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Pretreatment anti-thyroid autoantibodies indicate increased risk for thyroid autoimmunity secondary to alemtuzumab: A prospective cohort study

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ABSTRACT

Background: Alemtuzumab is approved for the treatment of active relapsing-remitting multiple sclerosis (RRMS). Alemtuzumab-related secondary autoimmune disorders (sAID) are common, with thyroid sAID being the most frequent, and fundamentally affect the risk-benefit ratio. Therefore, biomarkers indicating the development of sAID are urgently needed to instruct clinical decisions.
Methods: We evaluated whether the anti-thyroid autoantibodies (ThyAb) anti-thyroglobulin (anti-TG) and anti–thyroid-peroxidase (anti-TPO) detected at baseline by standard testing are able to indicate increased risk for thyroid sAID following alemtuzumab treatment in a multicentre prospective cohort of 106 alemtuzumab-treated RRMS patients. We here present an interim-analysis with a median follow-up of 36 months.
Findings: Baseline characteristics demonstrated no significant differences between patients with or without thyroid sAID. 29/106 (27·4%) patients developed thyroid sAID between 5 and 51 months following alemtuzumab treatment initiation. 14/29 patients (48·3%) were positive for ThyAb at baseline and developed thyroid sAID. Hazard ratio for time to thyroid autoimmunity was 12.15 (95% CI 4.73–31.2) indicating a highly increased risk for ThyAb positive patients. Baseline ThyAb were associated with shorter time to sAID, but not with a specific disease entity of thyroid sAID. Hazard ratios for age, sex, previous treatment, disease duration, disability and smoking status demonstrated no significant association with thyroid autoimmunity.
Interpretation: Standard ThyAb-testing for anti-TPO and anti-TG antibodies at baseline was able to indicate increased risk for clinically manifest thyroid sAID and should therefore be used in clinical decisions concerning alemtuzumab treatment initiation.
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1. Introduction

Alemtuzumab is used for the treatment of active relapsing-remitting multiple sclerosis (RRMS) and depletes B- and T-lymphocytes followed by a prolonged repopulation phase with profound quantitative and qualitative changes within immunological networks. Secondary autoimmune disorders (sAID) following alemtuzumab treatment are common and fundamentally affect the risk-benefit ratio and clinical decisions [1]. Hence, there is an urgent need for biomarkers instructing risk-estimation for sAID. Thyroid sAID are the most common and affects approximately 38% to 41% of alemtuzumab-treated patients peaking in year 3 after therapy initiation [2–4].

The most frequent alemtuzumab-associated sAID are mainly B-cell- and autoantibody-driven [1]. Therefore, it is currently believed that the disproportionately high B-cell over T-cell recovery leads to unregulated expansion of autoreactive B cells and consequently to sAID. However, alopecia, haemophagocytic syndrome, sarcoidosis and vitiligo after alemtuzumab infusion, which are all T cell-mediated, have been reported and hint to a more complex pathogenesis [5–8]. Previously, serum IL-21 concentrations have been shown to correlate with the incidence of sAID after alemtuzumab and were proposed as potential biomarker [9]. However, the findings have not been substantiated in

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Research in context

Evidence before this study

Secondary autoimmune disorders (sAID) are the most relevant risk related to alemtuzumab treatment in RRMS patients and thyroid sAID are by far the most common. However, biomarkers indicating increased risk for sAID in clinical routine are currently missing. We searched PUBMED, SCOPUS and COCHRANE databases from inception until May 21st 2019. In addition, ECTRIMS and ACTRIMS conference abstracts as well as reference lists of published articles were screened for further articles to be included. We combined search terms for multiple sclerosis, alemtuzumab, secondary autoimmune disorders, thyroid autoantibodies and thyroid autoimmunity. There were no language restrictions.
IL-21 serum levels were proposed as a biomarker for the risk of developing autoimmunity post alemtuzumab, however currently available detection kits show no predictive utility. A retrospective case-control-study demonstrated that anti-thyroid autoantibodies (ThyAb) precede the development of clinical overt thyroid autoimmune disorders in putative healthy controls. Two retrospective analyses of the CAMMS223 and a Welsh cohort support the indicative value of ThyAb for alemtuzumab-related thyroid sAID. However, data from prospective studies are missing.

Added value of this study

To our knowledge, this is the first study providing evidence for ThyAb detected by standard-testing as valuable tool for risk-estimation prior to alemtuzumab therapy from a prospective, multicentre observational study cohort. We found that anti-thyroglobulin (anti-TG) and anti–thyroid-peroxidase (anti-TPO) autoantibodies yielded good to strong performance for risk-estimation. ThyAb positivity was associated with a highly increased risk for and a shorter time to thyroid sAID.

Implications of all the available evidence

Thyroid sAID following alemtuzumab is often delayed by several years and the ability to reliably estimate the risk of individual patients would facilitate risk-benefit considerations in clinical decisions and allow for targeted monitoring as well as possibly early intervention in alemtuzumab-treated patients. In female patients with active family planning this might be of special relevance since thyroid disorders display a particular risk. Since the ThyAb testing applied in this study is readily available at most clinics in the world, this study can immediately instruct and improve clinical practice. However, positive ThyAb at baseline should not preclude alemtuzumab treatment per se, but instruct the informed consent building process before treatment initiation.

larger prospective cohorts and currently available IL-21 ELISA kits were not able to predict sAID after alemtuzumab [10]. A recent small retrospective study using custom-made high-sensitivity anti-TSHR (anti-thyroidea-stimulating-hormone-receptor) testing demonstrated that baseline measurement of anti-TSHR and anti–thyroid-peroxidase (anti-TPO) autoantibodies indicated increased risk for thyroid sAID following alemtuzumab treatment [11]. However, data from prospective studies evaluating the prognostic value of anti-thyroid autoantibodies (ThyAb) for alemtuzumab-related thyroid sAID have been missing so far.

We therefore evaluated whether baseline ThyAb including anti-thyroglobulin (anti-TG) and anti-TPO by standard tests are able to instruct risk-assessment for thyroid sAID in a prospective cohort of 106 alemtuzumab treated patients.

2. Materials and methods

2.1. Study design and participants

All patients were recruited at the Departments of Neurology at the University Hospital Münster (59 patients), University Hospital Essen (28 patients) and the Clinics Osnabrück (19 patients) in Germany. Patients were recruited starting from 1st April 2014 to 1st October 2018. All patients who received alemtuzumab were approached to participate in the study and 106 of 128 (83%) patients agreed to participate. Alemtuzmab patients received pre-treatments including azathioprine, beta interferons, glatiramer acetate, teriflunomide, fingolimod, natalizumab, mitoxantron, and siponimod (within a clinical trial). All patients were included in our prospective PROGRAM^{MS} ("Signatures of immune reprogramming in anti-CD52 therapy of MS: markers for risk stratification and treatment response") cohort study. Here, detailed clinical and MRI data are collected in combination with blood samples following established SOPs developed within the KKNMS (German competence network for multiple sclerosis). Patients are evaluated at baseline (before first course of alemtuzumab) and every 3 months thereafter including standardized neurological examination, assessments (e.g. EDSS, MSFC) and additional laboratory testing for a study duration of five years. In the current study anti-TG and anti-TPO are measured at baseline and thyroid sAID is monitored prospectively by clinical and laboratory assessments.

Alemtuzumab treatment and monitoring (creatinine, blood count and urine testing monthly and TSH every 3 months) was conducted according to summary of product characteristics (SmPC).

In case of clinical or laboratory signs indicating thyroid sAID patients were referred to a specialized endocrinologist. Diagnosis of Graves' disease was defined by anti-TSHR positivity and hyperthyroidism/hypothyroidism; autoimmune thyroiditis was defined by anti-TPO positivity and hypothyroidism (including cases with transient hyperthyroidism).

None of our patients showed (clinical or laboratory) signs of thyroid dysfunction before alemtuzumab initiation, and all were negative for anti-TSHR.

This study was performed according to the Declaration of Helsinki and approved by the local ethics committee of the University of Münster (Ethik-Kommission der Ärztekammer Westfalen-Lippe und der Westfälischen Wilhelms-Universität Münster, 2014-398-f-S). All patients gave written informed consent. The manuscript conforms to the STROBE reporting standards for cohort studies.

2.2. Detection of anti-thyroid autoantibodies

ThyAb were detected by ECLIA (ElectroChemiLumineszenzImmunAssay) on an automated Cobas® e801 analyzer (Roche) used in clinical routine in a blinded manner and in an independent certified laboratory. Analysis was performed using specific kits and following the manufacturer's instructions for anti-TG (Elecsys Anti-Tg, Roche; cut off >15 IU/ml), anti-TPO (Elecsys Anti-TPO, Roche; cut off 15 IU/ml) and anti-TSHR (Elecsys Anti-TSHR, Roche; cut off >1·1 IU/ml) testing.

2.3. Statistical analysis

Statistical analysis was performed using R 3.6.1. To determine development of thyroid sAID over time, we performed Cox proportional hazards model with the R packages survival and survminer [12]. First, we used a univariate Cox regression to evaluate the hazards over time for each thyroid antibody separately and combined. Next, we used a multivariate cox regression to adjust for possible confounders including sex, age, disease duration, number of previous DMT, type of last DMT,

baseline disability (EDSS) and smoking history. Statistical significance of each variable was evaluated by the Wald statistic value and the predictive value by Harrell's c statistic (concordance index). The global *p*-value was determined by the likelihood ratio test. Statistical significance between survival curves was tested by pairwise log rank test adjusted for multiple testing by Benjamini-Hochberg's false discovery rate correction. A *p*-value below 0·05 was considered statistically significant.

3. Results

We here present data of a safety-concern triggered interim analysis with a median follow-up of 36 months. Baseline characteristics demonstrated no significant differences between patients with or without thyroid sAID (Table 1). 46/75 (61·3%) of patients without sAID and 21/29 (72·4%) with thyroid sAID were female. Patients in the no sAID versus the thyroid sAID group had a median age of 35 versus 33 years at alemtuzumab initiation, a median disease duration of 5 versus 6 years and both had a median of 2 previous disease-modifying therapies (DMTs). No DMT was found at significantly higher frequencies in those groups. The proportions of patients with higher disability (EDSS step ≥3) and history of smoking were comparable.

31/106 (29·2%) patients developed sAID between 5 and 51 months following treatment initiation. While 29 patients presented with thyroid sAID, two patients developed non-thyroid sAID (vitiligo and immune-thrombocytopenia, respectively) and were therefore excluded from the analysis.

14/29 patients (48·3%) with thyroid sAID were positive for ThyAb at baseline (4× anti-TG, 3× anti-TPO, and 7× anti-TG + anti-TPO), whereas only 4/75 patients (5·3%) without sAID were ThyAb positive (Table 1) so far. Autoantibody titres seemed to be higher in the thyroid sAID group however not reaching statistical significance. A multivariate Cox regression for time to thyroid sAID showed no significant coefficients for sex, age, disability, number and type of previous DMT, disease duration and smoking history (Fig. 1a–c) suggesting negligible impact on thyroid sAID development. In contrast, anti-TG and anti-TPO or both combined had statistically significant coefficients in the multivariate Cox regression. We evaluated each antibody separately and then combined. Performance of anti-TPO (hazard ratio 12.52, 95% CI 5.15–30.4) suggested slightly superior performance to anti-TG (hazard ratio 7.23, 95% CI 2.88–18.1) without reaching statistical significance (Fig. 1a–c). The combination of anti-TPO and anti-TG (hazard ratio 12.15, 95% CI 4.73–31.2) yielded almost similar performance as anti-TPO only. The predictive ability of the multivariate Cox regression model with anti-TG (concordance index 0.71), was slightly lower than anti-TPO (concordance index 0.74) and the combination of both (concordance index 0.76), however indicating overall good to strong models. Comparing the survival plots of each antibody set showed a statistical significance between anti-TG and/or anti-TPO presence and the absence of both, but no statistical significance between all other possible comparisons (Fig. 1d).

ThyAb positivity was not associated with a specific disease entity of thyroid sAID, 14 patients developed autoimmune thyroiditis and 15 Graves' disease. 6/14 (42·9%) with baseline ThyAb positivity versus 4/15 (26·7%) without needed radioiodine therapy and/or thyroidectomy for successful treatment, however not reaching statistical significance. Remarkably, baseline ThyAbs were associated with shorter time to sAID (Table 2).

Table 1
Patient baseline characteristics.

	No sAID (n = 75)	*Thyroid sAID (n = 29)*	*p*
Female patients, No (%)	*46 (61.3)*	*21 (72.4)*	*0.36*[b]
Age at baseline, years, median (IQR)	*35 (29–43)*	*33 (27.5–39)*	*0.52*[a]
Disease duration, years, median (IQR)	*5 (2−10)*	*6 (4–11)*	*0.1*[a]
Previous disease-modifying therapies, No, median (IQR)	*2 (1–3)*	*2 (2–4)*	*0.15*[a]
EDSS step ≥ 3 at baseline, No (%)	*36 (48)*	*15 (51.7)*	*0.83*[b]
Months of follow-up, median (IQR)	*35 (25–44)*	*37 (32.5–52)*	*0.06*[a]
History of smoking, No (%)	*18 (24)*	*8 (27.5)*	*0.8*[b]
Last previous DMT, No (%)			*0.28*[b]
Naïve	*13 (17.3)*	*2 (6.9)*	
Natalizumab (escalation)	*29 (38.7)*	*6 (20.7)*	
Fingolimod (escalation)	*8 (10.7)*	*11 (37.9)*	
Dimethyl fumarate (basic)	*9 (12)*	*2 (6.9)*	
Interferon-beta (basic)	*8 (10.7)*	*4 (13.8)*	
Glatiramer acetate (basic)	*2 (2.7)*	*3 (10.3)*	
Teriflunomide (basic)	*3 (4)*	*0 (0)*	
Other	*3 (4)*	*1 (3.4)*	
ThyAb serostatus at baseline, No (%)			*<0.001*[b]
TPO−; TG−	*71 (94.7)*	*15 (51.7)*	
TPO+; TG−	*1 (1.3)*	*3 (10.3)*	
TPO−; TG+	*3 (4.0)*	*4 (13.8)*	
TPO+; TG+	*0 (0.0)*	*7 (24.1)*	
Titer level in ThyAb + patients, IU/mL, median (IQR)			
Anti-TPO	*61.1*	*75.3 (60–1064)*	*n/a*
Anti-TG	*47.8 (37.1–65.2)*	*84.1 (72.1–139)*	*0.09*

No: number; IQR: interquartile range; EDSS: expanded disability status scale; DMT: disease-modifying treatment.
[a] Significance evaluated using Mann-Whitney test.
[b] Significance evaluated using Fisher's exact test.

4. Discussion

sAID are common and the most relevant risk associated with alemtuzumab treatment in RRMS patients [1]. Therefore, biomarkers instructing risk-assessment for sAID are a big unmet need in clinical practice for alemtuzumab-related treatment decisions. This safety-triggered interim analysis of a multicentre, prospective cohort of alemtuzumab treated RRMS patients provides evidence that standard ThyAb-testing of anti-TPO and anti-TG antibodies at baseline is able to indicate increased risk for clinically manifest thyroid sAID. ThyAb positivity was not associated with a specific disease entity of thyroid sAID, but with shorter time to sAID.

Consistently, thyroid autoantibodies have been associated with hypothyroidism and autoimmune thyroid disease in individuals with a positive family history for these disorders [13,14]. Moreover, a previous retrospective case-control study of 522 female US military personnel described that anti-TG, anti-TPO, and anti-TSHR precede the development of spontaneous thyroid autoimmunity and reported odds ratios between 4·6 and 25 two to five years prior diagnosis [15]. However, these studies were small, retrospective in design or in case of the study on female US military personnel comprised a highly selected population. Therefore, no sufficient data exist allowing for a direct comparison of risk associations between our data and the general population. However, a large Canadian cohort comparing 4192 MS patients with 20,940 healthy controls has shown comparable incidence (422·8 vs. 407·7 per 100,000 persons/year) and prevalence (9·51% vs. 8·56%) of autoimmune thyroid disease [16]. Of note, the prevalence of thyroid autoimmunity in alemtuzumab-treated MS patients accounts for 38–41% as stated above [3]. Therefore, alemtuzumab seems to be related to a highly increased risk for thyroid autoimmunity with fundamental pathophysiological differences compared to the natural history.

Concerning alemtuzumab, two retrospective analyses evaluated the risk-indicative value of ThyAb prior to alemtuzumab treatment for the development of thyroid sAID. The analysis of the phase II CAMMS223 patient cohort (median follow-up of 57·3 months) demonstrated anti-TPO positivity in patients with thyroid sAID at baseline, in contrast to our cohort, only in 11/73 patients (15%) [17]. However, in this study only anti-TPO were measured and patients included were treatment-naïve with disease duration lower than 36 months, potentially

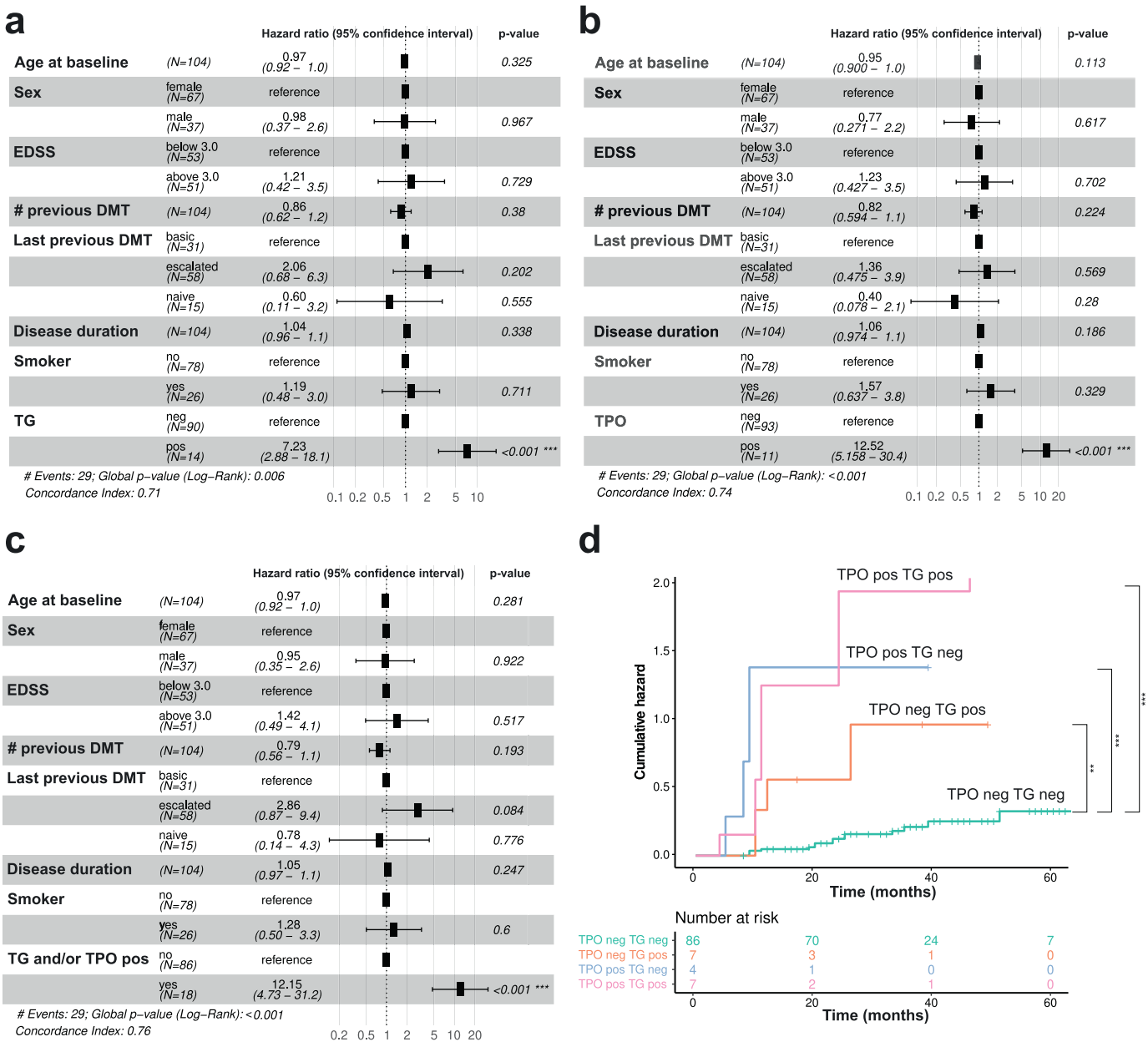

Fig. 1. Thyroid autoantibodies at baseline indicate increased risk for thyroid secondary autoimmunity following alemtuzumab treatment. Multivariate Cox proportional hazards model: forests plots of hazard ratios for time to thyroid sAID for age, sex, disability, number and type of previous disease modifying therapies (DMT), disease duration, smoking history and either (a) anti-TG (TG), (b) anti-TPO (TPO) or (c) both combined. (d) Survival plot displaying the cumulative hazard and patients at risk for time to thyroid sAID for each set of antibody. * $p < .05$, ** $p < .01$, *** $p < .001$. EDSS: expanded disability status scale.

Table 2
Thyroid sAID patient characteristics stratified according to baseline antibody status.

	$ThyAb^+$	$ThyAb^-$	p
Disease entity			0.715[b]
Autoimmune thyroiditis	6	8	
Graves' disease	8	7	
Treatment required			0.304[b]
Levothyroxine	6	8	
Levothyroxine + TPOI	2	3	
Levothyroxine + TPOI + RIT	2	1	
Levothyroxine + TPOI + surgery	4	3	
Onset from baseline, months (IQR)	10 (8.75–15)	23 (11—33)	0.041[a]

TPOI: thyroperoxidase inhibitor; RIT: radioiodine therapy.
[a] Significance evaluated using Mann-Whitney test.
[b] Significance evaluated using Fisher's exact test.

explaining the observed differences. In our cohort only 14% of patients were treatment naïve before alemtuzumab treatment, which might be a better representation of the current alemtuzumab prescription strategies in real-life [18].

In another smaller, retrospective cohort study using custom-made high-sensitivity anti-TSHR testing at baseline, anti-TSHR were present in 5/16 (31%) patients with thyroid sAID and in 0/14 (0%) without, supporting the indicative value of ThyAb at baseline [11]. In contrast, we used a standard electrochemiluminescence assay common in clinical routine. Moreover, anti-TSHR positive patients were excluded from the analysis since anti-TSHR are causatively involved in the pathogenesis of Graves' disease therefore precluding inclusion in our study [14]. However, implementation of high-sensitivity anti-TSHR testing might further improve the value of baseline ThyAb for risk-estimation.

Remarkably, a higher proportion of patients needing radioiodine therapy and/or thyroidectomy for successful treatment were ThyAb

positive at baseline potentially indicating a more severe disease course. However, differences were not statistical significant and need to be substantiated in larger studies or in the final analysis of our cohort.

Of note, thyroid disease represents particular risks in women who are pregnant and therefore prediction of thyroid sAID might especially instruct clinical decisions concerning alemtuzumab treatment in women with active family planning [19].

Strengths of our study are the prospective design and the use of standard ThyAb testing. Of note, the interim analysis, the relatively small cohort with only 29 positive thyroid sAID cases (as per the exploratory nature of the study) and potential centre effects might be limitations that should be considered for data interpretation. These limitations might also affect generalisability of our data, however real-life and trial cohorts of alemtuzumab-treated RRMS patients share many characteristics of our cohort [4,18]. Since autoimmune thyroid disorders have been diagnosed >7 years following the first alemtuzumab course our analysis might have missed further thyroid sAID cases becoming clinically manifest in the future [20].

Model performance of anti-TPO suggested slightly superior performance compared to anti-TG and the combination of anti-TPO and anti-TG yielded almost similar performance as anti-TPO only. However, since 4/29 (13·8%) patients developing thyroid autoimmunity were only anti-TG positive, we suggest a pragmatic approach testing both autoantibodies in clinical practice.

In conclusion, standard ThyAb-testing of anti-TPO and anti-TG at baseline was able to indicate increased risk for clinically manifest thyroid sAID and is therefore a valuable tool to facilitate risk-benefit considerations before alemtuzumab treatment initiation.

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Author contributions

TR: design and concept of study, analysis and interpretation of data, writing of the manuscript. ASM: analysis, and interpretation of data, writing of the manuscript. SP: analysis and interpretation of data, critical revision of manuscript for important intellectual content. MH: critical revision of manuscript for intellectual important content and further statistical analysis of data. LK: critical revision of manuscript for intellectual important content. SW + CK patient recruitment, critical revision of manuscript for important intellectual content. CCG: study concept, interpretation of data, and critical revision of manuscript for intellectual content. HW + SGM: study concept and design, study supervision and critical revision of manuscript for intellectual content. All authors read and approved the final version of the manuscript.

Declaration of Competing Interest

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References

[1] Ruck T, Bittner S, Wiendl H, Meuth SG. Alemtuzumab in multiple sclerosis: mechanism of action and beyond. Int J Mol Sci 2015;16(7):16414–39.
[2] Havrdova E, Arnold DL, Cohen JA, et al. Alemtuzumab CARE-MS I 5-year follow-up: durable efficacy in the absence of continuous MS therapy. Neurology 2017;89(11):1107–16.
[3] Tuohy O, Costelloe L, Hill-Cawthorne G, et al. Alemtuzumab treatment of multiple sclerosis: long-term safety and efficacy. J Neurol Neurosurg Psychiatry 2015;86(2):208–15.
[4] Coles AJ, Cohen JA, Fox EJ, et al. Alemtuzumab CARE-MS II 5-year follow-up: efficacy and safety findings. Neurology 2017;89(11):1117–26.
[5] Pfeuffer S. Sarcoidosis following alemtuzumab treatment: autoimmunity mediated by T cells and interferon-gamma. Mult Scler 2018;24(13):1783–4.
[6] Zimmermann J, Buhl T, Muller M. Alopecia Universalis following alemtuzumab treatment in multiple sclerosis: a barely recognized manifestation of secondary autoimmunity-report of a case and review of the literature. Front Neurol 2017;8:569.
[7] Saarela M, Senthil K, Jones J, et al. Hemophagocytic lymphohistiocytosis in 2 patients with multiple sclerosis treated with alemtuzumab. Neurology 2018;90(18):849–51.
[8] Ruck T, Pfeuffer S, Schulte-Mecklenbeck A, et al. Vitiligo after alemtuzumab treatment: secondary autoimmunity is not all about B cells. Neurology 2018;91(24)(e2233-e7).
[9] Jones JL, Phuah CL, Cox AL, et al. IL-21 drives secondary autoimmunity in patients with multiple sclerosis, following therapeutic lymphocyte depletion with alemtuzumab (Campath-1H). J Clin Invest 2009;119(7):2052–61.
[10] Azzopardi L, Thompson SA, Harding KE, et al. Predicting autoimmunity after alemtuzumab treatment of multiple sclerosis. J Neurol Neurosurg Psychiatry 2014;85(7):795–8.
[11] Muller I, Willis M, Healy S, et al. Longitudinal characterization of autoantibodies to the thyrotropin receptor (TRAb) during alemtuzumab therapy: evidence that

TRAb may precede thyroid dysfunction by many years. Thyroid 2018;28(12):1682–93.
[12] Therneau TM, Grambsch PM. Modeling survival data: extending the Cox model. New York: Springer; 2000.
[13] Vanderpump MP, Tunbridge WM, French JM, et al. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham survey. Clin Endocrinol (Oxf) 1995;43(1):55–68.
[14] Effraimidis G, Strieder TG, Tijssen JG, Wiersinga WM. Natural history of the transition from euthyroidism to overt autoimmune hypo- or hyperthyroidism: a prospective study. Eur J Endocrinol 2011;164(1):107–13.
[15] Hutfless S, Matos P, Talor MV, Caturegli P, Rose NR. Significance of prediagnostic thyroid antibodies in women with autoimmune thyroid disease. J Clin Endocrinol Metab 2011;96(9):E1466–71.
[16] Marrie RA, Yu BN, Leung S, et al. The incidence and prevalence of thyroid disease do not differ in the multiple sclerosis and general populations: a validation study using administrative data. Neuroepidemiology 2012;39(2):135–42.
[17] Daniels GH, Vladic A, Brinar V, et al. Alemtuzumab-related thyroid dysfunction in a phase 2 trial of patients with relapsing-remitting multiple sclerosis. J Clin Endocrinol Metab 2014;99(1):80–9.
[18] Frau J, Coghe G, Lorefice L, Fenu G, Musu L, Cocco E. Efficacy and safety of alemtuzumab in a real-life cohort of patients with multiple sclerosis. J Neurol 2019;266(6):1405–11.
[19] Yalamanchi S, Cooper DS. Thyroid disorders in pregnancy. Curr Opin Obstet Gynecol 2015;27(6):406–15.
[20] Twyman C, Oyuela P, Palmer J, Margolin D, Dayan C, et al. Neurology 2014;82(10 Supplement) (P2.199).