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SHORT REPORT

Predicting autoimmunity after alemtuzumab treatment of multiple sclerosis

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ABSTRACT

Objective We have previously shown that autoimmunity following alemtuzumab treatment of multiple sclerosis can be predicted by high baseline serum interleukin IL-21 (IL-21), as measured using a now 'redundant' enzyme linked immunosorbent assay (ELISA). Here we ask whether currently available ELISAs have similar prognostic value.

Design Serum IL-21 from 141 individuals with relapsing remitting multiple sclerosis was measured using the now 'redundant' IL-21 ELISA and five further currently available kits. All patients had been treated with alemtuzumab; 61/141 had developed secondary autoimmunity.

Results The 'redundant kit', and one current kit, confirmed higher baseline serum IL-21 in patients with autoimmunity (542 pg/mL vs. 222 pg/mL and 53.1 pg/mL vs. 9.3 pg/mL respectively) and showed positive correlation. However, only the 'redundant' kit had predictive utility.

Conclusions Currently available IL-21 ELISA kits should not be used to counsel individuals with multiple sclerosis considering treatment with alemtuzumab.

Alemtuzumab has proven efficacy as a treatment for relapsing-remitting multiple sclerosis (MS). In a phase II trial, compared with interferon β-1a, alemtuzumab reduced the risk of relapse and sustained accumulation of disability by more than 70% at 3 years, with sustained efficacy at 5 years.[1 2] Two phase III trials (CARE-MS I and CARE-MS II) have confirmed its efficacy in treatment-naïve patients and established superiority over interferon β-1a in patients who continue to relapse despite first-line therapy.[3 4] In September 2013, it was licensed for active relapsing-remitting MS in Europe.

Alemtuzumab's primary safety concern is secondary autoimmunity, occurring up to 5 years after treatment[2] and maximally at 2 years: at least 30% of patients develop thyroid autoimmunity and 2% idiopathic thrombocytopenic purpura. We have previously reported that pretreatment serum interleukin-21 (IL-21) may predict post-treatment autoimmunity[5]; in a cohort of 59 patients, pretreatment serum IL-21 was more than twofold higher in the 32 who subsequently developed autoimmunity compared with those who did not (430 pg/mL vs 206 pg/mL; p=0.001). Using receiver operating characteristic (ROC) curve analysis, a pretreatment cut-off value of 210 pg/mL represented a sensitivity of 66% and specificity of 67%, with a positive predictive value (PPV) of 70% and a negative predictive value (NPV) of 62% for secondary autoimmune disease. Our findings were replicated by Cossburn *et al*[6] in an independent cohort of 62 alemtuzumab-treated patients, of whom 14 developed secondary autoimmunity.

A reliable biomarker for autoimmunity after alemtuzumab would provide personalised risk–benefit assessments, informing treatment choices and post-treatment monitoring. However, due to the desire to switch from ascitic antibody production to a more ethical cell-based technique, the ELISA used to generate our original data has been withdrawn. Here, we replicate our original findings using the 'redundant assay', then go on to show that no currently available assay matches its ability to predict autoimmunity after alemtuzumab.

MATERIALS AND METHODS

Subjects

All subjects had relapsing-remitting MS as defined by McDonald's criteria and received treatment with alemtuzumab at either Cambridge University Hospital or University Hospital of Wales (table 1). Alemtuzumab was given by intravenous infusion for 5 days (12–24 mg/day) followed by retreatment at 12 months. Further infusions were guided by clinical relapse and/or radiological evidence of disease activity. All patients consented to venesection for research purposes (CAMSAFE REC 11/33/0007; SE Wales REC 05/WSE03/111). For at least 3 years, patients had monthly full blood counts and 3-monthly thyroid function tests, anti-nuclear antibodies, serum creatinine and urinalysis (to screen for Goodpasture's). Secondary autoimmunity was defined as any new autoimmune disease after treatment, with or without autoantibodies. No autoimmunity was defined as the absence of secondary autoimmunity for at least 2 years after last treatment.

IL-21 immunoassays

IL-21 was measured using three Ready-Set-Go ELISA kits obtained from eBioscience: (i) 'Kit A', used until 2011 (catalogue: 88-7216; capture antibody 3A3-N2, detection antibody 2B2-G20—made by ascites); (ii) 'Kit B', used until it was discontinued in 2012 (catalogue number and clones as per kit A, but detection antibody made by cell culture rather than ascitic fluid production) and (iii) 'Kit C' (catalogue: 88-8218; capture antibody 1308/12F3, detection antibody 1227/14F10). In addition, we tested the following commercially available kits:

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Table 1 Summary of patient clinical and demographic data according to autoimmune cohort

	Secondary autoimmunity	No secondary autoimmunity
Gender, the female to male ratio	3:1	1.5:1
Pretreatment annualised relapse rate	Mean 1.8 Range 0.5–3.5	Mean 1.9 Range 1.0–4.5
Age at first treatment	Mean 31.2 years Range 17–48 years	Mean 32.9 years Range 17–62 years
Type of secondary autoimmune disease (% of sum patients)	Thyroid 35% ITP 1.4% Goodpasture's 0.7% Others* 1.4%	–
Number of treatment cycles before onset of autoimmunity	Median 2 Range 1–3	–
Time from baseline treatment to onset of autoimmunity	Mean 33 months Range 9–79 months	–

*Others: autoimmune hepatitis and anti-neutrophil antibody neutropaenia.
ITP, idiopathic thrombocytopenic purpura.

eBioscience human IL-21 Platinum ELISA (catalogue: BMS2014), BioLegend human IL-21 ELISA Max Deluxe (catalogue: 433804) and Promokine human IL-21 ELISA kit (catalogue: PK-EL-62921). All kits were used according to manufacturer's instructions and plates were read using a microplate reader (Bio-Rad model 680) at 450 nm and wavelength subtraction 570 nm. We classified 'detectable' IL-21 as above the lower limit of detection, defined as 3 SDs greater than blank samples; undetectable samples were assigned a value of zero for the purposes of calculating group values.

Statistical analyses

Data were analysed using GraphPad Prism5. Unpaired two-tailed Student t tests were performed to compare serum IL-21 levels in patients with and without autoimmunity. The results from different kits were compared by Spearman correlation coefficient assuming a non-parametric distribution. A p value of <0.05 was considered statistically significant. 95% CIs are reported for the calculated mean values.

RESULTS

IL-21 predicts autoimmunity; replication of original result using the redundant ELISA kit A

Pretreatment sera from 141 patients (90 Cambridge patients, 59 of whom have been reported,[5] and 51 Cardiff patients, some of whom were presented) were tested using kit A. IL-21 was twofold higher in 61/141 patients with secondary autoimmunity compared with their non-autoimmune counterparts (542.4 ±91.3 pg/mL vs 222.5±32.8 pg/mL, $p=5\times10^{-9}$; figure 1A). To investigate the utility of IL-21 in predicting secondary autoimmunity, a threshold concentration of 230 pg/mL was selected using ROC curve analysis; chosen in order to minimise false-negative results. 230 pg/mL yielded a sensitivity of 81%, specificity of 67%, PPV of 66% and NPV of 84% for secondary autoimmunity (figure 1B; area under curve 0.8043, p=<0.0001).

Subsequent and currently available IL-21 ELISA kits have no utility as predictive tests

Kit B consisted of the same antibody clones as in kit A, but the detection antibody was produced in a cell culture system. IL-21 was undetectable in 52/59 pretreatment samples assayed. The seven positive samples did not differentiate secondary autoimmunity (n=3 autoimmune vs 4 non-autoimmune, mean IL-21: 112.5±102.4 pg/mL vs 193.9±107.7 pg/mL, respectively; p=0.1283). Three positive samples had been tested using kit A, which yielded threefold higher concentrations (404.6 pg/mL vs 122.0 pg/mL for kit B). Kit B has been discontinued.

Kit C consisted of new capture and detection antibodies, both made by cell culture. IL-21 was detectable in a higher proportion of pretreatment samples from patients who developed secondary autoimmunity than non-autoimmune samples (22/25 vs 12/27, respectively, Fisher's exact test p<0.0001) and mean IL-21 was significantly higher in the autoimmune cohort; 53.07 ±41.83 pg/mL versus 9.31±7.52 pg/mL (p=0.032, figure 1C). This difference persisted on longitudinal analysis of 18 patients (seven autoimmune vs 11 non-autoimmune) at month 3 (75.9 pg/mL vs 21.7 pg/mL, p=0.0702), month 6 (103.4 pg/mL vs 17.6 pg/mL, p=0.0097) and month 12 (57.08 pg/mL vs 18.18 pg/mL, p=0.0437). 44/52 of the samples assayed using kit C had been tested using kit A. Of these, IL-21 was detectable in 44/44 samples using kit A versus 28/44 samples using kit C (Fisher's exact test p<0.0001). The readings obtained using kit A were significantly higher than those obtained using kit C (364.4±90.6 pg/mL vs 49.6±37.3 pg/mL). However, despite the 10-fold difference in concentration, results obtained from the two kits were positively correlated (r=0.6793, 95% CI 0.4005 to 0.8430, p=<0.0001, figure 1D).

Other kits: Further samples were assayed using three additional commercially available IL-21 ELISAs: (i) eBioscience human IL-21 Platinum ELISA, (ii) BioLegend human IL-21 ELISA Max Deluxe and (iii) Promokine human IL-21 ELISA kit. IL-21 was undetectable in 23/33, 36/53 and 33/33 samples, respectively, so further testing was curtailed.

DISCUSSION

As alemtuzumab enters routine clinical practice for relapsing-remitting MS in Europe, and potentially the USA, patients and physicians may seek to measure pretreatment serum IL-21 to personalise the risk of its most significant adverse effect, secondary autoimmunity, following our provisional findings.[5] Here, we replicate our original findings using an ELISA kit which has now been withdrawn in order to switch from ascitic to cell culture-based antibody production. However, currently available IL-21 kits have little or no utility in predicting autoimmunity and we strongly advise against their use in counselling patients considering treatment with alemtuzumab; in particular, a low or undetectable IL-21 may falsely reassure patients that their risk of autoimmunity is low.

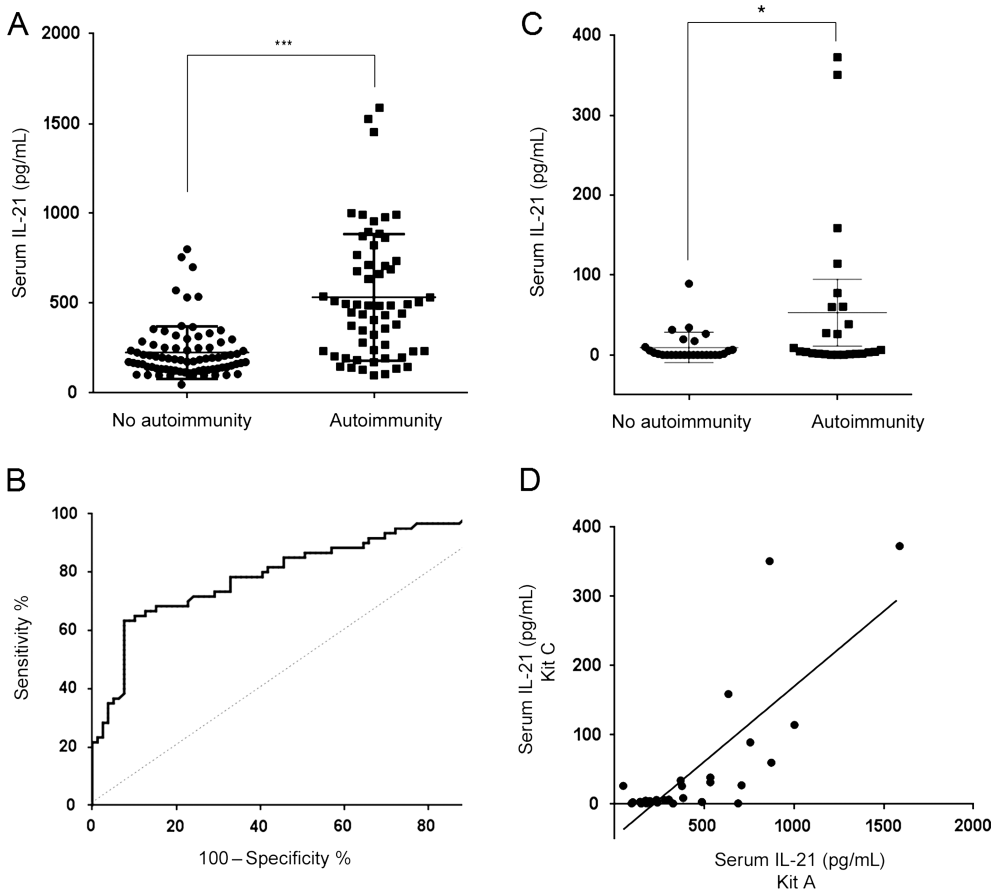

Figure 1 Pretreatment serum interleukin-21 (IL-21) is associated with development of secondary autoimmunity using kit A, but currently available IL-21 immunoassays are less able to detect serum IL-21 affecting the clinical predictive utility of the test. (A) Serum IL-21 levels before treatment with alemtuzumab are higher in patients who develop secondary autoimmunity. Samples were tested between 2009 and 2011 using eBioscience human IL-21 ELISA kit 'A' catalogue no. 88-7216, capture antibody 3A3-N2, detection antibody 2B2-G20. (B) Receiver operating characteristic curve analysis displaying the trade-off between sensitivity (true positive rate) and (100%–specificity) (false-positive rate). A cut-off value of 230 pg/mL gives a sensitivity of 81% and specificity of 67% for autoimmunity after alemtuzumab. (C) Serum IL-21 levels before treatment with alemtuzumab measured using the currently available IL-21 ELISA kit (kit C; catalogue no. 88-7216, purified antibody 1308/12F3, biotinylated antibody 1227/14F10) continue to demonstrate higher serum levels in patients who develop secondary autoimmunity. (D) Despite serum IL-21 values being 10-fold higher using kit A, results from kit C and kit A are positively correlated (Spearman r=0.6793, p=<0.0001).

Although there are several possible explanations for our findings, we postulate that the change in production of the detection antibody from ascites in kit A to a cell culture system in kit B resulted in loss of sensitivity to IL-21, perhaps because of differences in glycosylation patterns that influence the antigen-binding capacity of the antibody. The results from kit A positively correlate with those from kit C, which is made up of entirely different capture and detection antibodies, giving us confidence that they are detecting the same analyte. Furthermore, mean pretreatment IL-21 remains statistically different between those with and without secondary autoimmunity using kit C; however, with less spread between these groups, the kit has little utility as a predictive test. IL-21 remains an attractive candidate for autoimmunity; it is a member of the type 1 cytokine family with pleiotropic effects, including the expansion of cytotoxic T lymphocytes, inhibition of T regulatory cells and differentiation of B cells into immunoglobulin-producing plasma cells.[7–9] Moreover, IL-21 has been associated with various autoimmune conditions, including rheumatoid arthritis, inflammatory bowel disease and autoimmune diabetes.[10]

We conclude that IL-21 remains a potential biomarker for the development of autoimmunity after alemtuzumab treatment of MS; however, currently available IL-21 ELISA kits show little or no utility as predictive tests. Given the recent licensing of alemtuzumab in Europe, it is essential that treating neurologists are aware of this fact, so that clinicians and patients are not falsely reassured by low measured IL-21.

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Contributors LA collected and analysed data and wrote the manuscript. SAJT contributed to the collection and interpretation of data. KEH, MC and NR collected, analysed and interpreted data on the Cardiff cohort. AJC reviewed the manuscript. AJC and JLJ contributed to the conception and design of the study, data analysis and interpretation and writing of the manuscript.

Competing interests AJC is funded by the NIHR Cambridge Biomedical Research Centre. JLJ is a NIHR clinical lecturer.

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