Demographics of Populations at High Risk of Lung Cancer and Results of the *Early*CDT-Lung™ Test

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BACKGROUND

***Early*CDT-Lung**™ is a commercially-available blood test offered in the United States by Oncimmune USA LLC to aid in the early detection of lung cancer in a high-risk, asymptomatic population.

Evidence for the variation of autoantibodies (AAbs) in normal populations is limited. A study of autoimmune PAP (pulmonary alveolar proteinosis) (1) reported no strong link between CSF AAb levels and smoking. AAb levels are known to rise with age (2), but this may be due to increasing cancer incidence itself, and smoking may alter AAb levels without an overt tumour being diagnosed (3).

We demonstrate in three datasets that the ***Early*CDT-Lung**™ test remains valid across high-risk and all-risk population subgroups differentiated on the basis of demographics.

SAMPLE COLLECTION

Prospective blood collections were made from three community-based locations in two countries. Age, gender and smoking history were recorded at all sites (Table 1), plus ethnicity at the US sites and autoimmune (AI) disease at the UK site. No individual had a history of previous malignancy. There was some variation in smoking pattern, with fewer current smokers in the highest age group.

Table 1. Numbers of participants by site, age and smoking.

	UK-PAS n=2044 (565M,1479F)			US-FL n=319 (185M,134F)		US-MO n=1213 (575M,638F)		
Age	Non	Ex	Yes	Ex	Yes	Non	Ex	Yes
20-29	166	44	82	0	0	102	37	45
30-39	163	70	70	5	85	64	37	32
40-49	213	92	51	9	84	43	66	142
50-59	173	130	43	9	80	59	97	119
60-69	218	217	22	3	44	39	101	54
70-89	158	117	15	0	0	11	132	33

ASSAY PROCEDURE

Serum samples were evaluated for AAbs to a panel of six cancer-associated antigens (p53, NY-ESO-1, CAGE, GBU4-5, Annexin 1 and SOX2) using an ELISA (enzyme-linked immunosorbent assay) method where optical densities (OD) are converted to calibrated reference units (RU) (4).

STATISTICAL METHODS

During validation of the test, samples were compared and where possible individually matched by gender, age and smoking history. Matching allows ordinary group means to be compared without adjustment for the matched factors, but reduces the number of samples available for analysis. If matching was not feasible, unmatched analysis was performed where means were adjusted for factor imbalance.

To enable a valid analysis, several statistical issues needed addressing. See BOX below.

Statistical issue	Solution
High between-subj variance	> Use large dataset
Skewed OD distribution	> RU values on log scale
Analytical unevaluability	> Only analyze evaluable data
Imbalance in subgroup nos	> Sample matching
Low sub-group numbers	> Apply subsetting or pooling
Interaction between factors	> Analysis of variance terms
Run-to-run assay variation	> Careful study design
Multiplicity of testing	> Use 1% significance level

RESULTS

ETHNICITY: An unmatched analysis showed no significant differences between African-American, Caucasian and Hispanic groups (Table 2 for US-MO results).

AI DISEASE: An unmatched comparison between Rheumatoid Arthritis (RA), Diabetes Mellitus (DM) and Normals showed no significant differences (Table 2).

Table 2. Statistical summary for ethnicity and AI disease (unmatched data sets) [mean adjusted RU].

	Ethnicity (US-MO)			AI Disease (UK-PAS)			
	Afr-Am n=95	Caucas n=230	P-val	Normal n=1844	R/A n=57	DM n=74	P-val
p53	3.74	3.54	0.24	3.70	3.73	3.50	0.04
SOX2	2.29	2.33	0.67	2.61	2.56	2.57	0.26
CAGE	2.94	2.92	0.85	3.10	3.12	2.98	0.17
NY-ESO-1	1.47	1.44	0.83	1.76	1.68	1.75	0.58
GBU4-5	2.58	2.66	0.65	2.67	2.48	2.62	0.13
Annexin 1	5.87	5.86	0.91	6.16	6.16	6.10	0.51

Table 3. Summary for within-US [Mean RU].

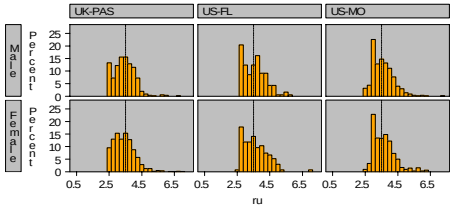
	Within-US		
	US-FL n=275	US-MO n=275	P-val
p53	4.00	3.98	0.77
SOX2	3.73	3.64	0.68
CAGE	3.38	3.45	0.27
NY-ESO-1	2.33	2.39	0.48
GBU4-5	2.80	2.72	0.19
Annexin 1	6.89	6.77	0.32

COUNTRIES/SITES: No significant difference was found between UK and US (n=353) (Figure 1 for p53), or within-US (US-FL vs US-MO) (n=275) (Table 3) for any antigen using matched analysis.

GENDER, AGE, SMOKING: There was complete information for 3576 (UK-PAS=2044, US-FL=319, US-MO=1213) individuals in the three unmatched datasets:-

• **GENDER:** There was no difference between males and females for any of the AAb assays, e.g. for p53 (Figure 1). Interactions were not significant.

Figure 1. Data distribution for sites and gender: p53



• **SMOKING:** No clear evidence for an effect of smoking history on AAb levels was found (Table 4).

[No=Non-smoker, Ex=Ex-smoker, Yes=Current smoker]

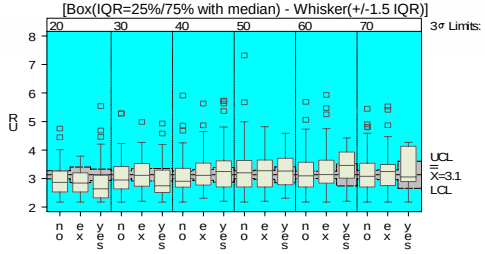
Table 4. Summary for smoking status [mean RU].

	Smoking status			
	No n=1409	Ex n=1166	Yes n=1001	P-val
p53	3.83	3.84	3.92	0.19
SOX2	3.60	3.60	3.46	0.49
CAGE	3.43	3.45	3.52	0.14
NY-ESO-1	2.24	2.21	2.31	0.38
GBU4-5	2.85	2.91	2.86	0.39
Annexin 1	6.83	6.85	6.85	0.92

• **AGE:** For p53, CAGE and GBU4-5, mean AAb levels showed an increase with age (Figure 2 for CAGE). The effect was most noticeable at the extremes. Note that cancer incidence also increases with age.

Figure 2. Age by smoking (mean RU): CAGE

[Box(IQR=25%/75% with median) - Whisker(+/-1.5 IQR)]



CONCLUSIONS

- The effect of demographics on the AAb test was investigated in three substantial datasets, including both all-risk and high-risk subjects.
- Apart from a possible effect of age on certain antigens at the extremes of the age range, no effects were found.
- Absence of demographic effects allows sample databanks to be pooled over the non-significant factors to obtain larger sample size for subsequent work.
- The absence of effects also means that no population subgroups investigated here need be excluded from AAb testing as an aid to early detection of lung cancer.
- **These results support the wider clinical applicability of AAb testing as an aid to the early detection of lung cancer in a high-risk population.**

REFERENCES

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