Improved Specificity of the *Early*CDT®-Lung Test Through Additional Autoantibody Assays

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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-(LCa) 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].

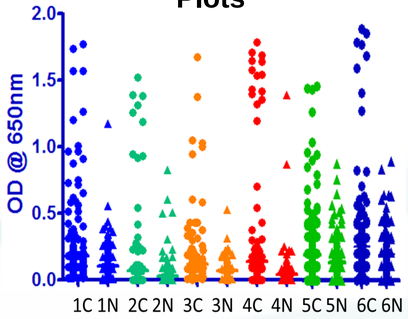
The original published test detected AAbs to a panel of 6 TAAs[1,2]. An improved test (introduced in November 2010) incorporated the addition of 2 new TAAs (and the removal of 1 of the original antigens). This change was investigated in both a case-control setting, and in a clinical setting via an audit of the test[4].

METHODS

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].

Two sets of samples were run: an optimization and a clinical population set.

Examples of ELISA Scatter Plots



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

Optimization Set: 235 patients with newly diagnosed LCa (68% early stage) 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 and the new 7 AAb panels were compared (Table 1).

Clinical Population Set: Two consecutive series of 776 and 836 individuals (deemed by their physician to be at an increased risk of developing LCa), were audited to provide a comparison of the 2 panels in a true clinical setting (Table 2).

Table 1. Frequency of AAbs to a Panel of TAAs in LCa

Percentage positivity and specificity in LCa shown

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

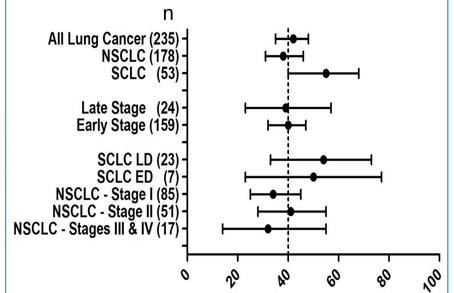
		n	p53	CAGE	NY-ESO-1	GBU 4-5	MAGE A4*	SOX2 BirA	HuD	Panel of 7
New Panel										
New cut-offs set	LCa	235	13	9	10	3	12	4	5	41
	Spec	266	97	99	98	98	96	99	99	91

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 cancers in the normal population gave an overall specificity and sensitivity of the new test of 93% and 41% (see Forest Plot).

Forest Plot



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

Table 2. Frequency of AAbs in the Clinical Setting

Clinical Population Sets:

Number, percentage positivity, sensitivity and specificity shown for 1612 sequential samples from a clinical audit of the *Early*CDT-Lung test.

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 → increase in specificity & sensitivity

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%).

REFERENCES

[1]Murray *et al* Technical Validation of an autoantibody test for lung cancer. Ann Oncol. 2010; 21:1687-93. [2]Boyle *et al* Clinical Validation of an autoantibody test for lung cancer. Ann Oncol. 2011; 22:383-9. [3]Zhong *et al* Profiling tumor-associated antibodies for early detection of NSCLC. J. Thor Oncol 2006; 1:513-9. [4]Chapman *et al* *Early*CDT-Lung test: improved clinical utility through additional autoantibody assays. Tumor Biol 2012 epub.

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