Signal Stratification of Autoantibody Levels in Serum Samples

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BACKGROUND & AIMS

The *Early*CDT®-Lung test (Murray *et al* 2010; Boyle *et al* 2011) which measures serum autoantibodies (AAbs) to 7 tumor-associated antigens (TAAs) (p53, NY-ESO-1, CAGE, GBU4-5, SOX2, MAGE A4 and HuD each at 50nM and 160nM concentration) in a calibrated ELISA assay (in RU reference units), is used by clinicians as an aid to early detection of lung cancer in a high risk population. Currently a single set of test cut-offs classifies the samples into two strata: positive or negative, indicating high and low risk for cancer respectively. We now add two new sets of low and high cut-offs to classify the samples into four risk strata indicating strength of evidence (Table 1). This should facilitate interpretation of the test for clinicians and allow more precise intervention for any particular patient.

Table 1. Definition of risk strata

Stratum	Rule for TAAs	Result	Risk
1	≥one >H	Strong positive	Very high
2	All <H, but ≥one >C	Positive	High
3	All <C, but ≥one >L	Negative	Low
4	All <L	Strong negative	Very low

Cut-offs: L = Low, C = Central, H = High

SAMPLE SETS

Validation set: A case-control set with 235 lung cancers (UK, US, Ukraine, Russia), obtained at confirmation of the tumor (NSCLC 76%, SCLC 23%), and 266 normals matched by age, gender and smoking history. **Post-validation set:** A case-control set comprising four groups of patients with 336 newly diagnosed lung cancers (prior to treatment), from UK, US, Canada, Ukraine and Russia (Lam *et al* 2011) (NSCLC 90%, SCLC 10%) matched by gender, age and smoking history to 415 control individuals with no previous history of malignant disease. **Population set:** A commercially derived sample set with 36 confirmed cancers and 811 normals collected since November 2010, from individuals at high risk of lung cancer. Clinical follow-up was carried out for up to six months. The demographics were summarised (Table 2)

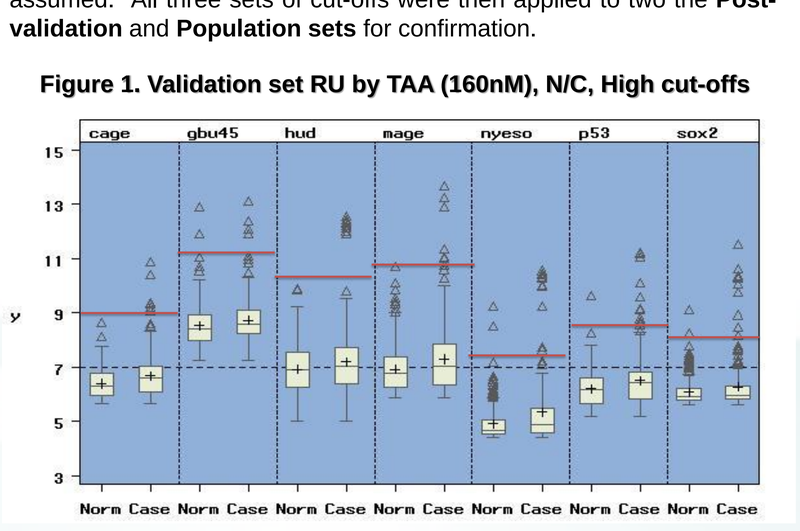
Table 2. Summary of demographics (Normals/Cancers)

	Males	Median age	Smoker	Ex-smoker
Validation	**70%/73%**	**65/65**	**35%/46%**	**54%/29%**
Post-validation	**64%/65%**	**62/67**	**19%/52%**	**57%/33%**
Population	**36%/42%**	**60/70**	**45%/50%**	**41%/44%**

DERIVATION OF NEW CUT-OFFS

For the **Validation** set, direct search over all antigens was used to locate the optimum increase over the original two-stratum cut-offs to obtain new high cut-offs maximizing the sensitivity for a specificity approaching 100% (Figure 1), thus giving a high positive predictive value (ppv). Similarly, the optimum decrease to obtain new low cut-offs maximizing the specificity for a sensitivity approaching zero, giving a high negative predictive value (npv). A prevalence of 2.7% was assumed. All three sets of cut-offs were then applied to two the **Post-validation** and **Population sets** for confirmation.

Figure 1. Validation set RU by TAA (160nM), N/C, High cut-offs



RESULTS

For the Validation dataset, cancer/normal (C/N) positivity for current (central) cut-offs gives a ppv of 1 in 9. The very high risk stratum gave an improved ppv of 1 in 4. C/N negativity for current cut-offs gave a pnpv of 1 in 57. The very low risk stratum improved this to a pnpv of 1 in 105. For the Combined dataset, ppv for current cut-offs was 1 in 11, which improved to 1 in 5 with the very high risk stratum, whilst a pnpv of 1 in 51 improved to 1 in 90 with the very low risk stratum. [pnpv = prob(positive-given-negative-result predictive value]

Table 3. Spec/sens and ppv/pnpv for four-stratum test

	Positivity (C/N) ppv	Negativity (C/N) pnpv	Positivity (C/N) ppv		Negativity (C/N) pnpv	
Dataset	**Current Positive[a]**	**Current Negative[b]**	**Strong Positive[c]**	**Positive[d]**	**Negative[e]**	**Strong Negative[f]**
Validation (235C/266N)	41%/9% 1 in 9.2	59%/91% 1 in 57	25%/2% 1 in 3.7	16%/7% 1 in 18	51%/68% 1 in 49	8%/23% 1 in 105
Post-validation (336C/415N)	30%/10% 1 in 13	70%/90% 1 in 47	17%/4% 1 in 8.8	13%/6% 1 in 19	58%/68% 1 in 43	12%/22% 1 in 65
Population (36C/811N)	36%/9% 1 in 10	64%/91% 1 in 53	19%/2% 1 in 4.4	17%/7% 1 in 16	47%/60% 1 in 47	17%/31% 1 in 67
Combined (607C/1492N)	34%/9% 1 in 11	66%/91% 1 in 51	20%/2% 1 in 5.2	14%/7% 1 in 18	55%/64% 1 in 43	11%/27% 1 in 90

CONCLUSIONS

The autoantibody signal level in the *Early*CDT®-Lung test can be used to stratify patients into risk categories, where those above the high cut-offs are deemed highest risk, and those below the low cut-offs are lowest risk. This stratification will enable individualization of subsequent screening and treatment programs for patients. We are now developing this by summarising the AAb results in a single Occurrence Score™ that can be translated into a continuous risk estimate, additive over demographic risk.

REFERENCES

1 Murray *et al*. Technical validation of an autoantibody test for lung cancer. *Ann Oncol* 2010;21:1687–1693. 2 Boyle P, *et al*. Clinical validation of an autoantibody test for lung cancer. *Ann Oncol* 2011;22:383–389. 3 Lam S. *et al*. *Early*CDT-Lung: an immunobiomarker test as an aid to early detection of lung cancer. *Cancer Prev Res (Phil)* 2011;4:1126–1134.