A Blood Test and Machine Learning Enhanced Algorithm to Aid in Screening for the Early Detection of Lung Cancer

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ABSTRACT

A blood test and associated algorithm has been developed to help enrich the population of patients most likely to benefit from low-dose computed tomography (LDCT) screening for lung cancer. Yearly LDCT screening has been demonstrated to save lives by accurately detecting lung cancer at a stage early enough for curative surgery. Among the factors limiting the impact of LDCT screening, however, are (i) low patient compliance, especially for annual screening, (ii) narrow eligibility criteria the excludes more than 50% of lung cancer victims, and (iii) unacceptable false positive rates. A blood test and associated algorithm that combines the values of several biomarkers (antigen and autoantibody) together with several clinical factors (age, smoking history, and prior lung ailments) has been shown to more accurately identify patients who could benefit from LDCT screening than merely looking at age and smoking history alone. Use of machine learning approaches appears to improve these algorithms with continuous improvements anticipated as more patient outcome data is made available to the algorithms.

BACKGROUND

Lung cancer (LC) is the leading cause of cancer mortality in the United States (US) and around the world, accounting for approximately 28% of all cancer deaths in the US and 18% of cancer deaths worldwide [1]. While the overall survival rate for LC is 15%, a survival rate approaching 90% can be achieved when LC is detected at an early stage through LDCT screening programs [2]. Unfortunately, fewer than 16% of LCs are diagnosed at that early stage [1].

Earlier detection of lung cancer can lead to curative surgery to thousands of patients otherwise doomed to present with advanced disease later in life. Despite its relatively good sensitivity for LC detection (93.8%) [3], LDCT scanning, the only recommended screening modality for lung cancer in the U.S. today, has many drawbacks that limits its applicability as a standalone detection methodology. These challenges include a high false-positive rate (including the inability to unambiguously distinguish benign nodules that can involve expensive invasive follow-up procedures); generally low patient compliance, the danger of cumulative diagnostic radiation exposure with repeated testing, and overdiagnosis which has been estimated to be more than 18.5% for all LCs [4-7]. An accurate early detection blood test could improve the efficiency of LDCT lung cancer screening, allowing more lung cancers to be found while minimizing the potential harms to those with a low likelihood of having lung cancer.

Since 2015, following an analysis by the U.S. Preventive Services Task Force (USPSTF) [8] the Center for Medicare and Medicaid Services (CMS), and most private insurance companies will cover yearly LDCT for patients that meet the following criteria: asymptomatic adults aged 55 to 77 years, having a 30 pack-year smoking history, and currently smoking or having quit within the past 15 years. However, according to 2016 study by the Mayo Clinic [9], only 37% of female and 50% of male patients diagnosed with lung cancer meet this USPSTF eligibility criteria. Thus, it would be desirable to have improved means to enrich the population of patients for recommended LDCT screening.

The combination of LDCT scans with a blood biomarker test and associated algorithm could reduce the false positive test rate from the LDCT scan alone and help direct at-risk patients to this screening paradigm. In addition, an accurate biomarker test has the potential to improve the management of imaging findings, expediting therapy for early stage cancers while minimizing the risks from evaluating those with benign disease. Therefore, a strong need exists for a rapid, accurate, simple-to-use blood test and algorithm for routine use with at-risk populations in the US and worldwide.

20/20 GeneSystems, Inc., (Rockville Maryland) has developed, validated, and makes available through its CLIA licensed Genesys Biolabs a blood test for the early detection of lung cancer [10, 11]. “PAULAs test” (an acronym for Protein Analytes Used for Lung cancer Algorithms) measures the levels of serum antigens, an autoantibody, and several clinical factors including patient age, smoking history, and prior lung disease. The test is intended to be used as an initial screen for non-small cell lung cancer (NSCLC) in asymptomatic individuals from a high-risk population (e.g. 20 pack-year current smokers or past smokers who quit less than 15 years ago, and are over the age of 50) who are not receiving annual CT scans. The test measures a panel of tumor proteins (carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA125), cytokeratin-19 fragment 21-1 (CYFRA 21-1)) and an autoantibody - New York esophageal cancer-1 (NY-ESO-1) - and previously utilized a MoM (multiple of medians) algorithm to combine the biomarker values and generate a score categorizing the risk that the patient has LC [10]. Our unique approach involves measuring two distinct types of analytes circulating in the blood: tumor antigens and autoantibodies (patents pending). The tumor antigens have individually and collectively been shown by dozens of outside investigators around the world to distinguish early LC from risk-matched controls [12-20]. The combination of “established” biomarkers with more unique analytes such as autoantibodies coupled with advanced statistical treatment provides an optimal panel for screening diverse, asymptomatic patient populations. The internal validation of this biomarker panel showed that the test had a sensitivity of 74% at a specificity of 80% and AUC of 0.81 [10].

Clinical factors plus serum biomarkers is superior to either method alone

Traditionally, physician judgment has formed the basis for lung cancer risk estimation, patient counseling, and decision making. However, clinicians' estimates are often biased due to both subjective and objective confounders. To mitigate this problem and to obtain more accurate lung cancer predictions, dozens of multi-biomarker panels have been developed over the last decade. They can provide additional information to the physicians to better estimate the presence of lung cancer. In several studies the panel of biomarker values and clinical features were incorporated into one algorithm using statistical methods [17, 20]. For example, the recent study by Molina *et al.* [20] compares the diagnostic accuracy of a traditional clinical model that considers nodule size, patient age, and smoking status with that resulting from the combination of this clinical model and the assessment of several tumor markers panel. This study clearly demonstrated the added value of biomarkers and clinical factors in the diagnostic evaluation of lung cancer patients.

Cognitive computing/ machine learning approaches models further improve discrimination accuracy in Lung Cancer Risk Estimation

Statistical models can provide assistance in processing a large number of variables (biomarker values and clinical factors). Several different statistical methods have been applied to discriminate between patients with and without lung cancer, such as multivariate logistic regression (MLR), random forest (RF), classification and regression trees, support vector machine (SVM), etc. These methods have been used to develop algorithms that combine measurements of the most predictive biomarkers in a panel to achieve the highest diagnostic accuracy. Recently, tools of artificial intelligence have been applied to lung cancer research [21-23]. These Artificial Neural Networks (ANNs) strategies allow the extraction of relevant information from large and noisy data sets, such as biomarker data collections. For example, Wu *et al.* [21] evaluated the diagnosis potential of an ANN model combined with six biomarker panel and three metal ions in 50 lung cancer patients and 90 controls, and achieved a prediction rate of 85% in the test phase. In the study by Feng *et a*l. [22] an ANN model with six serum tumor markers and 19 clinical parameters was used to distinguish lung cancer from benign lung disease and healthy people with 96.9% accuracy. Flores-Fernandez *et al.* [23] evaluated 14 biomarkers using ANN modeling to find an optimized panel composed of only four biomarkers. This panel showed a correct classification rate for lung cancer patients of 90% in a testing phase. Unfortunately, the format of the tests used in these publications is too expensive for deployment in world-wide medical systems constrained by costs. Therefore, the development of a similar system using a more cost-effective platform would be beneficial. Although these studies are preliminary and not validated, they show that cutting-edge data processing is likely to be applicable to the poorly-developed fields of early lung cancer detection and lung cancer risk assessment.

Deep Learning Neural Network (DNN), the cutting edge of machine learning, is gaining more and more attention in the artificial intelligence world. DNNs are flexible multilayer systems of connected and

interacting artificial neurons that perform various data transformations [24-26]. However, despite achieving superior classification accuracy over traditional ANNs, the adoption of DNNs in biomedicine has been remarkably slow [27,28].

The goals of the current study were to confirm the accuracy of this panel of biomarkers on an independent data set, to explore the accuracy relative to and in combination with clinical risk predictors with a focus on at risk patients relevant to lung cancer screening and to further investigate whether an advanced multi-parameter statistical algorithm can materially improve diagnostic accuracy of our lung cancer test.

METHODS

Training set serum samples.

All of the cancer and normal control samples used in the training set were IRB-approved, consented serum samples that were purchased from the Clinical Research Center of Cape Cod, Inc. (Cape Cod, MA), Asterand (Detroit, MI), Indivumed (Germany) or Bioreclamation IVT (New York, NY). All of the lung cancer samples were collected at physicians' offices or hospitals.

All lung cancer and control serum samples were from patients 50 years of age or older who were current or former smokers with a smoking history of greater than 20 pack years and less than 15 years of smoking cessation. Diagnosis of the lung cancer cohort was confirmed from surgical pathology reports. The control group had no evidence of current or prior cancer.

Testing set serum samples.

All of the cancer and normal control samples used in the testing set were obtained from an IRB approved blood biorepository at the Cleveland Clinic. All patients had provided written informed consent. All lung cancer cases were biopsy confirmed and untreated. Control patient samples were obtained from patients attending the lung cancer screening clinic or general Pulmonary clinic.

Sample analysis.

Multiplex magnetic bead-based immunoassay of CEA, CYFRA21-1, CA125 and HGF in patient sera was performed using reagents from EMD Millipore, Inc. as previously described (2). The MILLIPLEX® MAP Human Circulating Cancer Biomarker Magnetic Bead Panel 1 was used. Four tumor proteins (CEA, CYFRA21-1, CA125 and HGF) were measured using the MAGPIX® instrument (Luminex Corporation, Austin, TX) as previously described [8]. Using Median Fluorescent Intensity (MFI) values and a five-parameter logistic curve fitting method (xPONENT® software for the MAGPIX®) the concentrations of each tumor protein in the samples were calculated. The calculated protein concentration values were used for the subsequent analysis.

NY-ESO1 autoantibody detection was performed using an immunoassay developed at 20/20 Gene Systems, MD and the MAGPIX® reader as previously described [8]. Background subtracted MFI values were used for the subsequent analysis.

Statistical analysis.

The study cohort was divided into two groups based upon the outcome of cancer or control. The demographics, comorbidities, and cancer characteristics were described using sample mean with standard deviation or proportion as appropriate.

Multivariate logistic regression analysis: To determine the direction and statistical significance of the effect of each biomarker on the outcome, we performed multivariate logistic regression (MLR) analysis for the full data set. Each MLR model included the five biomarkers. The AUC was calculated for the ROC curves that were constructed based on the models. Exploratory MLR analyses were performed on the testing set, divided by stage and histology, and after including clinical variables. Clinical variables included age, sex, a clinical diagnosis of COPD, and smoking history.

Random forests analysis: Random Forest (RF) models were used to identify the variables that were associated with and predictive of cancer (4). To avoid the possible overfitting of the MLR models, we used the repeated random-split cross-validation procedure (5). Specifically, we randomly split the data into training (70 %) and testing (30 %) sets 100 times. The RF model was built on each training set and then evaluated on the corresponding test set. The validation results were reported as the average performance over all test sets. Exploratory RF analyses were performed on the testing set, divided by stage and histology, and after including clinical variables (as above).

RESULTS

The training set consisted of 604 patient samples (268 with lung cancer, 336 controls). 151 of those with lung cancer (56.3%) had adenocarcinoma and 144 of the 268 lung cancers (53.7%) were stage I. The testing set consisted of 400 patient samples (155 with lung cancer, 245 controls). 74 (47.7%) of those with lung cancer had adenocarcinoma and 52 of the 155 lung cancers (33.5%) were stage I (Table 1).

Table 1*. Clinical characteristics of the cancer and control patients in the training and testing sets*

	Training (604)		**Validation (400)**	
	Cancer (268)	**Control (336)**	**Cancer (155)**	**Control (245)**
Age	64.0	64.5	65.3	68.3
Sex (%F)	43.7	39.9	40	51.9
Smoking (C/F/N)	N/A	N/A	20/129/6	95/142/7
Pack years	> 20	> 20	43	35
Adenocarcinoma (%)	56.3		47.7	
Squamous (%)	33.2		39.4	
Stage I (%)	53.7		33.5	
Stage II (%)	24.3		12.3	
Stage III (%)	17.9		37.4	
Stage IV (%)	4.1		16.8	

Training set results showed that combination of the biomarkers studied was more accurate than the individual biomarkers considered alone (panel AUC 0.80 vs. individual AUC 0.45-0.71). A logistic regression model was built on the training set using the biomarker values and then applied to the validation set. The diagnostic accuracy of 4 biomarker panel in the validation set was comparable with that of the training set (AUC 0.81).
There was less meta-data available for the training samples for the algorithm development that combines clinical factors and biomarkers values. Therefore, to evaluate an algorithmic approach that combines biomarker and clinical data further analyses were performed only on the validation set samples (n=400).

Table 2**.** *Logistic Regression (LR) and Random Forest (RF) model performance using biomarker panels and clinical factors*

	LR model	**Random Forest model (70:30 Split)**		
Variable	AUC*	Sensitivity (%)	Specificity (%)	AUC*
Clinical factors	0.68	34	85	0.66
Biomarkers	0.81	66	86	0.84
Combined	0.86	80	80	0.87

*Area under Receiver Operating Characteristic Curve

In exploratory analysis, a Multivariate Logistic Regression (MLR) model built from clinical variables in the validation set (age, sex, COPD, smoking history) had an AUC of 0.68. When combined with the 4 biomarker panel the AUC was 0.86 (Table 2). Similarly, Random Forest (RF) modeling of the clinical factors and biomarker values alone yielded an average AUC of 0.66 and 0.84, respectively. When combined with the 4 biomarker panel, the AUC improved to 0.87 (Table 2).
The validation sample set from the Cleveland Clinic (n=400) has a significant number of samples that do not conform to the indication criteria of either the USPTF or PAULAs Test. Specifically, samples are included with variations in smoking history, including some never smokers. Some patients have smoking histories with less than 20 pack years (and <30 pack years as per USPTF). Some patients are under age 50 (and under age 55 or over age 80 as per USPTF).

Using random forest statistical analysis we evaluated the improvements yielded by single predictor and identified the panel of classifiers that seem most significant out of both the biomarkers and the clinical factors: CEA, CA-125, CYFRA and NYESO-1, age, smoking history, pack years and COPD. The performance of this panel in the population conforming to PAULAs test inclusion criteria was better than in a broader population that included smokers under age 50 and with less than 20 pack years (Table 3). At approximately the same specificity (79% vs 80%) the sensitivity falls from 81% to 74% in a broader population. It should be noted, however, that sample size (400 vs 216) may also effect the difference between the results.

Table 3**.** *Performance of the test in the population within PAULAs test inclusion criteria and in a broader population.*

	Cohort size	AUC*	Sensitivity %	Specificity %
All patients	n=400	0.845	74	79%
Patients within PAULAs test inclusion criteria	n=216	0.887	80	80

*Area under Receiver Operating Characteristic Curve

Fig.1 shows the distribution of test score in a patient cohort conforming to PAULAs test inclusion criteria. For this analysis, we excluded never smokers and those with missing info, and limited the patient cohort.to the PAULAs test inclusion criteria.

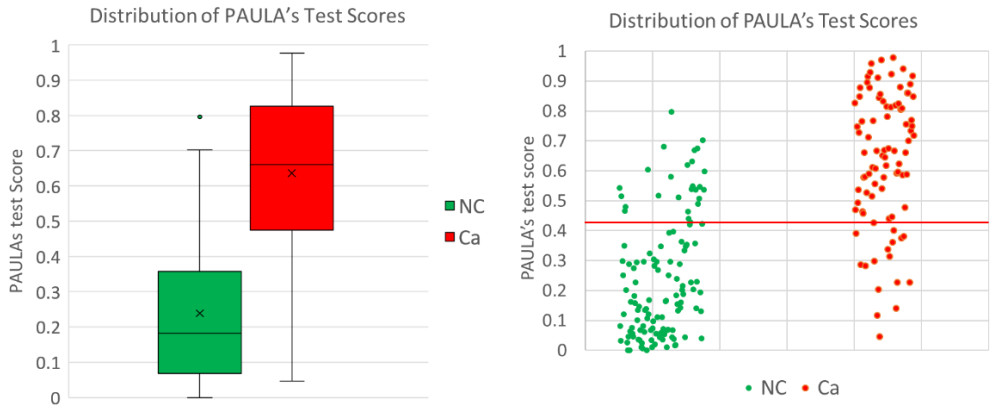

Fig.1 *Distribution of PAULAs test scores using RF model (CEA, CA-125, CYFRA and NYESO-1, age, smoking history, pack years and COPD): A. box-and whiskers plot. B. scatter dot plot. The red horizontal line shows the PAULAs test cut-off of 0.43 derived from the validation set results.*

Table 4**.** *Performance of the combined biomarker-clinical factors panel by lung cancer stage.*

	Sensitivity (%) at 80% Specificity	
	All patients	Patients within PAULAs test inclusion criteria
I	69.6%	82.4%
II	70.6%	84.6%
III	63.0%	75.0%
IV	82.4%	90.0%

Using the test cutoff that corresponds to 80% fixed specificity (0.43), we evaluated the accuracy of the combined panel by stage in both groups of patients. The detection sensitivity of the early stages (I and II) in patients corresponding to PAULAs test inclusion criteria was higher than in a broader population - 83.5% vs 70.1% (Table 4).

We also explored deep neural network (DNN) modelling approaches for the test performance evaluation using the entire validation set from Cleveland Clinic (n=400). To build a DNN model, we first identified the input variables, which included both clinical factors and biomarkers. We then applied 2 hidden layers, 1000 nodes in the first layer, and 5000 nodes in the second layer. Tanh activation function was adopted in the DNN method. With 70% data points as the training dataset and 30% of data points as testing set, the DNN model produced a higher AUC (0.89) than random forest (0.88) and logistic regression (0.87) models (Table 5).

Table 5**.** *Comparison of PAULAs test results using biomarkers and clinical variables and different modelling approaches (LR, RF and DNN)*

Method	**AUC***	**95% CI#**	**Sensitivity, %**	**Specificity, %**
Logistic Regression	0.86	0.80-0.94	75	80
Random Forest	0.88	0.81-0.95	80	80
Deep learning (DNN)	0.89	0.83-0.96	90	82

*Area under Receiver Operating Characteristic Curve; #95% Confidence Interval

DISCUSSION

The current study attempted to validate the clinical accuracy of a combined protein and antibody panel in a population at risk of having lung cancer and explore the impact of combining clinical and biomarker variables on test accuracy. The intended use population for this study was patients at risk of having lung cancer. The results suggest that the combination of markers is more accurate than any of the markers alone. In exploratory analysis, the highest accuracy was achieved by combining clinical features and biomarker results for patients within PAULAs test inclusion criteria (50 years of age or older who were current or former smokers with a smoking history of greater than 20 packs per year and less than 15 years of smoking cessation). Based on the Random Forest statistical algorithm the test yielded the following performance: 80% sensitivity, 80% specificity, 0.88 AUC when both biomarker values (CEA, CYFRA, CA125 and NY-ESO1) and clinical factors (age, smoking history, pack-years and COPD status) were considered.

To pursue clinical utility testing it should be determined if the results of this study support further development of this biomarker as an early detection tool. In order to justify moving forward, the accuracy of the test should support the potential application. To estimate the accuracy required to justify investment in a clinical utility study, a formula has been suggested that incorporates the accepted benefit : harm balance of current standard practice [29]. If we use this formula to determine a test accuracy that would allow us to use the results of this test to select patients for lung cancer screening from a population with a 0.2% incidence of lung cancer, and assume that we currently accept screening a population with a 0.83% incidence of lung cancer (the incidence during the screening years of the National Lung Screening Trial [3]), TPR (True positive rate, or Sensitivity)/ FPR (False positive rate, or (1-Specificity)) of the test would have to be at least 4. Based on this analysis, the accuracy of the biomarker panel in the current study (e.g. sensitivity of 80% at specificity of 80% (RF model) or sensitivity of 90% at specificity of 82% (DNN model) meets the minimal biomarker panel performance (TPR/FPR=4) and supports further development of the test as a screening tool. In addition, the cost of this test would be much lower than most omics-based testing platforms currently available. This is also important to consider when developing a screening test.

We also have developed a risk categorization tool based on the results from this study. The test generates a composite score form the Random Forest model comprising 4 clinical parameters and the levels of 4

biomarkers in patient serum. This score is an indicator of the level of risk for each patient of currently having lung cancer relative to others with a comparable smoking history. Using two cutoffs (0.43 and 0.62), the test results were broken down into three separate categories with increasing risk factors (Table 6). The table 6 indicates the probability of lung cancer for patients in a given score range at the time of testing. Positive predictive value (PPV) is the probability that person with a positive test score above the chosen cutoff truly have the disease. Unlike sensitivity and specificity, the PPV is dependent on the population being tested and is influenced by the prevalence of the disease. For the PPV calculation we used 0.83% lung cancer prevalence from the NLST study [3]. The Table 6 shows that the higher patient's score on PAULAs test the greater the likelihood that this patient does have cancer.

Table 6**.** *Test PPV in 3 separate score categories.*

Score Range	Sensitivity	Specificity	PPV
X≥0.62	55.1%	95.3%	8.89%
0.43≤X<0.62	62.2%	84.0%	3.16%
X<0.43	100.0%	0.0%	0.83%

Below the cutoff of 0.43 test will not differentiate between cancer and non-cancer. Individuals whose scores fall within this range have the same likelihood of having lung cancer as those people currently recommended for LCDT by the USPTF (0.83%). Individuals whose scores fall within the middle range are 3.8x more likely to have lung cancer than individuals currently recommended for LCDT by the USPTF. Finally, individuals whose scores fall within the high range are 10.7x more likely to have lung cancer than individuals currently recommended for LCDT by the USPTF. The result of the test presented using such categorization table will inform the physican about the degree of the lung cancer risk patient has after a positive result on the test.

The next steps in the development of this panel would include larger clinical validation studies in the intended use population, then clinical utility studies if supported by the validation results. Similarly, one might consider applying this test to help with the evaluation of indeterminate lung nodules. Though not directly assessed in this project, accuracies of the biomarker alone for stage I disease may approximate the accuracy within a nodule population. Combining the biomarker with clinical and imaging variables may optimize a nodule risk prediction tool.

The strengths of the current study include a reasonably large number of samples from a cohort relevant to the potential clinical application, with samples obtained from more than one source. The sample sets included a substantial portion of cases with early stage disease, and a diverse set of relevant patient comorbidities, supporting the robustness of the method. The results were compared to, and were more accurate than clinical prediction, and the combination of the marker results with clinical features improved the accuracy of both. A weakness of the study was that there was less information available about the quality of the training samples, and there was less meta-data available for these samples, so exploratory analysis was performed on only the validation set from the Cleveland Clinic.

In summary, this study validates the accuracy of a panel of proteins and an autoantibody in a population relevant to lung cancer screening, and suggests a benefit to combining clinical features with the biomarker results. Additional validation, followed by clinical utility testing, appears to be warranted.

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