Performance of a multi-analyte, multi-cancer early detection (MCED) blood test in a prospectively-collected cohort

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1 BACKGROUND

A multi-analyte blood test has the potential for robust sensitivity in detecting a broad range of cancer types and stages.

Previously, using retrospectively collected samples, we trained and independently assessed the performance of up to four different biomarker classes for the detection of cancers in a case-control study.[1, 2]

2 OBJECTIVES

The aim of this study was to further refine and assess calling algorithms, i.e. classifiers, for two of the four previously evaluated biomarker classes, methylation and protein, using samples from a multi-center, prospectively collected study: Ascertaining Serial Cancer patients to Enable New Diagnostic 2 (ASCEND 2).[3]

By measuring methylation and protein biomarkers that capture shared, cancer-associated signals, yet rely on different mechanism of release into the circulation, the objective was to show that these two biomarker classes can detect a broad range of cancer types while maintaining high specificity.

3 METHODS

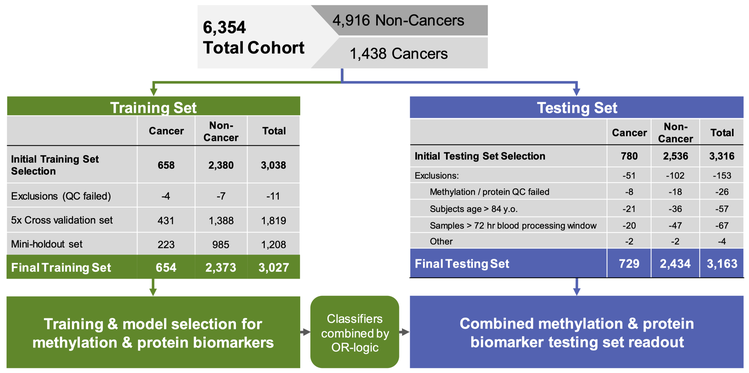
- For the methylation and protein classifier development a total of 6,354 blood samples (1,438 cancers and 4,916 non-cancers) collected in LBgard® tubes were selected from >11,000 subjects enrolled in ASCEND 2.[3]
- The selection of the samples was based on plasma volume availability, clinical eligibility criteria, availability of validated clinical data prior to testing initiation, and demographic matching requirements between the training and testing set cohort.
- Of the samples that were tested, additional exclusions were applied for training and the hold-out testing set, respectively (**Fig.1**).
- **Tab. 1** shows the training and testing cohort demographics. Training and testing set tumor organ sites and stage distributions are depicted in **Fig. 3**.
- Methylation and protein measurements were performed as described previously.[1]

Tab. 1. Demographics of training and testing cohorts

Characteristic	Training Set Cancer n=654	Training Set Non-Cancer n=2,373	Training Set Total n=3,027	Testing Set Cancer n=729	Testing Set Non-Cancer n=2,434	Testing Set Total n=3,163
Sex						
Female	354 (54.1%)	1,344 (56.6%)	1,698 (56.1%)	388 (53.2%)	1,392 (57.2%)	1,780 (56.3%)
Male	300 (45.9%)	1,029 (43.4%)	1,329 (43.9%)	341 (46.8%)	1,042 (42.8%)	1,383 (43.7%)
Age (years)						
Mean (SD)	66.9 (9.0)	64.8 (8.0)	65.3 (8.3)	66.3 (8.3)	64.9 (7.8)	65.2 (7.9)
(Min, Max)	(50, 92)	(50, 96)	(50, 96)	(50, 84)	(50, 84)	(50, 84)
Race / Ethnicity						
White	560 (85.6%)	1,899 (80.0%)	2,459 (81.2%)	587 (80.5%)	2,009 (82.5%)	2,596 (82.1%)
Non - Hispanic/Latino	530 (81.0%)	1,540 (64.9%)	2,070 (68.4%)	533 (73.1%)	1,697 (69.7%)	2,230 (70.5%)
Black or African American	49 (7.5%)	360 (15.2%)	409 (13.5%)	67 (9.2%)	327 (13.4%)	394 (12.5%)
Asian	24 (3.7%)	84 (3.5%)	108 (3.6%)	31 (4.3%)	55 (2.3%)	86 (2.7%)
American Indian or Alaska Native	2 (0.3%)	10 (0.4%)	12 (0.4%)	7 (1%)	10 (0.4%)	17 (0.5%)
Mixed Race	1 (0.2%)	3 (0.1%)	4 (0.1%)	1 (0.1%)	7 (0.3%)	8 (0.3%)
Native Hawaiian or Other Pacific Islander	0 (0.0%)	3 (0.1%)	3 (0.1%)	1 (0.1%)	2 (0.1%)	3 (0.1%)
Unknown / Missing	18 (2.8%)	14 (0.6%)	32 (1.1%)	35 (4.8%)	24 (1%)	59 (1.9%)
Ethnicity						
Not Hispanic/Latino	613 (93.7%)	1,969 (83.0%)	2,582 (85.3%)	650 (89.2%)	2,077 (85.3%)	2,727 (86.2%)
Hispanic/Latino	25 (3.8%)	390 (16.4%)	415 (13.7%)	49 (6.7%)	341 (14%)	390 (12.3%)
Unknown / Missing	16 (2.4%)	14 (0.6%)	30 (1.0%)	30 (4.1%)	16 (0.7%)	46 (1.5%)

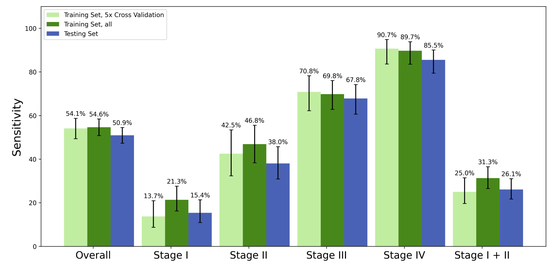
4 RESULTS

Fig. 1. Methylation & protein biomarker training & testing cohorts



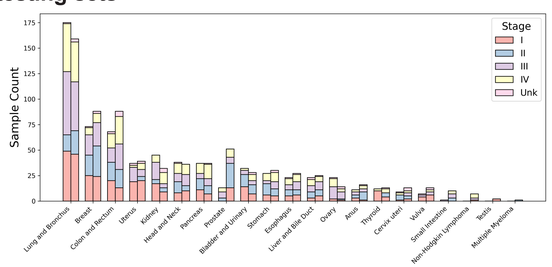
- Training and testing sets were split with stratification by age, race, ethnicity, collection site, cancer organ type, and stage.
- The training set was further divided into to a 5-fold cross validation (CV) set and a mini-holdout set.
- Learning curve analysis was used to determine minimum size of the CV set.
- The CV set was used to select model architecture, feature engineering, and transformation approaches as well as to perform hyperparameter tuning, and tuning of specificity thresholds.
- OR-logic was selected as the overarching methylation-protein classifier; other approaches did not offer significant advantages.
- A mini-holdout set was used to test multiple different models during the model selection process before evaluating two subsequent methylation – protein overarching classifier configurations in the test set, at 98.5% and >99.0% target specificity.
- The data shown here is based on the 98.5% specificity target and uses the less complex model.

Fig.2 Training & testing set performance comparison



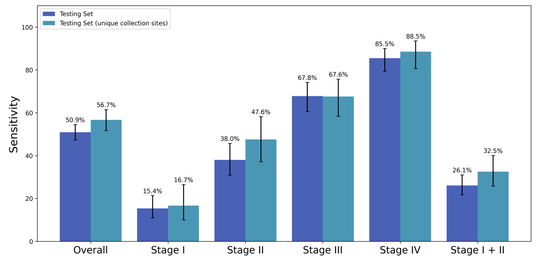
- The generalizability of the combined methylation and protein classifier was evaluated by comparing the performance between the 5-fold CV set, the full training set, and the testing set all targeted to 98.5% overall specificity.
- Training set 5-fold CV and full training set achieved 98.9% (95% CI, 98.1-99.3%) specificity and 98.8% (95% CI: 98.3-99.2%) respectively. Testing set specificity achieved 98.5% (95% CI: 97.9-98.9%).
- Taken together, no significant bias was observed between full training set, CV set, and testing set performance, indicating good generalizability of the classifiers.

Fig.3 Cancer organ type & stage distribution of training & testing sets



- The training (left bar) and testing (right bar) sets included subjects with cancers in 18 and 21 different organ sites, respectively.
- The number of cancers per organ site was targeted to represent cancers with high, common, and rare incidence and is not reflective of intended use population.
- Cancer stages and tumor organ sites in the cohort collectively represent >85% of incident cancers observed in the general population.[4]
- Overall, training and testing sets have similar distributions within organ sites. The overall distribution for stages I to IV was 27%, 21%, 26%, and 22 %, respectively.

Fig. 4. Evaluation of collection site impact on test set performance



- Testing set performance from all enrollment sites was compared to performance from sites that were unique in the testing set. 31% of subjects (non-cancers n=597; cancers n=383) in the test set were from these unique sites not present in the training set.
- The comparison of the unique collection site sensitivity (56.7%, 95% CI: 51.7-61.5) and specificity (99.3%, 95% CI: 98.3-99.7), as well as the sensitivity by stage indicates good model generalizability.

5 SUMMARY

Tab. 2. Performance of combined methylation & protein biomarker configuration in the testing set

Test Set Analysis	Estimate %	95% CI	n/N
Specificity	**98.5%**	**97.9%, 98.9%**	**2,397 / 2,434**
Sensitivity, all cancers	**50.9%**	**47.3%, 54.5%**	**371 / 729**
Stage I	15.4%	10.9%, 21.3%	28 / 182
Stage II	38.0%	30.9%, 45.7%	62 / 163
Stage III	67.8%	60.6%, 74.2%	122 / 180
Stage IV	85.5%	79.4%, 90.0%	147 / 172
Unknown stage	37.5%	22.9%, 54.7%	12 / 32
Stage I, II	26.1%	21.7%, 31.0%	90 / 345
Sensitivity, excl. breast & prostate cancers	**56.8%**	**52.8%, 60.7%**	**335 / 590**
Stage I	17.2%	12%, 24.2%	25 / 145
Stage II	48.6%	39.4%, 57.9%	53 / 109
Stage III	73.5%	66%, 79.9%	111 / 151
Stage IV	86.5%	80.2%, 91.0%	134 / 155
Unknown stage	40.0%	24.6%, 57.7%	12 / 30
Stage I, II	30.7%	25.4%, 36.6%	78 / 254
Sensitivity excl. breast, colorectal, prostate, & cervical	54.8%	50.4%, 59.2%	268 / 489
Sensitivity of pancreatic, esophagus, liver, lung, stomach, & ovarian	63.7%	58%, 69%	186 / 292

6 CONCLUSIONS

As shown in Tab.2, at a specificity of 98.5%, the combined methylation & protein configuration demonstrated:

- **50.9% sensitivity across all 21 cancer organ types, and 56.8% when breast and prostate cancers were excluded from the analysis.**
- **54.8% sensitivity excluding cancer organ types with average-risk standard of care screening (i.e. excluding breast, prostate, cervix, colon and rectum).**
- **63.7% sensitivity for the 6 most aggressive cancer organ types with the shortest 5-year survival rate (i.e. pancreas, esophagus, liver, lung and bronchus, stomach, and ovary).**

7 REFERENCES

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