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Assessment of background levels of autoantibodies as a prognostic marker for severe SARS-CoV-2 infection

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ABSTRACT

Background: Patients with more severe forms of SARS-CoV-2 exhibit activation of immunological cascades. Participants (current or ex-smokers with at least 20 years pack history) in a trial (Early Diagnosis of Lung Cancer, Scotland [ECLS]) of autoantibody detection to predict lung cancer risk had seven autoantibodies measured 5 years before the pandemic. This study compared the response to Covid infection in study participants who tested positive and negative to antibodies to tumour-associated antigens: p53, NY-ESO-1, CAGE, GBU4-5, HuD, MAGE A4 and SOX2.

Methods: Autoantibody data from the ECLS study was deterministically linked to the EAVE II database, a national, real-time prospective cohort using Scotland's health data infrastructure, to describe the epidemiology of SARS-CoV-2 infection, patterns of healthcare use and outcomes. The strength of associations was explored using a network algorithm for exact contingency table significance testing by permutation.

Results: There were no significant differences discerned between SARS-CoV-2 test results and EarlyCDT-Lung test results ($p = 0.734$). An additional analysis of intensive care unit (ICU) admissions detected no significant differences between those who tested positive and negative. Subgroup analyses showed no difference in COVID-19 positivity or death rates amongst those diagnosed with chronic obstructive pulmonary disease (COPD) with positive and negative EarlyCDT results.

Conclusions: This hypothesis-generating study demonstrated no clinically valuable or statistically significant associations between EarlyCDT positivity in 2013-15 and the likelihood of SARS-CoV-2 positivity in 2020, ICU admission or death in all participants (current or ex-smokers with at least 20 years pack history) or in those with COPD or lung cancer.

Keywords: COVID-19, Current or ex-smokers, Lung cancer, Mortality prediction, Serum biomarkers

Introduction

Patients infected with Covid-19 show a range of immune responses, from weaker immune responses in asymptomatic individuals, to symptomatic patients showing a varying degree of immune dysregulation. These may be manifested by increased levels of interleukins, C-reactive protein and D-dimer, along with lymphopenia, monocytosis and neutrophilia. Extremely high levels of proinflammatory cytokines can lead to a cytokine storm and macrophage activation syndrome in patients with severe SARS-CoV-2. This may cause harmful tissue damage, multiple organ failure and hypercoagulability, and is associated with poor clinical outcomes (1). Conversely it is known that people with immune deficiency have an increase in mortality when admitted to hospital with Covid-19 (2). A range of serum autoantibodies, such as nucleolar antinuclear antibodies (ANAs), antineutrophil cytoplasmic antibody (ANCA), anti-cyclic citrullinated peptide, and antiphospholipid autoantibodies, have already been detected in severe SARS-CoV-2 patients and linked to disease severity, reflecting immune system dysregulation in patients with severe SARS-CoV-2 lung disease (3-5). It is not yet clear, however, whether patients who exhibit such robust immune response to SARS-CoV-2 have higher background levels of antibody and autoantibody responsiveness when compared to patients who develop mild disease (6), and for how long the level of autoantibodies persist. One form of antibody response to the development of abnormal cell surface characteristics is tumour-associated autoantibodies. These proteins are produced early in tumorigenesis, being measurable up to 5 years before the development of clinical symptoms (7). They represent biologically amplified markers,

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increasing the detectable signal for the corresponding level of antigen (8). They persist in the circulation with half-lives of typically up to 30 days (9).

The EarlyCDT-Lung test is an enzyme-linked immunosorbent assay (ELISA) that measures seven autoantibodies, each with individual specificity for the following tumour-associated antigens (TAAs): p53, NY-ESO-1, CAGE, GBU4-5, HuD, MAGE A4 and SOX2 (10). A sample is positive if at least one autoantibody is elevated above a predetermined cut-off (11). The test has been developed throughout the pre-clinical, clinical assay validation and retrospective biomarker development pathway stages. In cohort studies, it has demonstrated a specificity of 91% and sensitivity of 41%. The Early Diagnosis of Lung Cancer Scotland (ECLS) study was a phase IV biomarker trial using EarlyCDT-Lung followed by imaging in 12,208 smokers and ex-smokers aged 50-75 at risk of developing lung cancer recruited from General Practices in Scotland (12,13). A total of 6,088 participants in the intervention arm received the EarlyCDT-Lung test at the baseline visit and 598 (9.8%) had a positive autoantibody result. In the 2-year analysis of the ECLS trial, EarlyCDT-Lung was shown to reduce late stage presentations of lung cancer.

We have investigated whether the production of autoantibodies in response to cell surface abnormalities in cancer, as measured by the baseline EarlyCDT-Lung test in the ECLS trial, was associated with more severe disease in at-risk participants (current and former smokers) who then developed a SARS-CoV-2 infection 5-6 years later.

Methods

Participants aged 50-75 who were current or ex-smokers with at least 20 years pack history were recruited to ECLS between December 2013 and April 2015, and all baseline assessments of plasma antibody levels occurred during this time (14). SARS-CoV-2 status and outcome data for ECLS participants during 2020 were obtained from the EAVE II database, which is a national, real-time prospective cohort using Scotland's health data infrastructure, to describe the epidemiology of SARS-CoV-2 infection, patterns of healthcare use and outcomes (15,16). Data from both sources was linked using Scotland's Community Health Index (CHI) number at the University of Dundee's Health Informatics Centre (HIC) (17,18).

The strength of associations was explored using a network algorithm for exact contingency table significance testing by permutation. This approach is appropriate for the sparseness of the data here, where an approximate chi-squared analysis would provide severely discrepant outputs. (For 2 × 2 contingency tables, the network algorithm reduces identically to Fisher's exact test.)

Results

There were no significant differences discerned between SARS-CoV-2 test results and EarlyCDT-Lung test results (positive/negative) (p = 0.734); or likewise between SARS-CoV-2 test results and EarlyCDT-Lung test results (positive/negative/control) (p = 0.779); or finally between SARS-CoV-2 test results and Treatment (tested/not tested) (p = 0.587). An additional analysis of intensive care unit (ICU) admissions detected no significant differences between those who tested positive and negative.

There was no difference in COVID-19 positivity or death rates amongst those diagnosed with lung cancer with positive and negative EarlyCDT-Lung test results (Tab. I).

Table I - SARS-CoV-2 test results by EarlyCDT-Lung test result

Result of SARS-COV-2 test	Positive		Negative		Control	
	N	%	N	%	N	%
Positive	9	6.7	86	7.8	84	7.0
Negative	126	93.3	1021	92.2	1110	93.0
Total	**135**	**100**	**1107**	**100**	**1194**	**100**
Patient deceased						
No	131	97.0	1072	96.8	1155	96.7
Yes	4	3.0	35	3.2	39	3.3
Total	**135**	**100**	**1107**	**100**	**1194**	**100**

In Table II, nil significance was found.

Table II - Outcomes in at-risk participants (current and former smokers) with lung cancer

	EarlyCDT-Lung test result							
	Test-positive		Test-negative		Not tested		Total	
Stage	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Stage 3	0	(0.0)	1	(14.3)	4	(44.4)	5	(27.8)
Stage 4	0	(0.0)	1	(14.3)	0	(0.0)	1	(5.6)
Other	2	(0.0)	5	(71.4)	5	(55.6)	12	(66.7)
Total	**2**	**(100)**	**7**	**(100)**	**9**	**(100)**	**18**	**(100)**
Covid result	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Test-positive	0	(0.0)	1	(14.3)	1	(11.1)	2	(11.1)
Test-negative	2	(100.0)	6	(85.7)	8	(88.9)	16	(88.9)
Total	**2**	**(100)**	**7**	**(100)**	**9**	**(100)**	**18**	**(100)**
OR** = 0.00 (0.00, 66.5) p = 1.0								
Hospitalized*	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
No	0	(0.0)	4	(57.1)	3	(33.3)	7	(38.9)
Yes	2	(100.0)	3	(42.9)	6	(66.7)	11	(61.1)
Total	**2**	**(100)**	**7**	**(100)**	**9**	**(100)**	**18**	**(100)**
OR** = 0.00 (0.00, 4.20) p = 0.44								
Death*	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
No	2	(100.0)	6	(85.7)	9	(100.0)	17	(94.4)
Yes	0	(0.0)	1	(14.3)	0	(0.0)	1	(5.6)
Total	**2**	**(100)**	**7**	**(100)**	**9**	**(100)**	**18**	**(100)**
OR** = 9999 (0.015, 9999) p = 1.0								

*Event within 28 days of a Covid test.
**Odds ratio (Test-positive vs Test-negative).

Table III shows no difference in COVID-19 positivity or death rates amongst those diagnosed with chronic obstructive pulmonary disease (COPD) with positive and negative EarlyCDT results.

Table III - Outcomes in at-risk participants (current and former smokers) with COPD

	EarlyCDT-Lung test result							
	Test-positive		Test-negative		Not tested		Total	
Covid result	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Positive	1	(6.6)	3	(2.6)	9	(8.3)	13	(5.4)
Negative	15	(93.8)	113	(97.4)	100	(91.7)	228	(94.6)
Total	**16**	**(100)**	**116**	**(100)**	**109**	**(100)**	**241**	**(100)**
OR** = 2.51 (0.0913, 24.13) p = 0.407								
Hospitalized*	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
No	9	(56.3)	68	(58.6)	58	(53.2)	135	(56.0)
Yes	7	(43.8)	48	(41.4)	51	(46.8)	106	(44.0)
Total	**16**	**(100)**	**116**	**(100)**	**109**	**(100)**	**241**	**(100)**
OR** = 0.908 (0.312, 2.858) p = 1.0								
Death*	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
No	16	(100.0)	110	(94.8)	105	(96.3)	231	(95.9)
Yes	0	(0.0)	6	(5.2)	4	(3.7)	10	(4.1)
Total	**16**	**(100)**	**116**	**(100)**	**109**	**(100)**	**241**	**(100)**
OR** = 9999 (0.176, 9999) p = 1.0								

*Event within 28 days of a Covid test.
**Odds ratio (Test-positive vs Test-negative).

Discussion and conclusions

No clinically valuable or statistically significant associations between EarlyCDT-Lung positivity in 2013-15 and the likelihood of SARS-CoV-2 positivity in 2020, ICU admission or death were found. This was true for the entire study cohort and in subgroup analyses of at-risk participants (current and former smokers) with lung cancer and COPD. This is in contradistinction to those exhibiting the nucleolar immunofluorescence pattern where a significant association with interstitial lung SARS-CoV-2 disease has been demonstrated (19).

Strengths of the study include the community-based sampling of the ECLS cohort, large numbers of the cohort who had a Covid test validated by laboratory and outcome assessment. Weaknesses include the time which had elapsed between the initial trial and the onset of the pandemic, as well as small numbers of study subjects who were in the subgroup analyses.

Some studies have shown that some routine clinical laboratory tests, such as lymphocyte count, lactate dehydrogenase and D-dimer are known to be affected in patients with COVID-19 (20), with lymphopenia, raised lactate dehydrogenase and elevated D-dimer being associated with worse disease severity and outcomes (21-23). Other studies have shown significant differences in inflammatory markers amongst patients who required ICU admission compared to patients who have not, and markers of infection and inflammation such as C-reactive protein, procalcitonin, and ferritin which are, as expected, correlated with severe disease (24-27).

This hypothesis-generating study did not find a clear association between the expression of tumour-associated antibodies in the ECLS cohort of at-risk participants (all current and former smokers) and the development of SARS-CoV-2 infection and its complications 5 years later.

Author disclosures

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The authors confirm that all appropriate ethical guidelines for the use of human subjects have been followed and ethics committee review has been obtained. The authors confirm that all necessary patient/participant consent or assent has been obtained, and the appropriate institutional forms have been archived.
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