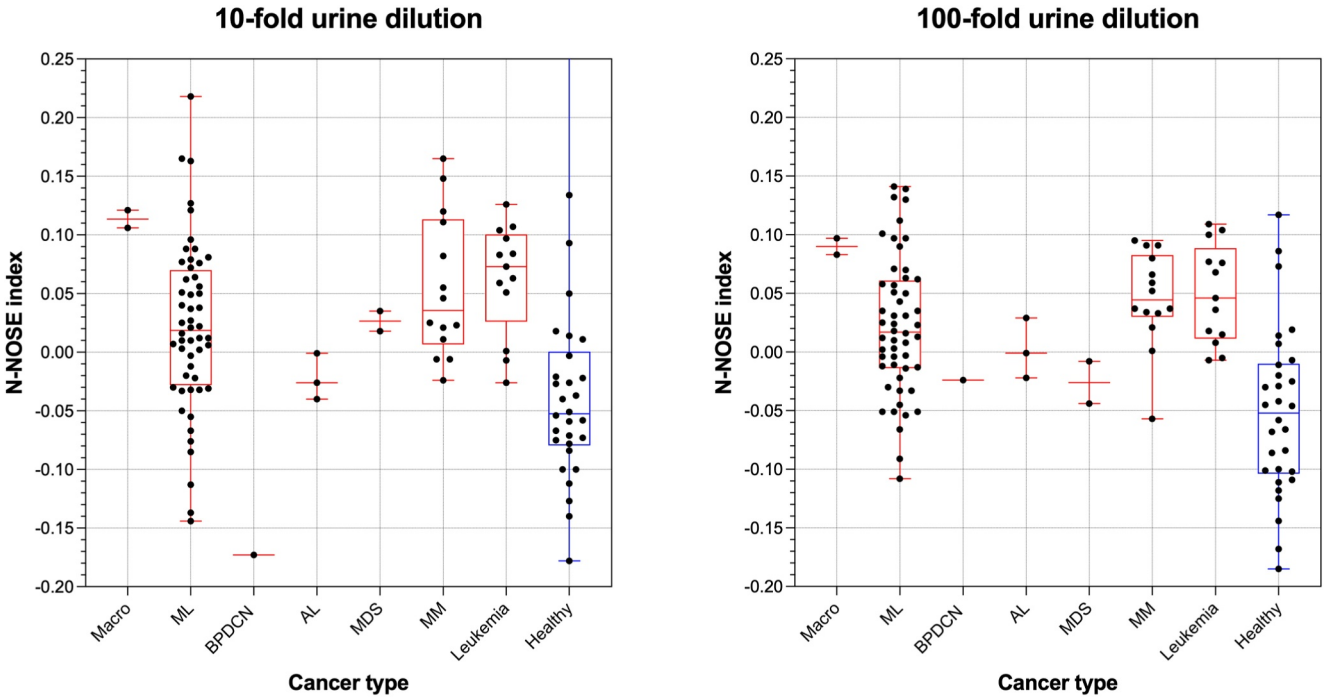

FIGURE 3 | N-NOSE index in hematological malignancies. AL: AL amyloidosis, BPDCN: blastic plasmacytoid dendritic cell neoplasm, Macro: Waldenström's macroglobulinemia, MDS: myelodysplastic syndrome, ML: malignant lymphoma, MM: multiple myeloma.

TABLE 2 | Cancer-type-specific sensitivities of N-NOSE in hematological malignancies.

Cancer type	Total	Positive	Negative	Sensitivity / Specificity (Healthy)
10-fold urine dilution				
ML	53	39	14	73.6%
MM	15	14	1	93.3%
Leukemia	13	12	1	92.3%
AL	3	1	2	33.3%
Macro	2	2	0	100.0%
MDS	2	2	0	100.0%
BPDCN	1	0	1	0.0%
Total	89	70	19	Sensitivity: 78.7%
Healthy	30	8	22	73.3% (Specificity)
100-fold urine dilution				
ML	53	42	11	79.2%
MM	15	14	1	93.3%
Leukemia	13	13	0	100.0%
AL	3	3	0	100.0%
Macro	2	2	0	100.0%
MDS	2	1	1	50.0%
BPDCN	1	1	0	100.0%
Total	89	76	13	85.4%
Healthy	30	9	21	70.0% (Specificity)

Abbreviations: AL: AL amyloidosis, BPDCN: blastic plasmacytoid dendritic cell neoplasm, Macro: Waldenström's macroglobulinemia, MDS: myelodysplastic syndrome, ML: malignant lymphoma, MM: multiple myeloma.

TABLE 3 | Stage-specific sensitivities of N-NOSE in hematological malignancies.

Stage	Total	Positive	Negative	Sensitivity
10-fold urine dilution				
I	11	9	2	81.8%
II	12	8	4	66.7%
III	19	17	2	89.5%
IV	24	17	7	70.8%
Undefined	23	19	4	82.6%
Total	89	70	19	78.7%
100-fold urine dilution				
I	11	10	1	90.9%
II	12	9	3	75.0%
III	19	16	3	84.2%
IV	24	19	5	79.2%
Undefined	23	22	1	95.7%
Total	89	76	13	85.4%

NOSE, 2 had administered prednisolone before urine collection, 1 had received hemodialysis, and 1 had cirrhosis. The sensitivities in detecting leukemia and MM were very high (over 90%), whereas that in detecting ML was slightly lower than 80%. There was no relationship between the N-NOSE index and the stage of ML ($p = 0.9848$ at 10-fold dilution; $p = 0.8959$ at 100-fold dilution) or MM ($p = 0.6590$ at 10-fold dilution; $p = 0.8242$ at 100-fold dilution), and no association between the N-NOSE index and ML histological subtypes ($p = 0.7123$ at 10-fold dilution; $p = 0.8820$ at 100-fold dilution). Furthermore, high N-NOSE sensitivities were also maintained at all stages, suggesting that N-NOSE can successfully detect various types of hematological malignancies with high sensitivity, even at earlier stages, and in the case of malignant solid tumors.

Additionally, no significant correlations were found between the N-NOSE index and the underlying disease and comorbidities (diabetes mellitus, hyperlipidemia, or hypertension), hepatic function, renal function, and urine test results, as calculated by single regression analysis (Table 4), indicating that the N-NOSE index only associates with the presence of cancer ($p = 0.0001$ at 10-fold dilution; $p < 0.0001$ at 100-fold dilution).

3.3 | N-NOSE Index After Treatment for Hematological Malignancies

We also investigated whether the N-NOSE can respond to the change in pre-/post-chemotherapy for patients with hematological malignancies. Among 89 patients, we collected urine from 32 patients who received chemotherapy and achieved at least partial response (CR, $N = 23$; PR, $N = 3$; VGPR, $N = 6$), including 21 ML, 7 MM, 3 leukemia, and 1 other subtype (Supporting Information Table S1). Similar trends for decrease in the N-NOSE index after chemotherapy were observed in both urine conditions of 10-fold and 100-fold urine dilution, and a statistically significant difference was confirmed in 100-fold diluted urine samples ($p = 0.0006$) when compared to 10-fold dilution ($p = 0.0674$) (Figure 4A). We then evaluated the transition of N-NOSE index before/after chemotherapy for all 32 patients who received chemotherapy (Figure 4B). The decreased N-NOSE index, which indicates the reduction of hematological malignant cells, was confirmed (22 out of 32 patients at 10-fold dilution; 24 out of 32 patients at 100-fold dilution). We therefore conclude that N-NOSE is also utilized as a clinical evaluation tool for monitoring the therapeutic efficacy.

TABLE 4 | Regression analysis of the underlying disease and comorbidities, hepatic function, renal function, and urine test.

10-fold urine dilution: Single analysis		*p*-value
Age		0.7660
Sex		0.1726
Disease	Diabetes mellitus	0.8247
	Hyperlipidemia	0.4355
	Hypertension	0.6840
Hepatic function	ALT	0.4061
	AST	0.1078
Renal function	Creatinine	0.4778
	BUN	0.8211
Urine qualitative test	Glucose	0.8247
	Protein	0.9721
	Ketone bodies	0.0615
	Occult blood	0.2601
100-fold urine dilution: Single analysis		***p*-value**
Age		0.3670
Sex		0.3111
Disease	Diabetes mellitus	0.1675
	Hyperlipidemia	0.7142
	Hypertension	0.1268
Hepatic function	ALT	0.3939
	AST	0.1222
Renal function	Creatinine	0.2481
	BUN	0.5363
Urine qualitative test	Glucose	0.1675
	Protein	0.6565
	Ketone bodies	0.9393
	Occult blood	0.3590

4 | Discussion

Hematological malignancies often manifest with subtle, nonspecific symptoms, posing significant challenges for early recognition and diagnosis in primary care settings. Acute leukemia may be suspected from persistent abnormalities in blood counts, whereas malignant lymphoma (ML) typically requires invasive tissue biopsy, and multiple myeloma (MM) is often identified only after characteristic end-organ damage—such as anemia,

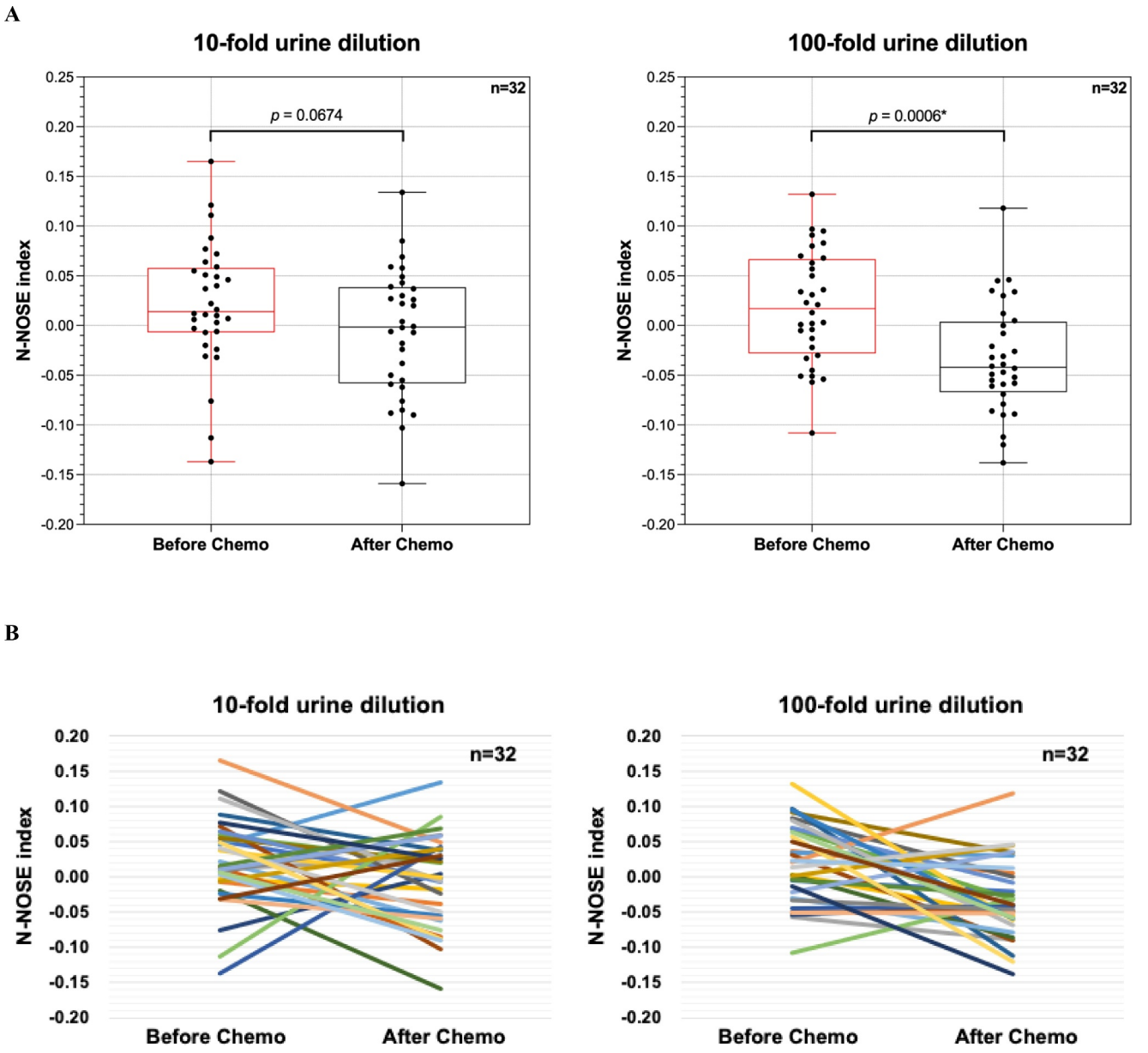

FIGURE 4 | (A) The transition of N-NOSE index measured by two different conditions of 10-fold (left) and 100-fold (right) urine dilution before and after chemotherapy. The difference of index between before and after chemotherapy was analyzed by Wilcoxon signed-rank test. (B) The transition of N-NOSE index before/after chemotherapy for all 32 patients who received chemotherapy.

bone lesions, renal failure, or hypercalcemia—has occurred. These diagnostic hurdles highlight the urgent need for simpler, more accurate, and earlier detection strategies that can be applied broadly across various hematological malignancies.

Our study demonstrates that N-NOSE, a nematode-based chemotaxis assay, provides a promising solution to this need. By leveraging the highly sensitive olfactory system of *C. elegans*, we achieved a high rate of detection across multiple hematological malignancies, including MM, ML, and leukemia. Importantly, N-NOSE maintained robust sensitivity even at earlier disease stages, which are notoriously difficult to identify using current clinical approaches. Moreover, the N-NOSE index was not influenced by sex, comorbidities (including diabetes mellitus, hyperlipidemia, or hypertension), or routine laboratory parameters, underscoring its versatility and potential for broad clinical application. This non-invasive, urine-based test could easily be integrated into a wide range of clinical settings—from primary care clinics to specialized hematology-oncology units.

Beyond its diagnostic utility, N-NOSE also demonstrated potential as a tool for monitoring therapeutic response. In our study, patients who achieved clinical remission or a favorable response to chemotherapy showed a notable decrease in the N-NOSE index. These findings align with our previous reports in other cancer types on the N-NOSE index changes of pre-/post-chemotherapy in patients with esophageal cancer [14], suggesting that N-NOSE may serve as a dynamic biomarker capable of reflecting changes in tumor burden. As a result, it could support early detection and aid in long-term surveillance, identifying relapse or progression at a stage when intervention may still be effective. Such a non-invasive monitoring method is particularly appealing

in older or frail patients for whom repeated invasive procedures, such as biopsies, are less tolerable.

While our sample size was relatively limited, and certain hematological malignancies—such as myeloproliferative disorders and adult T-cell leukemia/lymphoma—were not included, these preliminary data are encouraging. Larger, more comprehensive studies are now needed to fully establish N-NOSE's place in the diagnostic and monitoring algorithm for hematological malignancies, ensuring that it meets established screening guidelines such as those proposed by Pepe et al. [15]. Such investigations should also further elucidate the underlying biological mechanisms—specifically, which volatile organic compounds (VOCs) guide *C. elegans* chemotaxis, and whether these VOCs are universal cancer markers or vary by malignancy subtype. Understanding these nuances will refine the specificity and sensitivity of N-NOSE and may lead to the development of even more accurate, mechanistically informed screening tools.

In an era when cancer screening often relies on tests tailored to individual tumor types, N-NOSE stands out as a broadly applicable approach. Using a single-voided urine sample, N-NOSE could potentially identify hematological and solid tumors, simplifying the screening landscape. As part of a multi-tiered diagnostic strategy, N-NOSE may aid clinicians in evaluating patients with unexplained cytopenia, lymphadenopathy, or persistent fever, thereby accelerating referral for definitive diagnostic procedures and treatment. Ultimately, integrating N-NOSE into routine clinical practice could promote earlier detection, improve patient outcomes, and contribute to reducing mortality from these complex malignancies.

In conclusion, our findings highlight N-NOSE as a non-invasive, highly sensitive, and potentially game-changing tool for both the detection and monitoring of hematological malignancies. By bridging the gap between subtle early presentation and timely intervention, N-NOSE has the potential to transform the diagnostic landscape, leading to earlier interventions, better prognosis, and improved long-term patient care.

Author Contributions

Shingen Nakamura: conceptualization, sample acquisition and processing, writing – original draft preparation, manuscript supervision, project administration. **Hideyuki Hatakeyama:** data acquisition, writing – original draft preparation, writing – revised draft and editing. **Sumiko Yoshida:** conceptualization. **Umbhorn Ungkulpasvich:** writing – revised draft and editing. **Takaaki Hirotsu:** conceptualization, project administration. **Eric di Luccio:** writing – revised draft and editing, manuscript supervision. **Masahiro Abe:** conceptualization, manuscript supervision. All authors have read and agreed to the published version of the manuscript.

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Ethics Statement

Institutional Review Board Statement. The study was conducted per the Declaration of Helsinki and approved by the Ethics Committee of the Tokushima University (Approval number 3045-1, 3045-4).

Consent

Institutional Review Board Statement Informed Consent Statement. Informed written consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Conflicts of Interest

T.H. is the CEO and founder of Hirotsu Bio Science Inc.; E.d.L., H.H., U. U. are Hirotsu Bio Science Inc. employees.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/hon.70062.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.