Autoantibodies as Immunobiomarkers in Lung Cancer and their use in Early Cancer Detection

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BACKGROUND & AIMS

A humoral immune response in the form of autoantibodies (AAbs) to tumor-associated antigens (TAAs) has been reported in individuals with evidence of solid tumors.

These AAbs have been shown to be present in the circulation of individuals with lung cancer, and in some cases up to 5 years before the cancer presented clinically[1-3]. These AAbs may therefore represent the earliest markers of carcinogenesis.

The *Early*CDT®-Lung test, which detects AAbs to a panel of lung cancer-associated antigens, has a previously reported sensitivity of 40% and a specificity of 90% for the detection of lung cancer, and has been shown to aid in the detection of both early-stage and late-stage disease in high-risk individuals[1,2].

The original published test detected AAbs to a panel of 6 TAAs[1,2]. An improved test (introduced in November 2010) incorporated the addition of 2 new TAAs (and the removal of 1 of the original antigens). This change was investigated in both a case-control setting, and in a clinical setting via an audit of the test.

PATIENTS & METHODS

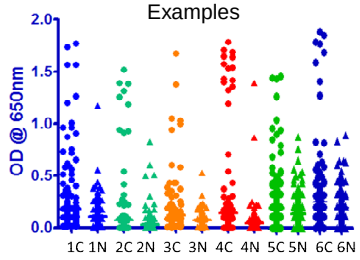
Optimization Set: Samples from 235 patients with newly diagnosed lung cancer (68% with early stage disease) and matched controls were measured for the presence of AAbs to 8 TAAs (p53, NY-ESO-1, CAGE, GBU4-5, Annexin I, SOX2, MAGE A4, HuD). The sensitivity and specificity of the original 6 (p53, NY-ESO-1, CAGE, GBU4-5, Annexin I, SOX2) and the new 7 (p53, NY-ESO-1, CAGE, GBU4-5, SOX2, MAGE A4, HuD) AAb panels were compared.

Clinical Validation Set: In addition independent clinical sample sets, comprising two consecutive series of 776 and 836 individuals who were deemed by their clinician as being at an increased risk of developing lung cancer, were audited to provide a comparison of the 2 panels in a true clinical setting.

ASSAY PROCEDURE

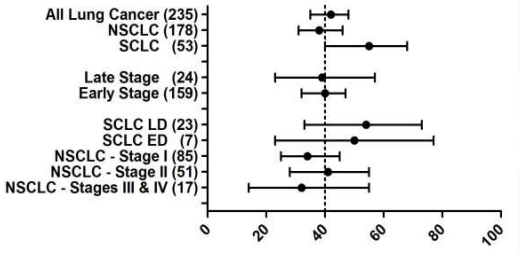
The presence of AAbs was evaluated using a semi-automated ELISA method where optical densities (OD) (see ELISA scatter plots) were converted to calibrated reference units (RU)[1]. Full assay details are described elsewhere[1].

ELISA Scatter Plots - Examples



C: cancer, N: Normal
1. p53, 2: SOX2, 3: CAGE, 4: NY-ESO-1, 5: MAGE A4, 6: HuD

Forest Plot – Optimization Set: sensitivity by cancer stage



Positivity defined as the presence of raised levels of one or more AAbs (New panel of 7)

RESULTS

Optimization Set: The addition of MAGE A4 and HuD to the *Early*CDT®-Lung panel, and the removal of Annexin I improved both the sensitivity and the specificity of the assay (Table 1). Adjustment for occult cancer in the normal population give an overall specificity and sensitivity of the new test of 93% and 41% respectively.

The positivity rates for the new panel by stage of disease for non-small cell lung cancer (NSCLC) and for extensive vs limited disease for small cell lung cancer (SCLC) were similar for all stages (see Forest Plot).

The positivity rate for individual AAb assays in the new panel ranged in NSCLC from 2%-10% and in SCLC from 6%-21% with specificity for each antigen being at least.

Table 1. Frequency of AAbs to a Panel of TAAs in Lung Cancer

Optimization Set : Percentage positivity and specificity in lung cancer (LCa) shown

Former Panel

	n	Ann I	p53	CAGE	NY-ESO-1	GBU 4-5	SOX2 NusA	Panel of 6
LCa	235	9	10	12	11	4	8	39
Spec	266	95	98	99	98	98	97	89

New Panel

New cut-offs set

	n	p53	CAGE	NY-ESO-1	GBU 4-5	MAGE A4*	SOX2 BirA	HuD	Panel of 7
LCa	235	13	9	10	3	12	4	5	41
Spec	266	97	99	98	98	96	99	99	91

Conclusion : new panel →increase in **specificity** & sensitivity

Clinical Validation Set: Analysis of the two different panels in the clinical sample sets confirmed that the 7 antigen panel maintained the sensitivity of the test, but also resulted in a highly significant increase in its specificity from 82% (for the original 6 AAb panel) to 90% (with the 7 AAb panel) p<0.0001 (Table 2).

Table 2. Frequency of AAbs in the Clinical Setting

Clinical High Risk Population Sets:

Number, percentage positivity, sensitivity and specificity shown for 1612 sequential samples from a clinical audit of the *Early*CDT-Lung test. (Data from May 2009-August 2011, *data last updated August 2011)

Clinical Audit data	Number	Lung Cancer confirmed* N (%)	Lung Cancer free* N (%)
Panel of 6 AAb Assays 5.09-11.10			
Total	776	25 (3.2)	751 (96.8)
Positive AAb assay result	145	10 (6.9)	135 (93.1)
Negative AAb assay result	631	15 (2.4)	616 (97.6)
Sensitivity or Specificity (Original Panel)		Sensitivity 40%	Specificity 82%
Panel of 7 AAb Assays 11.10-8.11			
Total	836	19 (2.3)	817 (97.7)
Positive AAb assay result	87	9 (10.3)	78 (89.7)
Negative AAb assay result	749	10 (1.3)	739 (98.7)
Sensitivity or Specificity (Improved Panel)		Sensitivity 47%	Specificity 90%

Conclusion : new panel → **highly significant increase in specificity of the test in the clinical setting**

CONCLUSIONS

These data confirm that the change of the *Early*CDT®-Lung test from a 6 to a 7 panel of AAb assays increased the accuracy of the test for the detection of all stages of lung cancer.

For a lung cancer incidence in a high risk population of 2.4%, this improvement, particularly in terms of the specificity of the test, would result in a positive predictive value (PPV) of **12.5%** (1 in 8), a negative predictive value (NPV) of **98.5%,** and an overall accuracy of the test of **92**% (based on a specificity and sensitivity of the test of 93% / 41%).

Differences in the AAb profiles may in the future be useful in identifying what subtype or stage of lung cancer a patient is most likely to have.

CLINICAL IMPLICATIONS

The *Early*CDT®-Lung assay can be used as part of the armamentarium of tests, available to the clinician, to aid the detection of early stage lung cancer. The change from a six to seven AAb assay improved the overall specificity of the test, thereby increasing its clinical usefulness.

REFERENCES

1. Murray *et al* Technical Validation of an autoantibody test for lung cancer. Ann Oncol. 2010;21:1687-93.
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