Elemental analysis by Metallobalance provides a complementary support layer over existing blood biochemistry panel-based cancer risk assessment

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ABSTRACT

Despite the benefit of early cancer screening, Japan has one of the lowest cancer screening rates among developed countries, possibly due to there being a lack of "a good test" that can provide sufficient levels of test sensitivity and accuracy without a large price tag. As a number of essential and trace elements have been intimately connected to the oncogenesis of cancer, Metallobalance, a recent development in elemental analysis utilizing the technique of inductively coupled plasma mass spectrometry has been developed and tested as a robust method for arrayed cancer risk screening. We have conducted case-control epidemiological studies in the prefecture of Chiba, in the Greater Tokyo Area, and sought to determine both Metallobalance screening's effectiveness for predicting pan-cancer outcomes, and whether the method is capable enough to replace the more conventional antigen-based testing methods. Results suggest that MB screening provides some means of classification potential among cancer and non-cancer cases, and may work well as a complementary method to traditional antigen-based tumor marker testing, even in situations where tumor markers alone cannot discernibly identify cancer from non-cancer cases.

INTRODUCTION

Despite being the leading cause of death since 1981 (*Ministry of Health, Labour, and Welfare, Annual Vital Statistics Report Main Statistics, 2020*), cancer, a number of types of which can be prevented with early screening and monitoring, often does not appear on most people's radar until later in life. Japan, for instance, has one of the lowest cancer screening rates (*Editorial Board of the Cancer Statistics in Japan & Cancer Statistics in Japan, 2021*; *OECD stat, 2020*) in developed countries, yet only a few limited healthcare facilities are dedicated to screening and preventative medicine (*Okuhara et al., 2018b*).

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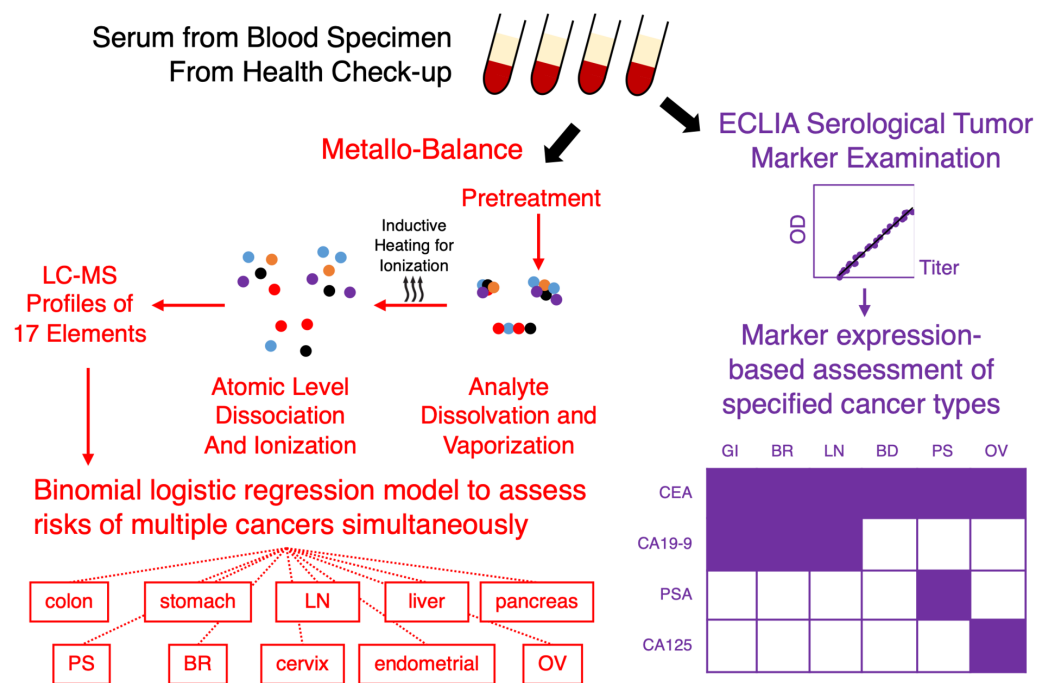
Figure 1 Metallobalance (MB) as a complementary test for pan-cancer screening. Conventional serological examinations of tumor markers by methods such as electrochemiluminescence immunoassays (ECLIA), is only limited to a small known panel of markers, *e.g.*, CEA, CA19-9, PSA and CA125; conversely, only a limited number of cancer susceptibility may be reportable (bottom right example; shaded grid indicates reportable type of cancer per marker; GI, gastrointestinal; BR, breast; LN, lung; BD, bladder; PS, prostate; OV, ovarian). Metallobalance (highlighted in red) on the other hand, utilizes induced collision plasma assisted mass spectrometric analysis to ionize serological analytes and produces a profile of 17 elemental levels. When combined cancer risk outcomes can generate logistic regression models to characterize susceptibility to a large number of cancer types simultaneously.

While multiplexed reasons, such as anti-cancer screening sentiments (*Okuhara et al., 2018b*), may have led to this current development, the lack of "a good test" is perhaps be the largest hurdle to clear to-date. In absence of such a test with sensitivity, reproducibility and low costs of entry, a bottleneck for the expansion rate for effective large-scale screening remains. Even as recent trends in biomedical advances have introduced to the public a barrage of modern instrumentation and testing such as genetic risk screening by next generation sequencing, or even methods that exploit a number of decided novel oncotargets such as MicroRNA, nucleosomes and even apoAII (*PDQ Screening and Prevention Editorial Board, 2019*; *Sugimoto et al., 2010*; *Ito et al., 2015*; *Honda et al., 2015*), the "bread and butter" of cancer screening remains the use of blood biochemistry panels (Fig. 1, purple) for common cancer antigens such as CEA, CA19-9, CA125, etc. (*Hall et al., 2018*), which are highly limited in the scope of coverage for screening cancer of various types.

In analytical chemistry, elemental analysis is an essential tool for compound characterization; as elements not only compose the macromolecules that provide for life, their essential role in homeostasis and biological processes it is not difficult to make a natural connection for using elemental analysis to characterize human health and perhaps
diseases such as cancer. Essential elements such as metals are intimately connected to carcinogenesis and metastasis; metalthioneine, for instance, is a protein involved in the regulation of metal ions, and has also been associated with tumor growth, differentiation and even drug resistance as others such as *Manfei & Lang (2018)* have investigated and concluded in a recent review. Additionally, interactions between metals such as Zn and Cu have also been said to cause dramatic effects on zinc-containing enzymes involved in processes leading up to cancer (*Masoudreza et al., 2018*). There is also association between serum elemental levels and cancer *via* events such as iron deficiency and oxidative stress, or baseline serum selenium levels with association to deaths from gastric cancer (*Hu et al., 2018*; *Zhou et al., 2018*).

A recent development in elemental analysis is Metallobalance (MB), an inductively coupled plasma mass spectrometry (ICP-MS)-based method to evaluate the comprehensive serum profile of 17 elements (*Okamoto et al., 2020*) for cancer screening (Fig. 1, red). A feature of MB is its ability to characterize, in addition to free elements in serum, elements in bound states such as the fundamental makeup of macromolecules. The test itself only requires small amounts of serum sample, can be stored and transported in absence of complex storage and protective measures, and thus can easily be implemented as part of the annual checkup procedure along with other tumor markers; additionally, the high-throughput nature and inexpensive preparation of ICP-MS also allows costs to be greatly reduced. On paper, however, while MB may be that one good test to promote large-scale screening, to-date MB only has risk prediction models for a handful of cancer types, partly due to an initial cohort of limited cancer type makeup. To characterize the applicability of MB as a pan-cancer screening tool, we have conducted case-control epidemiological studies in the prefecture of Chiba, in the Greater Tokyo Area, and sought to determine whether MB may be able to replace or supplement the more conventional antigen-based biomarker tests.

MATERIALS & METHODS

Study subjects

The ethics review committee at Chiba Cancer Center granted IRB approval (Cc# 902) for this study, and we obtained verbal informed consent from participants who later provided a signed consent form agreeing to specimen deposition at the Chiba Cancer Center Biobank. As part of a larger study, during the period of 2006 to 2013 we recruited and invited over 8,000 participants (M:F = 2,918:5,176) between the ages of 35 to 69 who resided in cities of Inzai, Abiko and Kashiwa in the prefecture of Chiba to participate in a cancer screening study which included blood specimen submission for multi-biomarker panel of CEA, CA 19-9, CA125 and PSA screening; remnants of the specimens were preserved and used for MB screening at a later time. In addition, these participants also volunteered responses to an epidemiological survey detailing their health histories and lifestyles, such as demographics, education, alcohol consumption, smoking, sleeping, exercise, food intake frequency, medication and supplement use, personal and family disease history, psychological stress, and female reproductive history. We selected those whose past medical histories had no

Table 1 Cohort statistics.

	P_0	P_1	Excluded
N	5,327	1,856	1,491
M: F	1,696: 3,631	697: 1,159	544: 947
Age [Mean]	35∼69 [53.0]	15∼94 [62.7]	35∼69 [55.4]
<40	733 (13.8)	106 (5.7)	112 (7.5)
40–49	1,332 (25.0)	234 (12.6)	315 (21.1)
50–59	1,489 (28.0)	257 (13.8)	452 (30.3)
60–69	1,773 (33.2)	623 (33.6)	612 (41.1)
>69	0	636 (34.3)	0
CEA	2,090	1,331	258
CA19-9	2,795	980	683
CA125	2,003	521	435
PSA	748	231	184

Notes.

P_0, non-cancer individuals; P_1, cancer subjects; N, number of subjects within cohort; M:F, male:female ratio; Age, age range with estimated mean within bracket; value inside parentheses for individual age groups indicate percent of the subjects within cohort.

indications of cancer as healthy individuals (cohort P_0 henceforth; Table 1 left) based on records in the prefectural cancer registry kept and curated by the Cancer Prevention Center at Chiba Cancer Center.

For the cohort of cancer cases P_1 (Table 1 center) we selected a cohort of patients who visited our medical center for inpatient or outpatient services from 2013 to 2019, and consented to a biobank deposit of blood specimens for the study. P_1 subjects all received diagnoses of cancer at our center and provided informed consent for the study. Cancer types among P_1 subjects included cancers of the colon and rectum ($N = 100$, M:F = 60:40), stomach ($N = 90$, M:F = 150:68), liver ($N = 117$, M:F = 89:28), bile duct ($N = 45$, M:F = 22:68), pancreas ($N = 233$, M:W = 112:121), thyroid ($N = 90$, M:F = 34:56), prostate ($N = 200$), breast ($N = 322$), ovary ($N = 79$), cervix ($N = 197$), and uterine body ($N = 148$), and from their medical histories we recorded gender, age as well as cancer history; while some in P_0 self-reported history of cancer, those ("Excluded" in Table 1) were excluded from subsequent analysis. In the case of P1 subjects who had more than one biomarker reading as a result of multiple visits to our hospital, we used the median value as the representative biomarker level.

Metallobalance screening by ICP-MS

After specimen collection, the operation of ICP-MS for MB screening was performed by Renatech Corporation (Isehara, Kanagawa, Japan), following the procedure for sample pretreatment and spectrometric analysis by inductively coupled plasma mass spectrometry as previously described in *Okamoto et al. (2020)*. Briefly, 50 μl of serum samples were washed with ultra-pure water and 10% nitric acid along with cycles of heating for digestion; analytes were then treated with 61% nitric acid (v/v HNO_3: analyte = 2.5:1) and 30% hydrogen peroxide (v/v 0.5:1) for 16 h at 70 °C in preparation for in-line plasma ionization and mass spectrometric characterization. The 17 different elemental levels acquired on a
Agilent 7800 ICP-MS system under high-frequency output mode (1550 W) were as follows: Na (ppm), Mg (ppm), P (ppm), S (ppm), K (ppm), Ca (ppm), Fe (ppb), Co (ppb), Cu (ppb), Zn (ppb), As (ppb), Se (ppb), Rb (ppb), Sr (ppb), Mo (ppb), Ag (ppb) and Cs (ppb) against an internal standard of 50 µg/L beryllium, 5 µg/L yttrium, 1 µg/L rhodium and 50 µg/L tellurium at flow rates of 15 L/min and 1.05 L/min for plasma and nebulizer gases.

Data analysis

Statistical analysis was performed in R (ver. 4.1) with packages ggplot2 (ver. 3.3.3), effsize (ver. 0.8.1), caret (ver. 6.0.86), umap (ver. 0.2.6.0), RColorBrewer (ver. 1.1.2), smotefamily (ver. 1.3.1), pROC and gplots (ver. 3.0.4) (*Wickham, 2020*; *Marco, 2020*; *Kuhn et al., 2008*; *Siriseriwan, 2019*; *Robin et al., 2011*). Logistic regression models of the outcome of cancer history from MB elemental levels were generated to determine the odds ratio for individual elements. Due to the relative imbalance in population size, oversampling by SMOTE with 5 nearest neighbors was performed; results reported herein were averages over 1000 randomized samplings. Random forest classifier models associating MB levels with cancer history were trained on resultant SMOTE datasets with a 10% holdout and 500 trees; receiver operating characteristics were calculated from out of bag classification votes for each data point. Exploratory analysis *via* UMAP was performed using data obtained from MB for cohorts P_0 and P_1 after a parameter search for the number of nearest neighbors from 5 to 20 (final 5). Effect size comparisons were performed after log-transformation of MB data and subgroup stratification by gender and cohort; Cohen's *d* score was then determined per cancer type *via* comparison to P_0, where $d > 0$ indicated that there existed some measurable level of difference between the two cohort means, as normalized by the standard deviation of each respective elemental level. Generalized Gaussian linear regression models with identity link functions were used to create association models between elements included in the MB panel with common tumor markers. MB elemental levels were min-max scaled, while tumor biomarker levels were either log- (CA19-9, CA125, PSA) or square-root-transformed (CEA). For regression model building, CA19-9 readings beyond the cut-off of 2.0 U/ml were excluded as a precaution to ensure data quality. Reference ''normal'' thresholds of CEA < 5.0 ng/mL, CA19-9 < 37.0 U/mL, CA 125 < 35.0 U/mL and PSA < 4.0 ng/mL were established from a literature survey (*Bast et al., 1983*; *Coric et al., 2015*; *Scara, Bottoni & Scatena, 2015*; *Konishi et al., 2018a*), in line with cutoffs used in typical clinical serological examinations. A predefined alpha level of $p < 0.05$ was used for significance comparison, unless otherwise specified. Multiple comparison adjustments were not explicitly considered due to the exploratory nature of this study, however as a precaution for overfitting we adopted the alpha level of $p < 0.0001$ for regression models to account for cohort sizes.

RESULTS

A number of elements such as Mg, Ca, Fe, Co, Cu, Sr, Mo, Ag and Cs demonstrated some likelihoods of in their ability to discern cancer from non-cancer cases (Fig. 2A, log $OR > 0$). Pan-cancer logistic regression models (Fig. 2B) suggested cancer cases could be sufficiently identified from P_0 at an AUC of 0.830 (0.731 when only elements with log $OR > 0$ were
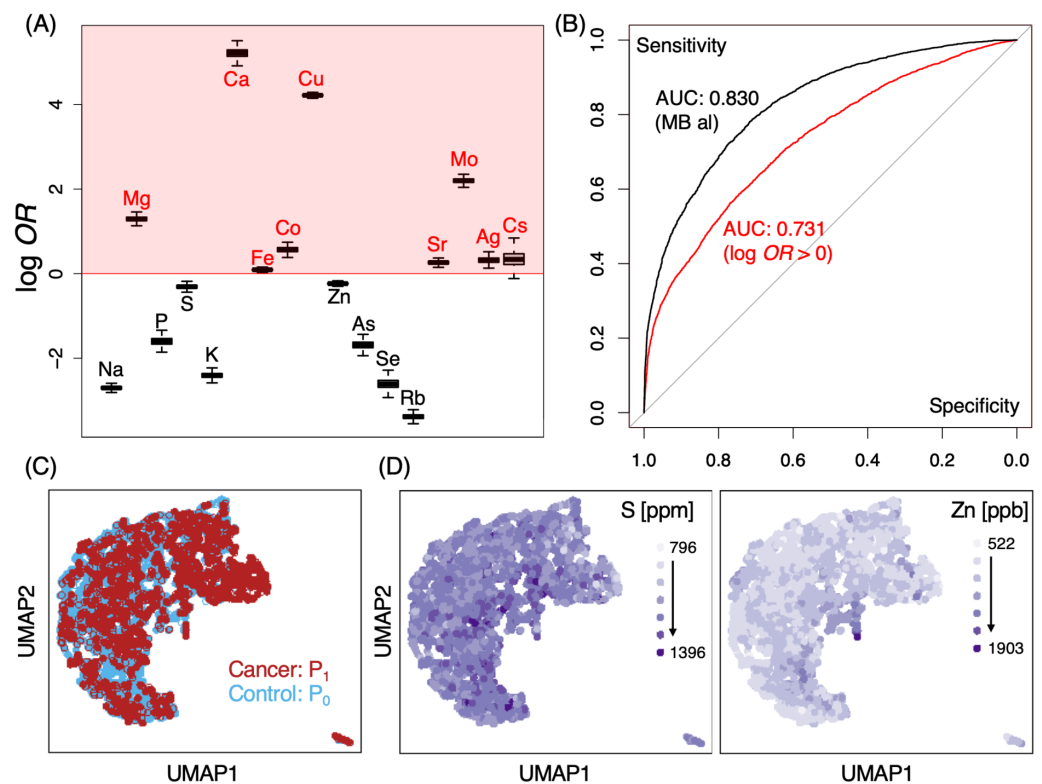
Figure 2 **ICP-MS levels of certain elements may provide well differentiable indicators for cancer occurrence.** (A) log odds-ratios (OR) of elements tested in MB screening. Red shaded regions indicate those with log $OR > 0$ for cancer occurrence; results shown of average ORs from 1000 random SMOTE sampling trials. (B) ROC analysis of MB logistic regression classifier models for P_0 and P_1; black, all elements; red, model constructed only from those with $\log_{10} OR > 0$; AUC, area under curve. (C, D) Distributional overlap of study cohorts by UMAP. Labeled colors indicate various conditions, either by cohort population (C) or by elemental levels (D). S (left) and Zn (right) are shown for representative purposes, gradients of light to dark purple indicate low to high elemental levels; P_0, non-cancer control; P_1, cancer cases.

used). At the same time, however, we also observed a large portion of overlap in P_0 and P_1 MB profiles in two-dimensional UMAP (Fig. 2C) and poorly defined separation boundaries; while certain gradients in elemental levels existed (Fig. 2D), straight-forward identification of cancer (P_1) from healthy subjects (P_0) was not trivial, indicating that data collection might be insufficient with a cohort of this size. To evaluate whether the overlap also occurred at the individual elemental level, we examined the effect size distributions *via* Cohen's *d* for each element among different cancer types in comparison to P_0 (Fig.3A; Fig. S1). Cohen's *d* provided a metric to highlight the distributional differences by standardized means free from influences of sample size, a feature in which the cancer cases are typically dwarfed by the size of the control cohort. We qualitatively evaluated effect sizes (Table 2) using the following boundaries: a difference of $|d| < 0.2$ was considered negligible, while values lower than 0.5, 0.8 and those above 0.8 were evaluated as small, medium and large differences, respectively. Effect sizes were also determined along gender lines, with men exhibiting a range of -3.25 to 1.70 and women from -2.5 to 1.77 (Fig. 3B). Elements found to have
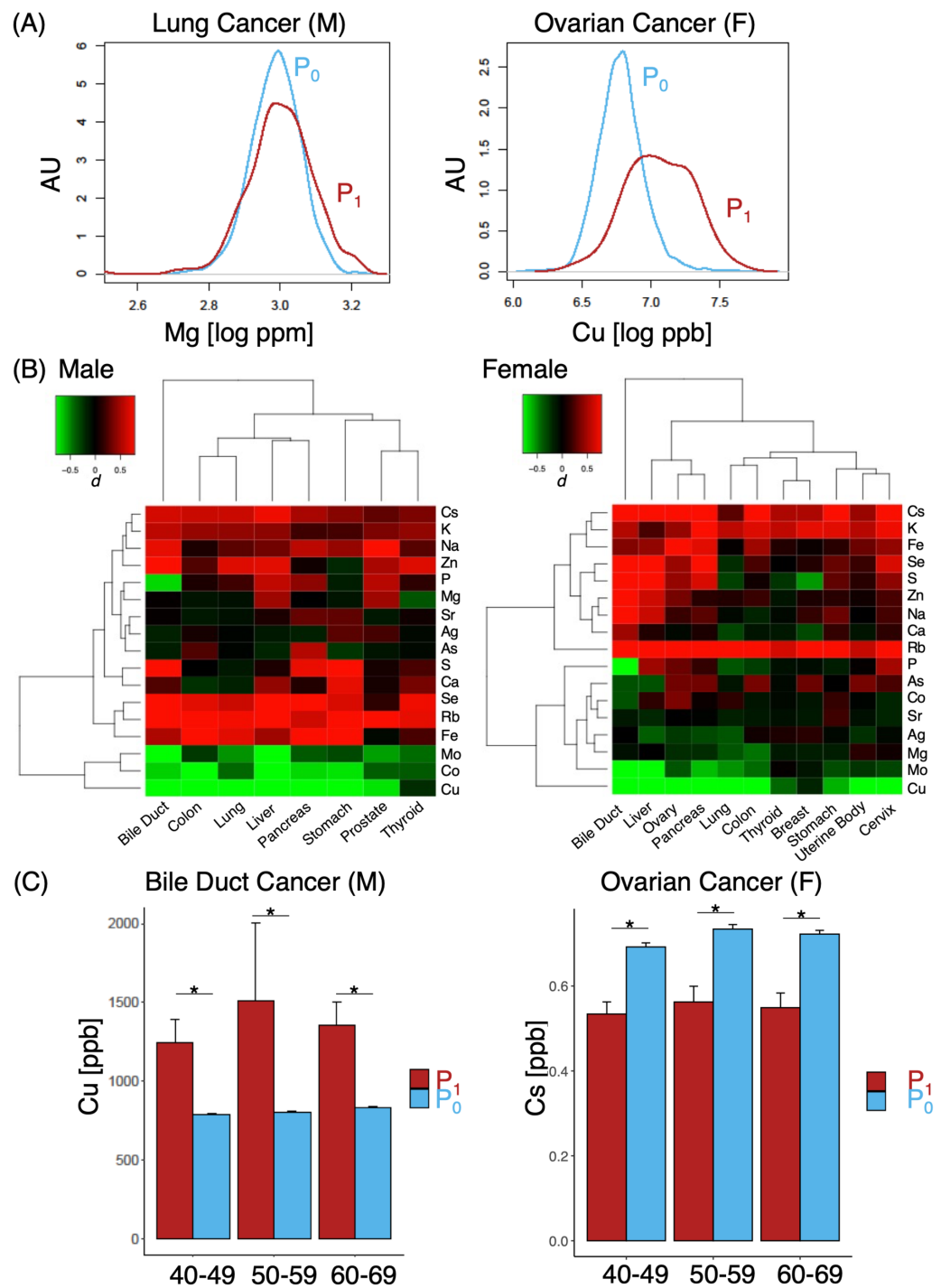
Figure 3 Differences in effect size in MB profiles. (A) Representative cases of elements with small (lung cancer, left) and large (ovarian cancer, right); (B) heat map of various different cases and corresponding effect sizes of P1, with color gradients of d indicated in the upper left corner; (C) comparison of Cu (left) and Cs (right) levels between P_0 and P_1 cohorts. M, male; F, females. Asterisk, $p < 0.05$.

Table 2 Ranges of effect sizes for various cancers.

	Stomach	Liver	Thyroid	Colon/Rectum	Bile duct	Lung	Pancreas
d_{low}	−0.72	−1.54	−0.29	−0.01	−2.64	−1.22	−1.80
d_{high}	1.24	1.10	0.66	0.93	1.37	0.75	0.99

Notes.

d_{low}, minimum Cohens d observed; d_{high}, maximum Cohens d observed.

Table 3 Elements with large effect sizes ($|d| < 0.8$) in men. Italicized elements are also found with large effect sizes in women.

Element	Stomach	Liver	Prostate	Colon	Bile duct	Lung	Pancreas
S	1.36				0.85		
Fe	1.10			1.03			1.06
Cu	−1.04	−1.86		−1.19	−3.25	−1.66	−2.18
Rb	0.97	1.13	0.81	0.82	1.23		
Se		1.33			1.70		1.22
Mo		−0.94			0.85		
Na			0.81				
Co		*1.14*		*−1.37*			

Table 4 Cancers exhibiting three or more elements with large effect sizes ($|d| < 0.8$) in women per cancer type.

Element	Ovary	Liver	Pancreas	Colon	Bile duct
S					1.38
Fe	0.88				
Cu	−1.65	−1.90	−1.75	−1.31	−2.50
Rb	1.01	1.15	1.37	1.17	1.77
Se		1.01	0.94		1.37
Mo		−1.12			−0.9
Na					1.07
P					*−2.51*
K			*0.86*		
Cs	*0.95*	*0.81*	*0.96*	*0.91*	*1.12*

an odds ratio of log $OR > 0$ also exhibited statistically significant differences across various cancers, for instance Cu in bile duct cancer in men and Cs in ovarian cancer for women (Fig. 3C). Effect sizes were markedly different among different cancer sites, suggesting that a comparison of multiplexed elemental levels had the potential for cancer risk evaluation. Several elements in P_1 had large effect sizes in a number of cancers (Table 3) in men, while a slightly different pattern was observed in women (Table 4). Comparisons of elemental levels in various age-matched groups (40–49, 50–59, 60–69; Table 5) revealed that a handful of elements appeared to have less overlap in the younger subpopulations in the two cohorts. As a whole, serum elements exhibited enough differences in their distribution as evidence of indicator effectiveness for characterizing cancer risks.

Table 5 Matched-age group comparison of elemental levels with large effect sizes per cancer site (gender separated).

Sex	Cancer site	Element	Age group	P_1/P_0 (p-value)
M	Prostate	Na[ppm]	40–49	3171/3227 (0.02)
			50–59	3170/3228 (<0.001)
			60–69	3264/3232 (<0.001)
		K[ppm]	40–49	164/169 (0.0192)
			50–59	160/168 (0.0496)
			60–69	165/172 (<0.001)
		Rb[ppb]	40–49	180/177 (0.0004)
			50–59	158/167 (0.0046)
			60–69	149/165 (<0.001)
	Bile duct	Cu[ppb]	40–49	1245/793 (0.0229)
			50–59	1512/808 (0.0219)
			60–69	1357/834 (0.0136)
	Pancreatic	Cu[ppb]	40–49	1341/793 (0.01769)
			50–59	1319/808 (0.0017)
			60–69	1223/834 (<0.001)
F	Ovarian	Rb[ppb]	40–49	151.3/172.4 (0.014)
			50–59	147.6/164.8 (0.019)
			60–69	131.6/158.8 (0.035)
		Cs[ppb]	40–49	0.53/0.69 (0.013)
			50–59	0.56/0.73 (<0.001)
			60–69	0.55/0.72 (<0.001)
	Uterine body	K[ppm]	40–49	159.4/166.2 (0.0166)
			50–59	157.4/165.5 (0.017)
			60–69	160.8/168.0 (<0.001)
	Cervical	K[ppm]	40–49	156.6/166.2 (0.028)
			50–59	155.1/165.5 (0.037)
			60–69	152.4/168.0 (<0.001)
		Se[ppb]	40–49	134.3/138.6 (0.079)
			50–59	132.6/144.5 (<0.001)
			60–69	134.0/145.6 (0.002)

Tumor markers such as CEA, CA19-9, CA125, PSA were routinely measured for cancer risk screening during annual health checkups, and while these were reasonable indicators on some level, we found that there was a great overlap in marker levels deemed normal in both P_0 and P_1 cohorts (Table 6); essentially, tumor markers alone could not sufficiently differentiate the two cohorts (Fig. 4), hinting that a limited serological panel of tumor markers were insufficient for cancer risk assessment. To validate MB's performance as a supplemental tool for risk assessment, we created 2 generalized linear regression models, to identify potential associations, using MB profiles to compare correlations between marker expression in these two cohorts. A number of elements appeared to have more distinguished influences in model performance in the cancer cohort (Fig. 5A); for those identified with normal values of CEA, elements such as Na, K, Cu, Fe Co and Mo exhibited

Table 6   Number of overlap cases with marker levels within normal values in cohorts P_0 and P_1.

Marker	P_0	P_1
CEA	2,015	1,147
CA 19-9	2,367	747
CA125	1,949	456
PSA	714	208

Notes.

$N_0 = 5,327$; $N_1 = 1,856$.

visible differences in mean in P_1 over the non-cancer P_0 cohort; similarly, Zn, Cs, Ag, and Mo appeared to be significantly different in mean for normal CA19-9 readings, while for CA125 P, As, Cu, Fe, Mo and Cs and for PSA K, Zn, Rb, Mo and Cs (Fig. 5B), notably intersecting with our earlier observations in Fig. 2A. Someone with a normal tumor biomarker reading, for instance, could potentially benefit from a supplemental MB screening for abnormal levels of elements such as Cu, Mo, Ag and Cs as support indicators for cancer risks.

DISCUSSION

While observations here largely affirmed some of our previous findings (*Okamoto et al., 2020*), a direct multivariate analysis of neither biomarker levels nor MB results alone curiously provided sufficient degrees of separation between the cancer and non-cancer cohorts surveyed with ~7,200 participants. There were nonetheless elements with abundance differences indicative of statistical significance, when grouped together or otherwise as seen in Fig. 2A. In consideration of some of the associations to cancer outcome as seen in Fig. 2, the use of MB should be able to supplement more conventional serological exams for a number of cancer types simultaneously. These odds ratios also provide a way to aggregate results statistically (*e.g.*, *via* geometric mean) from a cluster of elements to assess against reference values in a similar manner marker expression or activity levels provide. This is perhaps also advantageous for result interpretation, since the new aggregated scores can be normalized for different populations, and similarly monitored by tracing trends in the rate of changes in score over time.

Gender differences also existed to some extent, although it was not as pronounced as we expected; additionally, element with significantly different abundances across cohorts were also seen in groups where expressions of commonly used tumor markers failed to discern cancer from non-cancer individuals. While serum elemental levels had been implicated in estimating risks of susceptibility for a handful of cancers (*Okamoto et al., 2020*), it was perhaps, whilst erring on the side of conservatism, fitting to conclude that a comprehensive elemental profile would see better performance alongside tumor marker levels in a serological examination. An ICP-MS based elemental analysis method, when performed as a companion screening test to the usual tumor marker panels, could potentially improve the sensitivity and specificity of existing cancer screening methods. As an additional screening, it could potentially help physicians make decisions about whether a follow-up and more invasive or costly method of screening, *i.e.,* colonoscopy
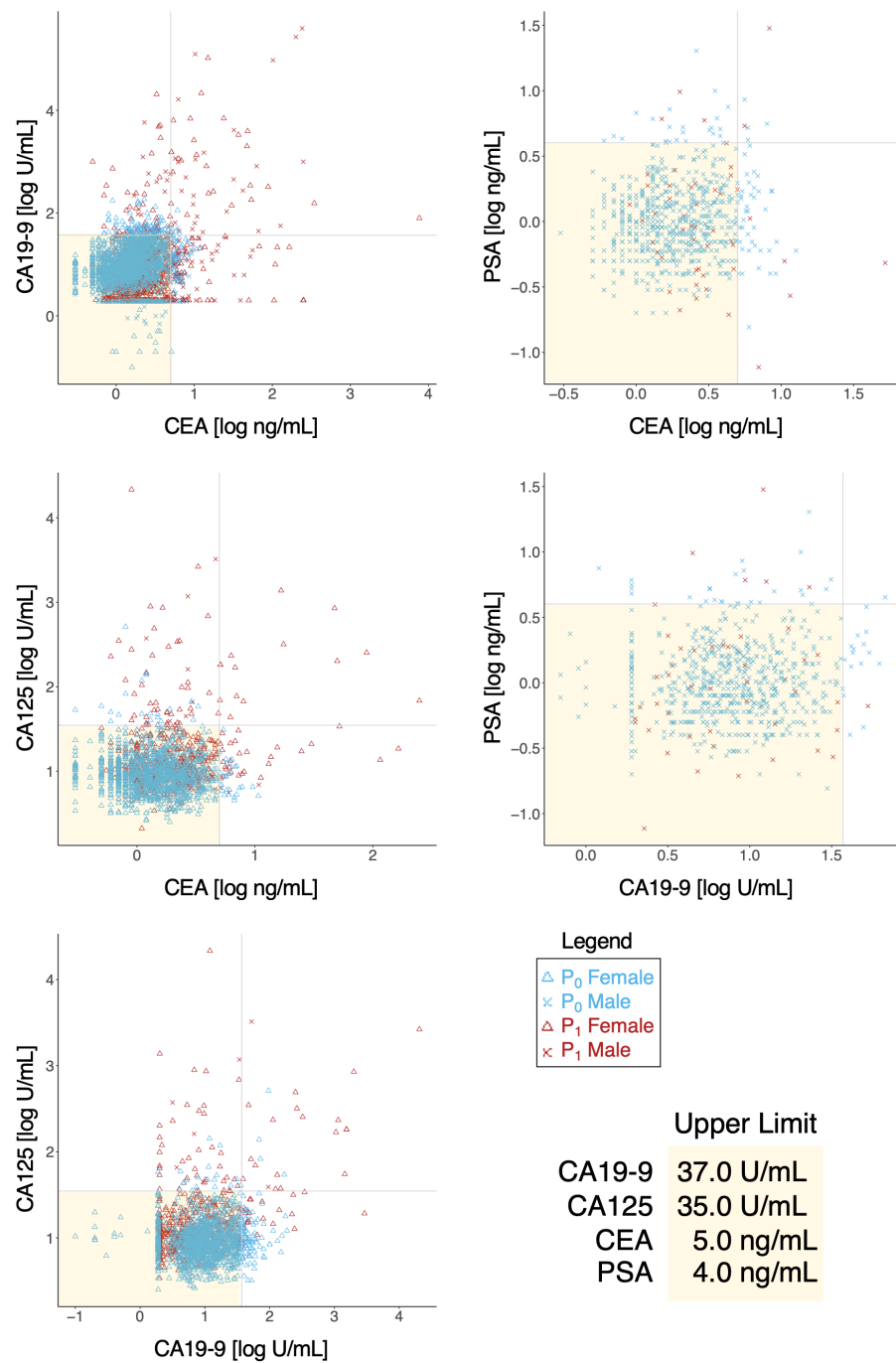
Figure 4 **Behavior of tumor biomarkers in cancer and non-cancer cases.** Shaded yellow region indicates normal thresholds (upper limit, bottom right) for each respective marker. Vertical and horizontal axes indicate pairings of tumor biomarkers and concentration.
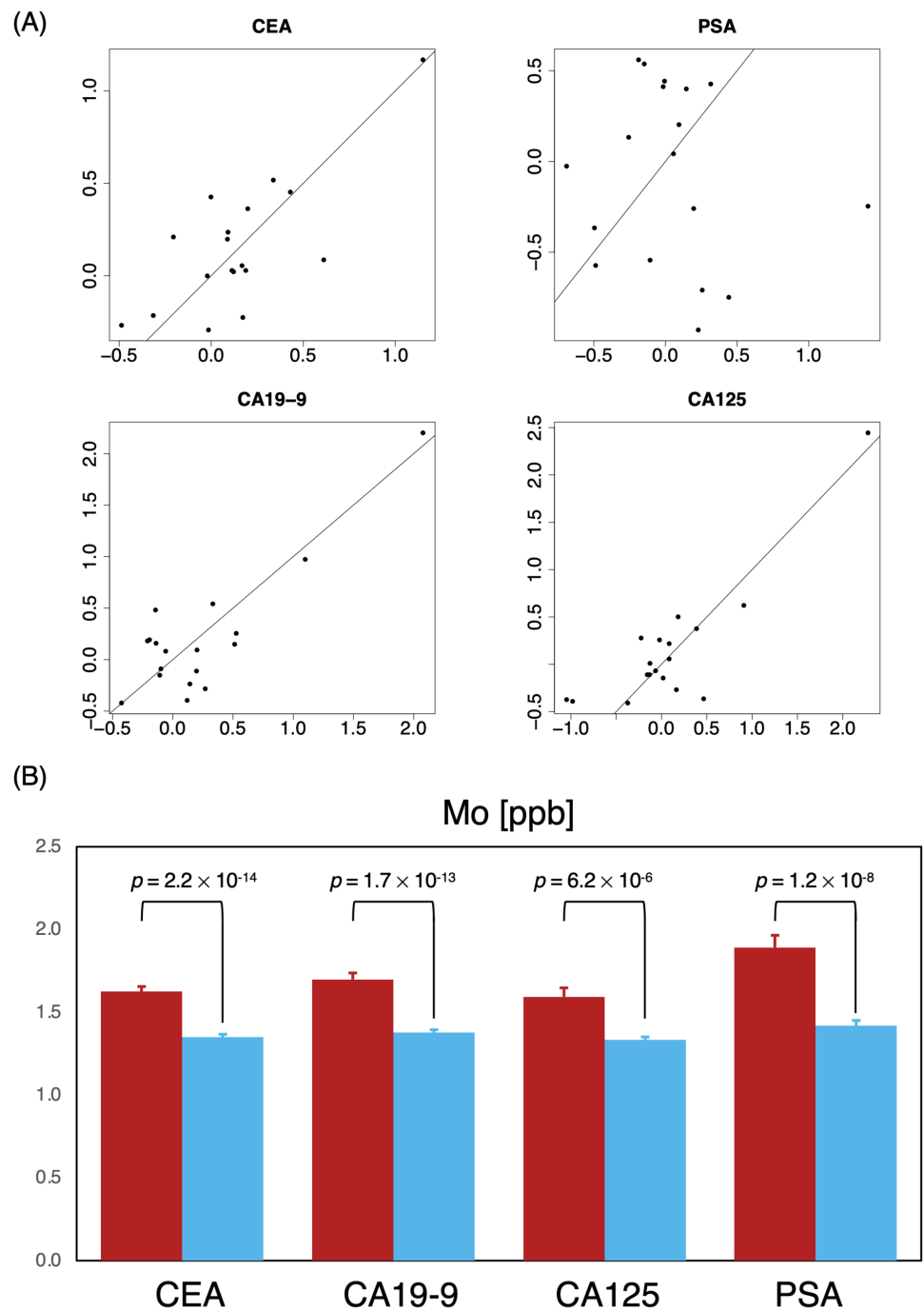
Figure 5 Identifying critical elements as support indicators for tumor biomarkers. (A) Two-dimensional comparison of generalized linear regression coefficients non-cancer ($\mathbf{P_0}$, horizontal axes) *vs.* cancer ($\mathbf{P_0}$, vertical axes) of MB elements; solid reference line has a slope of 1. (B) Comparison of mean Mo levels among subjects with normal marker ranges; $\mathbf{P_0}$ red; $\mathbf{P_0}$, blue.

or gastroendoscopy, would be necessary for someone who had an above-normal CEA reading (*Lennon et al., 2020*). Element with large effect sizes were particularly effective in distinguishing cancer from non-cancer subjects, for example in the case of cancers of the bile duct and liver in both men and women. For Cs, we found that the effect size was exclusively elevated in women, leading us to posit that gender-specific risk models could be a necessity in future investigations or model construction. Some of the less common cancer types such as thyroid and endometrial cancers also had sufficiently large enough effect sizes for a similar conclusion. Typically, thyroid cancer relied on ultrasound and percutaneous fine needle aspiration for testing (*Wang et al., 2020*); at the same time, endometrial cancer would require an extensive panel of protein markers, *e.g.*, *MUC16*, *TP53*, *PTEN* and *CDH1* (*Coll-de la Rubia et al., 2020*), all of which in various degrees (and often conflictingly) had also been implicated in other types of cancer. In comparison, a single panel of MB should be able to predict discernible profiles that indicated potential risks prior to more invasive testing methods.

For new cancer screening methods, it is common to ponder whether the test itself provides any added value over existing ones; while ICP-MS has been extensively deployed in the field of chemistry and engineering, the as-is deployment of such an instrumentation technique in healthcare as a direct conduit for cancer screening may raise eyebrows. However, ICP-MS's notable resistance to specimen degradation, when compared to other analytical tools, has led itself to be utilized in various degradation monitoring studies (*Kannamkumarath et al., 2004*; *Heuckeroth et al., 2021*; *Brunnbauer et al., 2020*), including biological specimens (*Hann et al., 2003*; *Theiner et al., 2015*; *Neumann et al., 2020*; *Meyer et al., 2018*; *Theiner et al., 2020*) even as far as single-cell-based studies. The high sensitivity of elemental analysis also ensures high levels of reproducibility; since MB can monitor simultaneously 17 elemental levels, the probability of all of the elements being affected by external factors is likely lower than that of a single or even a small panel of cancer biomarkers. For long-term monitoring studies involving specimens in frozen storage such as tissue banks, MB may be superior over antigen-based marker examinations that are highly dependent on the preservation of secondary and tertiary structural conformations of said targets.

The performance of MB also appeared to improve when oversampling techniques such as SMOTE and machine learning methods such as random forests or neural networks were used; this could potentially aid the algorithmic development for situations where significant imbalance between case and control subjects could occur. For instance, a random forests classifier model trained on the two cohorts using MB element levels alone achieved an AUC of 0.909 (Fig. 6). When compared to a recent report (*Kawasaki et al., 2021*) comparing the performance of various clinical procedures for colorectal cancer, the AUC of MB outperformed methods such as barium enema (0.836) and even colonoscopy (0.768); in other similarly sized comparisons, MB also appeared to offer above-par performance than other non-invasive tests such as in-stool DNA and fecal immunochemical test (FIT) testing (*Imperiale et al., 2014*) (0.73 and 0.67, respectively). Especially in "needle in a haystack" situations such as credit card fraud detection or identifying cancer cases in a general population, where incidence rates are typically fractions of a percent, the
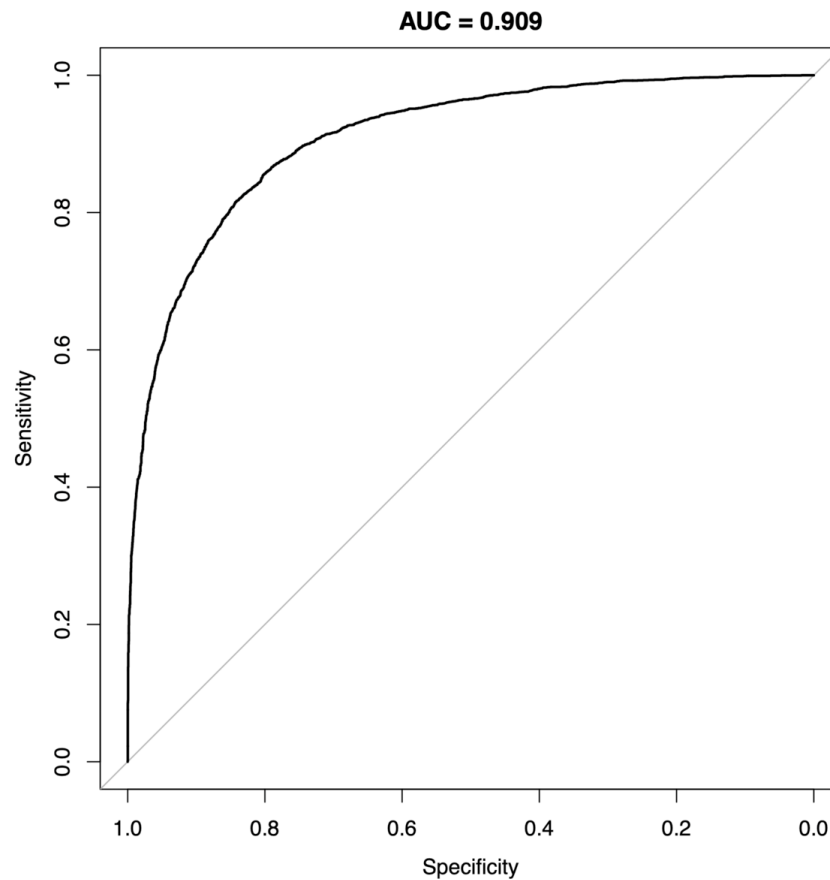
Figure 6 **Receiver-operating characteristics of a random forest classifier model for P_0 and P_1.** Representative result from one SMOTE sampling trial. AUC, area under curve.

performance of a model trained with tumor marker panels alone may be highly limited without increasing the dimension of the feature space beyond the typical handful of markers such as CEA, CA19-9 and the like, cohort size constraints will hamper the modeling process. In comparison, MB's comprehensive ICP-MS profile can be readily expanded at the data reporting stage, thus in a decent-sized cohort fine graining of the feature space may provide improved performance. While next-generation sequencing tools such as whole-exome, whole-genome or even single-molecule real-time (SMRT) sequencing have vastly expanded feature spaces compared to MB, the issue of feature distribution overlap can still exist (*Sud, Turnbull & Houlston, 2021*), and multigene assays *via* these sequencing techniques often also involve significantly more human, monetary and infrastructural resources such as multiple sequencers, cloud storage space, technicians and bioinformaticians, etc. (*Ignatiadis, Sledge & Jeffrey, 2021*) SMRT analysis, for instance, recommends the use of a full computational cluster with upwards of 384 processing cores, 3 terabytes of volatile memory for a single run of sequencing analysis to complete in nearly 7 h (*Pacific Biosciences, 2021*); at this scale, run time for a study of 8,000 participants by

SMRT sequencing-based methods alone will require 6 years of computing time, greatly eclipsing the analytical process of MB.

In addition to the process of establishing case-control discovery studies for the effectiveness of MB screening in a number of different cancer types, we are also contemplating ways to investigate whether MB-like methods can be used for other types of monitoring experiments in specimens such as patient-derived xenografts, or experiments in which connections between particular trace elements with oncogenesis can be confirmed. Aside from bench-scale studies, recently Renatech also independently launched "Metallo-Clinic," an effort to promote MB screening through Japan. At the time of writing, clinics and testing sites offer MB screening in a number of prefectures covering approximately 52% of the Japanese population (*Statistics Bureau of Japan, 2021*); if successful, the use and incorporation of MB profiles from Metallo-Clinic may provide a clearer snapshot on true cancer incidence rates in Japan.

CONCLUSIONS

In summary, cohort analyses suggested that MB screening was a capable supplementary test to serological tumor marker examinations for a number of cancer types. While the method alone might not be able to discern cancer from non-cancer cases in all situations, depending on the combination of elements, some degree of differentiation still appeared to be possible for cancer type classification. Even in cases where a positive assessment could not be sufficiently determined by the four commonly used tumor markers alone, a number of elements exhibited statistically significant odds between P_0 and P_1, making the case for referring MB for a secondary confirmation in said situations. Additionally, for both P_0 and P_1, subpopulations in which tumor markers were within normal ranges, the noticeable distributional differences in elemental levels would again bring emphasis to the implementation of MB in a typical health screening regimen.

As with most epidemiological studies, validation of the collected data remains a difficult hurdle to overcome. While the law of large numbers ensures some degree of reliability and "proximity" of the expected value to the universal truth one seeks, the practical difficulty of having to sift through nominally invariant or average data also increases. Between cohorts P_0 and P_1, there were significant overlaps in tumor biomarker panel results to make risk assessment based on these markers alone a difficult feat. While cancer biologists and epidemiologists may balk at the idea of utilizing non-conventional observables such as elemental analysis for risk evaluation due to their relative "abstractness" compared to "standards" such as tumor biomarkers or histology reports; nevertheless, it is worthwhile to recognize that those so-called standards may not always be great indicators for all types of cancer. For instance, it has been said that there is lack of sensitivity for CEA during early-stage oncogenesis (*Goldstein & Mitchell, 2005*), and CA19-9 is not expressed in nearly 5% of the general population (*Lamerz, 1999*); but as cohort sizes continue to grow, the expandable feature space in novel profiling tools such as MB may finally provide a mean to fill the previously unoccupied void of the early cancer screening feature space.

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Competing Interests

The authors declare that there are no competing interests.

Author Contributions

- Miho Kusakabe and Masahiro Sato analyzed the data, prepared figures and/or tables, and approved the final draft.
- Yohko Nakamura performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

- Haruo Mikami conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Jason Lin conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Hiroki Nagase conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

Human Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

Prior to experimentation, we obtained informed consent and received approval of the study protocol from the ethics review committee at Chiba Cancer Center (Cc# 902).

Data Availability

The raw data is available in the Supplemental File.

Supplemental Information

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REFERENCES

Brunnbauer L, Mayr M, Larisegger S, Nelhiebel M, Pagnin L, Wiesinger R, Schreiner M, Limbeck A. 2020. Combined LA-ICP-MS/LIBS: powerful analytical tools for the investigation of polymer alteration after treatment under corrosive conditions. *Scientific Reports* **10**:12513 DOI 10.1038/s41598-020-69210-9.

Bast Jr RC, Klug TL, St.John E, Jenison E, Niloff JM, Lazarus E, Berkowitz RS, Leavitt T, Griffiths CT, Parker L, Zurawski Jr VR, Knapp RC. 1983. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. *New England Journal of Medicine* **309**(15):883–887 DOI 10.1056/NEJM198310133091503.

Coll-de la Rubia E, Martinez-Garcia E, Dittmar G, Gil-Moreno A, Cabrera S, Colas E. 2020. Prognostic biomarkers in endometrial cancer: a systematic review and meta-analysis. *Journal of Clinical Medicine* **9**(6):1900 DOI 10.3390/jcm9061900.

Coric J, Mujic J, Kucukalic E, Ler D. 2015. Prostate-Specific Antigen (PSA) and prostate volume: better predictor of prostate cancer for bosnian and herzegovina men. *Open Biochemistry Journal* **9**:34–36 DOI 10.2174/1874091X01509010034.

Editorial Board of the Cancer Statistics in Japan, Cancer Statistics in Japan. 2021. 1st ed. Tokyo, Japan: FPCR; 2021. ISSN: 2433-3212. *Available at https://ganjoho.jp/public/qa_links/report/statistics/2021_en.html* (accessed on 3 September 2021).

Goldstein MJ, Mitchell EP. 2005. Carcinoembryonic antigen in the staging and follow-up of patients with colorectal cancer. *Cancer Investigation* **23**(4):338–351 DOI 10.1081/CNV-58878.

Hall IJ, Tangka FKL, Sabatino SA, Thompson TD, Graubard BI, Breen N. 2018. Patterns and Trends in Cancer Screening in the United States. *Preventing Chronic Disease* **15**:E97 DOI 10.5888/pcd15.170465.

Hann S, Koellensperger G, Stefanka Zs, Stingder G, Furhacker M, Buchberger W, Mader RM. 2003. Application of HPLC-ICP-MS to speciation of cisplatin and its degradation products in water containing different chloride concentrations and in human urine. *Journal of Analytical Atomic Spectrometry* **18**:1391–1395 DOI 10.1039/B309028K.

Heuckeroth S, Nxumalo TN, Raab A, Feldmann J. 2021. Fluorine-specific detection using ICP-MS helps to identify PFAS degradation products in nontargeted analysis. *Analytical Chemistry* **93**(**16**):6335–6341 DOI 10.1021/acs.analchem.1c00031.

Honda K, Kobayashi M, Okusaka T, Rinaudo JA, Huang Y, Marsh T, Sanada M, Sasajima Y, Nakamori S, Shimahara M, Uneo T, Tsuchida A, Sata N, Ioka T, Yasunami Y, Kosuge T, Miura N, Kamita M, Sakamoto T, Shoji H, Jung G, Srivastava S, Yamada T. 2015. Plasma biomarker for detection of early stage pancreatic cancer and risk factors for pancreatic malignancy using antibodies for apolipoprotein-AII isoforms. *Scientific Reports* **5**:15921 DOI 10.1038/srep15921.

Hu A, Li L, Hu C, Zhang D, Wang C, Jiang Y, Zhang M, Liang C, Chen W, Bo Q, Zhao Q. 2018. Serum concentrations of 15 elements among helicobacter pylori-infected residents from lujiang county with high gastric cancer risk in eastern China. *Biological Trace Element Research* **186**(**1**):21–30 DOI 10.1007/s12011-018-1283-4.

Ignatiadis M, Sledge GW, Jeffrey SS. 2021. Liquid biopsy enters the clinic –implementation issues and future challenges. *Nature Reviews Clinical Oncology* **18**:297–312 DOI 10.1038/s41571-020-00457-x.

Imperiale TF, Ransohoff DF, Itzkowitz SH, Levin TR, Lavin P, Lidgard GP, Ahlquist DA, Berger BM. 2014. Multitarget stool DNA testing for colorectal-cancer screening. *New England Journal of Medicine* **370**:1287–1297 DOI 10.1056/NEJMoa1311194.

Ito H, Hasegawa K, Hasegawa Y, Nishimaki T, Hosomichi K, Kimura S, Ohba M, Yao H, Onimaru M, Inoue I, Inoue H. 2015. Silver nanoscale hexagonal column chips for detecting cell-free DNA and circulating nucleosomes in cancer patients. *Scientific Reports* **5**:10455 DOI 10.1038/srep10455.

Kawasaki K, Nakamura S, Eizuka M, Tanaka Y, Kumei T, Yanai S, Toya Y, Urushikubo J, Torisu T, Moriyama T, Umeno J, Sugai T, Matsumoto T. 2021. Is barium enema examination negligible for the management of colorectal cancer? Comparison with conventional colonoscopy and magnifying colonoscopy. *Japanese Journal of Radiology* DOI 10.1007/s11604-021-01157-x.

Kannamkumarath SS, Wuilloud RG, Stalcup A, Caruso JA, Patel H, Sakr A. 2004. Determination of levothyroxine and its degradation products in pharmaceutical tablets by HPLC-UV-ICP-MS. *Journal of Analytical Atomic Spectrometry* **19**:107–113 DOI 10.1039/b307970h.

Konishi T, Shimada Y, Hsu M, Tufts L, Jimenez-Rodriguez R, Cercek A, Yaeger R, Saltz L, Smith JJ, Nash GM, Guillem JG, Paty PB, Garcia-Aguilar J, Gonen M,

Weiser MR. 2018a. Association of preoperative and postoperative serum carcinoembryonic antigen and colon cancer outcome. *JAMA Oncology* **4(3)**:309–315 DOI 10.1001/jamaoncol.2017.4420.

Kuhn M. 2008. Building predictive models in R using the caret package. *Journal of Statistical Software* **28(5)**:1–26 DOI 10.18637/jss.v028.i05.

Lamerz R. 1999. Role of tumour markers, cytogenetics. *Annals of Oncology* **10(Suppl 4)**:145–149 DOI 10.1023/A:1008386130283.

Lennon AM, Buchanan AH, Kinde I, Warren A, Honushefsky A, Cohain AT, Ledbetter DH, Sanfilippo F, Sheridan K, Rosica D, Adonizio CS, Hwang HJ, Lahouel K, Cohen JD, Douville C, Patel AA, Hagmann LN, Rolston DD, Malani N, Zhou S, Bettegowda C, Diehl DL, Urban B, Still CD, Kann L, Woods JI, Salvati ZM, Vadakara J, Leeming R, Bhattacharya P, Walter C, Parker A, Lengauer C, Klein A, Tomasetti C, Fishman EK, Hruban RH, Kinzler KW, Vogelstein B, Nickolas P. 2020. Feasibility of blood testing combined with PET-CT to screen for cancer and guide intervention. *Science* **369(6499)**:eabb9601 DOI 10.1126/science.abb9601.

Marco T. 2020. effsize: Efficient Effect Size Computation. *Available at https://cran.r-project.org/web/packages/effsize/index.html* (accessed on 18 June 2021).

Manfei S, Lang J. 2018. The roles of metallothioneins in carcinogenesis. *Journal of Hematology & Oncology* **11**:107 DOI 10.1186/s13045-018-0645-x.

Masoudreza S, Gholami A, Azar MH, Yaghoobi M, Shahi MM, Shirmardi S, Nikkhah M, Kohi Z, Salehpour D, Khoonsari MR, Hemmasi G, Zamani F, Sohrabi M, Ajdarkosh H. 2018. Trace element and heavy metal levels in colorectal cancer: comparison between cancerous and non-cancerous tissues. *Biological Trace Element Research* **183(1)**:1–8 DOI 10.1007/s12011-017-1099-7.

Meyer S, Lopez-Serrano A, Mitze H, Jakubowski N, Schwerdtle T. 2018. Single-cell analysis by ICP-MS/MS as a fast tool for cellular bioavailability studies of arsenite. *Metallomics* **10**:73–76 DOI 10.1039/C7MT00285H.

Ministry of Health, Labour, and Welfare, Annual Vital Statistics Report Main Statistics. 2020. *Available at http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/suii09/deth7.html* (accessed on 21 April 2021).

Neumann B, Hosl S, Schwab K, Theuring F, Jakubowski N. 2020. Multiplex LA-ICP-MS bio-imaging of brain tissue of a parkinsonian mouse model stained with metal-coded affinity-tagged antibodies and coated with indium-spiked commercial inks as internal standards. *Journal of Neuroscience Methods* **334(15)**:108591 DOI 10.1016/j.jneumeth.2020.108591.

OECD stat. 2020. Health Care Utilisation: Screening. *Available at https://stats.oecd.org/*.

Okamoto N, Mikami H, Nakamura Y, Kusakabe M, Yamamoto N, Takiguchi N, Nabeya Y, Soda H, Fukasawa S, Kishida T, Shiozawa M, Yoshida A, Shimizu T, Fujimoto S, Ueda M, Inagaki S, Miyagi Y, Nagase H. 2020. A nobel multivariate index for cancer Risk detection based on the serum trace elements: metallo-balance method. *Journal of Cancer Epidemiology and Prevention* **5**:1 DOI 10.36648/cancer.5.1.4.

Okuhara T, Ishikawa H, Okada M, Kato M, Kiuchi T. 2018b. Beliefs underlying messages of anti-cancer-screening websites in Japan: a qualitative analysis. *Asian Pacific Journal of Cancer Prevention* **19(2)**:427–433.

Pacific Biosciences. 2021. Appendix C, SMRT Link Software Installation (v10.1). *Available at https://www.pacb.com/wp-content/uploads/SMRT_Link_Installation_v10.1.pdf* (accessed on 26 August 2021).

PDQ Screening and Prevention Editorial Board. 2019. *PDQ Screening and Prevention Editorial Board. PDQ Cancer Screening Overview.* Bethesda: National Cancer Institute (US).

Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, Muller M. 2011. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinfo* **12**:77 DOI 10.1186/1471-2105-12-77.

Scara S, Bottoni P, Scatena R. 2015. CA 19-9: biochemical and clinical aspects. *Advances in Experimental Medicine and Biology* **867**:247–260 DOI 10.1007/978-94-017-7215-0_15.

Siriseriwan W. 2019. smotefamily: a collection of oversampling techniques for class imbalance problem based on SMOTE. *Available at https://cran.r-project.org/*.

Statistics Bureau of Japan. 2021. Population by prefecture and population increase decrease rate. *Available at https://www.stat.go.jp/data/nihon/02.html* (accessed on 24 June 2021).

Sud A, Turnbull C, Houlston R. 2021. Will polygenic risk scores for cancer ever be clinically useful? *Npj Precision Oncology* **5**:40 DOI 10.1038/s41698-021-00176-1.

Sugimoto M, Wong DT, Hirayama A, Soga T, Tomita M. 2010. Capillary electrophoresis mass spectrometry-based saliva metabolomics identified oral, breast and pancreatic cancer-specific profiles. *Metabolomics* **6**:78–95 DOI 10.1007/s11306-009-0178-y.

Theiner S, Kornauth C, Varbanov HP, Galanski M, VanSchoonhoven S, Heffeter P, Berger W, Egger AE, Keppler BK. 2015. Tumor microenvironment in focus: LA-ICP-MS bioimaging of a preclinical tumor model upon treatment with platinum(IV)-based anticancer agents. *Metallomics* **7(8)**:1256–1264 DOI 10.1039/C5MT00028A.

Theiner S, Loehr K, Koellensperger G, Mueller L, Jakubowski . 2020. Single-cell analysis by use of ICP-MS. *Journal of Analytical Atomic Spectrometry* **35**:1784–1813 DOI 10.1039/D0JA00194E.

Wang W, Chang J, Jia B, Liu J. 2020. The blood biomarkers of thyroid cancer. *Cancer Management and Research* **12**:5431–5438 DOI 10.2147/CMAR.S261170.

Wickham H. 2020. ggplot2: Create elegant data visualisations using the grammar of graphics. *Available at https://cran.r-project.org/web/packages/ggplot2/index.html* (accessed on 18 June 2021).

Zhou L, Zhao B, Zhang L, Wang S, Dong D, Lv H, Shang P. 2018. Alterations in Cellular Iron Metabolism Provide More Therapeutic Opportunities for Cancer. *International Journal of Molecular Sciences* **19(5)**:1545 DOI 10.3390/ijms19051545.