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29 **ABSTRACT**

30 **Context:** Alemtuzumab, a highly effective treatment for multiple sclerosis (MS), predisposes to  
31 Graves' disease (GD) with a reportedly indolent course.

32 **Objective:** To determine the type, frequency and course of thyroid dysfunction (TD) in a cohort of  
33 alemtuzumab-treated MS patients in the UK.

34 **Design:** Case records of alemtuzumab-treated patients who developed TD were reviewed.

35 **Results:** 41.1% (102/248; 80F, 22M) of patients developed TD, principally GD (71.6%). Median  
36 onset was 17 months (range 2-107) following last dose; the majority (89%) within 3 years. Follow-up  
37 data (range 6-251 months) was available in 71 cases, of whom 52 (73.2%) developed GD: 10 of these  
38 (19.2%) had fluctuating TD. All 52 GD patients commenced anti-thyroid drugs (ATD): 3 required  
39 radioiodine (RAI) due to ATD side-effects, drug therapy is ongoing in 2; of those who completed a  
40 course, 16 are in remission, 1 developed spontaneous hypothyroidism, and 30 (64%) required  
41 definitive or long-term treatment (RAI n=17, thyroidectomy n=5, long-term ATDs n=8). 3 cases of  
42 thyroiditis and 16 cases of hypothyroidism were documented; 5 with anti-TPO antibody positivity  
43 only, 10 with positive TRAb, 1 hypothyroidism (uncertain aetiology). Bioassay confirmed both  
44 stimulating and blocking TRAb in a subset of fluctuating GD cases.

45 **Conclusions:** Contrary to published literature, we have recorded frequent occurrence of GD that  
46 required definitive or prolonged antithyroid drug treatment. Furthermore, fluctuating thyroid status in  
47 GD and unexpectedly high frequency of TRAb-positive hypothyroidism suggested changing activity  
48 of TRAb in this clinical context; we have documented the existence of both blocking and stimulating  
49 TRAb in these patients.

50

51 **Key terms:** Alemtuzumab, Graves' disease, Drug-induced thyroid disease

52

## 53 INTRODUCTION

54 Alemtuzumab, a monoclonal antibody that binds CD52, a membrane glycoprotein on T and B  
55 lymphocytes and monocytes, leads to lysis and depletion of CD52+ cells (1). Its therapeutic effect is  
56 mediated by the alteration in immune repertoire that accompanies subsequent lymphocyte  
57 reconstitution (2). Alemtuzumab decreases relapse rate and disability progression in relapsing remitting  
58 multiple sclerosis (RRMS), either in treatment naïve patients (3), or in patients previously treated with  
59 interferon beta or glatiramer (4). Given its proven efficacy, alemtuzumab has been licensed for the  
60 treatment of RRMS in many regions, including the US and EU. It is administered intravenously, with  
61 treatment usually consisting of two courses; 12mg/day for five consecutive days, followed by the same  
62 dose for three consecutive days 12 months later. Additional treatment courses may be considered.

63 The principal adverse effect of alemtuzumab is development of autoimmunity, occurring most  
64 frequently at 16 months following last date of drug administration (5). Thyroid autoimmunity is most  
65 common, with most studies reporting its occurrence in 17 to 34% of patients (41% in one study, 5).  
66 Graves' disease (GD), occurring in 60-70% of cases, comprises the commonest cause of thyroid  
67 dysfunction (5, 6, 7). It has been suggested that individual risk is modified by smoking (3 fold greater  
68 risk) and family history (7 fold greater risk, 8); the role of gender is uncertain, with studies suggesting  
69 no difference (8) or doubling of risk in females (9). Total alemtuzumab dosage and frequency of  
70 intervals between treatments do not appear to influence development of autoimmunity (8). The  
71 mechanism of alemtuzumab-induced autoimmunity is not fully understood, but has been attributed to a  
72 breakdown in self-tolerance during immune reconstitution post alemtuzumab, with homeostatically  
73 expanding autoreactive T cells driving a humoral autoimmune response (10). Autoimmunity is also a  
74 recognised phenomenon following immune reconstitution in other contexts including bone marrow  
75 transplantation (11) or HIV antiretroviral therapy (12, 13); moreover, GD is the commonest form of  
76 TD seen during recovery from lymphopaenia (14).

77 The course of alemtuzumab-induced thyroid disease is not well described, but reports suggest that GD  
78 occurring in this context may be less aggressive than the conventional disorder. In one case series  
79 (n=31 GD), definitive treatment following failed response to anti-thyroid drug (ATD) therapy was only

80 required in 26%, compared to ~50% in conventional GD (8). Detailed analysis of TD in a large, phase  
81 2 clinical trial showed that 23% of alemtuzumab-induced GD patients became euthyroid spontaneously,  
82 15% developed hypothyroidism, with only 36% requiring **radioiodine** (RAI) or surgery (7, 9). In a  
83 subsequent phase 3 trial, only 2 out of 28 hyperthyroid patients required RAI or surgery (15); in  
84 another observational study 17 of the 22 patients with GD responded to drug therapy with only 3 cases  
85 requiring RAI (5). In contrast, anecdotal case reports suggest a poor response to anti-thyroid drug  
86 therapy (16) and GD with a fluctuating and unpredictable course has been noted (9).  
87 Here, in one of the largest case series of alemtuzumab-induced TD, followed for over 20 years, we  
88 have documented the type, frequency and course of alemtuzumab-induced thyroid dysfunction and  
89 determined whether response to treatment differs compared to that reported in conventional thyroid  
90 disease.

91

## 92 **SUBJECTS AND METHODS**

93 We undertook detailed **analysis** of all MS patients who developed TD after **treatment with**  
94 **alemtuzumab in clinical trials prior to its licensing** at Addenbrooke's Hospital in Cambridge and  
95 University Hospital Wales in Cardiff over a 20 year period (1993 to 2013).  
96 Alemtuzumab was administered intravenously on consecutive days for one or more cycles (five  
97 consecutive days for the first cycle; three consecutive days for subsequent cycles). The initial dose  
98 (20mg/day) was increased to 24mg/day in 2003 following a change in supplier. From 2006, the dose  
99 was reduced to 12mg/day to conform with the phase III study protocol. All patients receiving  
100 alemtuzumab at either centre had baseline thyroid function tests (TSH, FT4) prior to commencement of  
101 the drug, with TSH, FT4 and anti-thyroid peroxidase (TPO) antibody measurement 3 monthly for 2  
102 years, 6 monthly for 2 years and then annually, or sooner if symptoms of TD developed. At each time-  
103 point patients also underwent clinical review including **directed enquiry for** thyroid-related symptoms.  
104 One patient with pre-existing thyroid disease (**in her past medical history**), was excluded from this  
105 analysis.  
106 All patients who developed thyroid dysfunction (defined below) underwent evaluation by an

107 endocrinologist. We have reviewed clinical features at presentation, all thyroid function test and  
108 autoantibody data and management, including response to treatment. The bioactivity of anti-TSH  
109 receptor antibody (TRAb) was measured in a subset of patients with fluctuating Graves' disease (as  
110 defined below).

111

## 112 **Laboratory measurements**

113 Serum free T4, free T3 and TSH were measured using automated immunoassay systems (Advia  
114 Centaur, Siemens in Cambridge throughout and in Cardiff until 2010, with Abbott Architect thereafter).  
115 In Cambridge, TRAb was measured initially using a first generation ELISA assay, then Brahms  
116 Lumitest TRAK assay (RR 1-2 IU/L equivocal, >2 positive) from 2002. Cardiff also used the Brahms  
117 Lumitest TRAK assay (1-1.5 IU/L borderline, >1.5IU/L positive), changing to the Roche Cobas assay  
118 (RR 0.9-1.6 IU/L borderline, >1.6 IU/L positive) in 2014. Note that, as the upper, accurately  
119 quantifiable, limit of these assays is 40 IU/L (with levels greater than this being reported as ">40"  
120 IU/L), in calculations for this study we have used 40 whenever TRAb levels of >40 were reported.  
121 Information from Thermoscientific confirms that human TSH does not interfere with TRAb  
122 measurement in the lumitest TRAK assay, up to TSH values of at least 500mU/L.  
123 Anti-TPO antibody was measured using various assays over the study period: in Cambridge Serodia  
124 agglutination assay (positive or negative) up to 2002, then Phadia ELISA (RR <100 iu/ml) until 2007  
125 and Phadia immunocap (RR <100 iu/ml) till 2014 and then Siemens Centaur (RR <60 iu/ml)] until the  
126 present; in Cardiff the Advia Centaur assay (RR <60 kU/L) until 2010 then the Abbott Architect (RR  
127 <6 U/ml) until the present.  
128 The bioactivity of anti-TSH receptor antibodies (TRAb) was measured using a Chinese Hamster Ovary  
129 (CHO) cell line stably transfected with the human TSH receptor and a cAMP responsive luciferase  
130 reporter, classifying them into receptor stimulating (TSAb) or blocking (TBAb) activities, as described  
131 previously (17, 18).

132

## 133 **Definitions of Thyroid dysfunction**

- 134 • Thyroid dysfunction (TD): abnormal TSH on two or more occasions at least 3 months apart.
- 135 • Graves' disease (GD): hyperthyroidism (low TSH +/- elevated FT4) with positive TRAb
- 136 and/or increased tracer uptake (>1.5%) on technetium scan.
- 137 • Hashimoto's thyroiditis (HT): raised TSH with positive anti-TPO antibody and negative TRAb.
- 138 • Thyroiditis: thyrotoxicosis followed by spontaneous euthyroidism or hypothyroidism, with
- 139 negative TRAb and/or reduced or absent tracer uptake on technetium scan.
- 140 • Fluctuating GD: GD with unexpected fluctuations from hyper- to hypothyroidism (or vice
- 141 versa), which could not be explained by omission or changes in therapy.
- 142 • TRAB positive hypothyroidism: raised TSH with positive TRAb (+/- positive anti-TPO
- 143 antibody).

144

## 145 **RESULTS**

146 From May 1993 to October 2013 249 patients received at least one course of alemtuzumab therapy for  
147 MS in Cambridge and Cardiff. Following this, new TD was diagnosed in 102/248 (41.1%) of patients.  
148 Detailed follow up data (mean 67 months, range 6-251 months) was available in 71 of these cases  
149 (Figure 1).

### 150 **Patient Characteristics**

151 The age range of patients (n=102) was between 20 and 60 years (mean 37.6 years), with a  
152 preponderance of females (female n=80 (78%); male n=22 (22%); female to male ratio 3.6:1) (Table  
153 1a). Most patients received more than one course of alemtuzumab treatment [Courses (Number of  
154 Patients): 1 (n=10); 2 (n=55); 3(n=25); 4 (n=10); 5 (n=2)]. 34 (46.6%) patients had not received other  
155 therapy prior to alemtuzumab, 39 (53.4%) had received other therapies (usually steroids, IFN-beta or  
156 glatiramer), with no prior treatment information in 29 cases. With the exception of a single case in  
157 which IFN-beta was commenced four months before, none of the patients had received  
158 immunomodulatory therapy within one year prior to onset of TD. Anti-TPO antibody levels were  
159 checked prior to alemtuzumab in 50 patients, being negative in 42 and positive (mostly weakly) in 8  
160 cases; TRAb levels were not tested prior to alemtuzumab.

161

## 162 **Characteristics of Thyroid Dysfunction**

163 Overall, 41.1% (102/248; 80F, 22M) of patients developed thyroid dysfunction (TD). The onset of TD,  
164 calculated in months from the date of alemtuzumab dose immediately prior to the onset of TD, was  
165 very variable (mean onset ( $\pm$  SD)  $23 \pm 18.2$  months; range 2 to 107 months), with the majority of  
166 patients (89 of 100, 89%;) developing TD within three years of last treatment (in two patients the  
167 timing of onset was unknown).

168 73 patients (71.6%) developed GD, 12 patients (11.7%) exhibited hypothyroidism with positive TRAb,  
169 HT occurred in 6 patients (5.8%), thyroiditis in 5 patients (4.9%), hypothyroidism (TRAb negative,  
170 anti-TPO antibody negative or not tested) and hyperthyroidism (TRAb negative or not tested;  
171 technetium scan not done) of unknown aetiology each occurred in 2 patients; the cause of TD in 2  
172 patients was unknown and they were lost to follow-up (Table 1a).

173 TRAb levels, ascertained in 72 of the 73 GD patients, were recorded as either “positive” in 11 or  
174 quantified (Mean ( $\pm$ SD) TRAb level 19.2 IU/L ( $\pm$ 14.7) in 60 cases. In two patients with negative or  
175 unknown TRAb status, tracer uptake in isotope scans was diffusely increased.

## 176 **Fluctuating Graves’ Disease**

177 12 of the 73 (16.4%) GD cases exhibited fluctuating thyroid status, transitioning from hypo to  
178 hyperthyroidism and vice versa after a variable period of time (Table 2). Measurement of TRAb  
179 bioactivity in 8 of these patients showed the presence of both stimulating (TSAb) and blocking  
180 (TBAb) circulating TRAb activities (Table 2).

181 The course of fluctuating thyroid status in one such case (Patient 6, Table 2) is detailed in Figure 2.  
182 Following three cycles of alemtuzumab treatment (2006, 2007, 2011) a 44 year-old female developed  
183 subclinical hyperthyroidism (TSH  $<0.03$ mU/L, FT4 19.5 pmol/L) in 2013, 25 months after her last  
184 treatment; she then became hypothyroid (TSH 18 mU/L, FT4 10 pmol/L) spontaneously 3 months  
185 later. Following thyroxine replacement for one year, she developed hyperthyroidism (TSH  $<0.03$   
186 mU/L, FT4 36.6pmol/L), which persisted (TSH  $<0.03$ mU/L, FT4 32 pmol/L) despite thyroxine  
187 withdrawal and was associated with elevated TRAb levels (initially 4.3 mU/L, then  $>40$  mU/L): she

188 then commenced carbimazole. Despite compliance with a block & replace (carbimazole 40mg,  
189 thyroxine 25mcg) regimen, she remained persistently thyrotoxic (TSH <0.03 mU/L, FT4 28.6pmol/L)  
190 and has opted to continue on high dose thionamide (carbimazole 30mg) therapy rather than have  
191 definitive treatment.

## 192 **TRAb positive Hypothyroidism**

193 12 patients (11.7%) developed hypothyroidism associated with surprisingly high (mean 30.4 IU/L,  
194 range 3.9 - >40 IU/L) TRAb levels and variable anti-TPO antibody status (positive n=6, negative n=4,  
195 unknown n=2). Measurement of TRAb bioactivity showed circulating blocking TRAb (TBAb) in 3  
196 out of 4 such cases (Table 3).

## 197 **Outcome of Thyroid Dysfunction**

198 To determine the course of thyroid dysfunction, we analysed a dataset from 71 patients in whom  
199 detailed information from follow-up (median follow up 67 months, range 6-251 months) was  
200 available. The demographics of this subset of cases was similar to that of the whole TD cohort (Table  
201 1b), as was the type and frequency of TD; the majority (n=52, 73.2%) of patients developed GD, 10  
202 patients (14.1%) exhibited hypothyroidism with positive TRAb, HT occurred in 5 patients (7.0%),  
203 thyroiditis in 3 patients (4.2%) and hypothyroidism of unknown aetiology in one case. 7 (13.4%)  
204 patients (3 smokers, 4 non-smokers) developed **clinically overt** ophthalmopathy which was  
205 particularly severe in two cases (both non-smokers), possibly linked to RAI treatment without steroid  
206 cover in one individual, requiring immunosuppressive treatment or surgical decompression. Pretibial  
207 myxoedema or acropachy was not recorded in any cases.

208 All 52 patients with GD were treated initially with ATD; 3 patients intolerant (rash, fatigue) of drug  
209 therapy underwent RAI within 6 months of diagnosis; 49 patients were treated with either block and  
210 replace (n=30) or titration (n=19) regimens (Figure 3a) with the first course of ATD therapy ongoing  
211 in 2 patients (Figure 3b) at time of data analysis.

212 Of 47 patients completing ATD treatment of appropriate duration (block & replace regimen at least 6  
213 months; titration regimen at least 12 months), 30 (64%) individuals ultimately required definitive  
214 treatment (Figure 3b). Of these, 17 had RAI (**n=14**, one treatment; n=2, two treatments; n=1, three

215 treatments), 5 underwent thyroidectomy and 8 opted to remain on ATDs long-term (average duration  
216 45 months, range 15-90 months). Reasons for RAI included relapsed (n=10), fluctuating (n=4) or  
217 difficult to control (n=3) GD; thyroidectomy was undertaken in either difficult to control (n=2) cases  
218 or patients requiring prolonged ATD treatment (n=3; 3, 4, 6 years on ATDs).  
219 16 patients being followed **after discontinuation of antithyroid drug treatment** (average duration 82.6  
220 months, range 28 to 137 months), remain in remission. One patient with fluctuating disease (patient 3  
221 in Table 2) developed hypothyroidism spontaneously. Prior to discontinuation of anti-thyroid drug  
222 treatment TRAb levels were only checked in 7 patients (6 TRAb negative - 1 relapsed; 1 TRAb  
223 positive – relapsed).

224

## 225 **DISCUSSION**

226 Thyroid dysfunction occurred frequently (41%) in our cohort of alemtuzumab-treated multiple  
227 sclerosis patients, with GD (72%) being the most frequent thyroid disorder, in accordance with  
228 previous studies (8, 9). **Although TD was more commonly seen in women (F:M ratio 3.6:1), we**  
229 **acknowledge that the known excess female preponderance of MS could have influenced this gender**  
230 **distribution. Virtually all of our patients had not been treated with other MS therapies in the year**  
231 **preceding onset of TD.**

232 The onset of TD was highly variable, but 89% occurred within 36 months of last administration of  
233 alemtuzumab, and 91% within four years, the period recommended for regular thyroid surveillance. In  
234 a previous clinical trial, risk of autoimmune dysfunction peaked at 12-18 months **after last**  
235 **alemtuzumab treatment**, with no recorded autoimmunity beyond 5 years after therapy (8). In contrast,  
236 in our cohort, 9 patients exhibited late-onset TD (n=2 at 5yrs, n=4 at 6yrs, n=1 at 7yrs, n=2 at 9 yrs)  
237 following the last dose of alemtuzumab. Whilst such dysfunction might be unrelated to alemtuzumab  
238 treatment, it may be prudent to consider surveillance for thyroid dysfunction (e.g. annual TSH  
239 measurement) for a longer period following alemtuzumab therapy.

240 A significant proportion (16.4%) of our patients developed GD with a fluctuating and unpredictable  
241 course and it is conceivable that this is an underestimate as frequent use of a block and replace ATD

242 regimen may have masked additional cases. Fluctuating course in alemtuzumab-induced GD has been  
243 noted anecdotally previously, with one study documenting hypothyroidism followed by  
244 hyperthyroidism in 4 patients and unusual spontaneous transition of GD to euthyroidism or  
245 hypothyroidism (9). Measurements of TRAb bioactivity, documenting the presence of both  
246 stimulating (TSAb) and blocking (TBAb) TRAb activities in our patients, supports the notion that  
247 changes in the circulating proportions of TSAbs and TBAb species over time with resultant stimulation  
248 or inhibition of thyroid hormone production, lead to fluctuation in thyroid status. This phenomenon  
249 has been described previously (19) but in other contexts, with switching between TBAb and TSAbs (or  
250 vice versa) being documented in rare patients following levothyroxine therapy for hypothyroidism or  
251 after ATD treatment of conventional GD (20). We have also recorded a higher prevalence (11.7%) of  
252 hypothyroidism with positive TRAb in alemtuzumab-treated patients than in conventional  
253 Hashimoto's thyroiditis (5%) (21), suggesting that TBAb activity may also operate in this context.  
254 Similar to the management of conventional GD in our centres, the majority (49/52) of our  
255 alemtuzumab-induced GD patients were treated with ATDs, but a higher proportion (64%) of patients  
256 proceeded to either definitive treatment (RAI or thyroidectomy) or opted to remain on ATDs long-  
257 term, compared to the proportion (50%) of conventional GD patients exercising these options (22). In  
258 our retrospective analyses, reasons for long-term ATD treatment were not always documented, but it  
259 is likely that relative refractoriness to drug treatment or presence of a fluctuating course prompted  
260 many physicians to not withdraw ATDs at completion of a course of standard duration. Such  
261 requirement for either definitive or long-term ATD treatment and a lower remission rate (34%)  
262 compares unfavourably with the remission rate (50%) in conventional GD (22). Our observations  
263 differ from the published literature, which suggests that alemtuzumab-induced GD has a more  
264 favourable outcome, with a high rate of spontaneous remission and good response to medical  
265 treatment, than the conventional disorder (23). In conventional GD, higher TRAb levels at cessation  
266 of ATD therapy are known to be associated with greater risk of relapse following drug withdrawal  
267 (24). In our study TRAb levels were only recorded in a minority of patients at cessation of ATDs; in  
268 future studies of ATD therapy in alemtuzumab-induced GD, serial TRAb measurement could

269 determine whether lower remission rates correlate with differences in change of TRAb levels or  
270 activity following treatment.  
271 13.4% of patients exhibited Graves' orbitopathy (GO), but this could be an underestimate as patients  
272 did not undergo routine ophthalmological assessment or MRI imaging, such that mild or subclinical  
273 dysthyroid eye disease might not have been recorded. Development of GO following alemtuzumab  
274 therapy is documented infrequently with occurrence of 6.25% of patients in one study (9). In the  
275 published literature of over 1000 alemtuzumab-treated patients 6 cases of GO have been recorded, but  
276 this incidence (0.6%) is also likely to be an underestimate as ophthalmopathy was not screened for  
277 routinely. Nevertheless, sight-threatening ophthalmopathy seems to be a rare complication of  
278 alemtuzumab treatment.

279 Our study has several limitations. Due to its retrospective nature, data including type and onset of TD  
280 in 2 cases and its aetiology in 4 cases is missing. Our study was limited to two tertiary centres, such  
281 that complex or difficult cases could be over-represented in the cohort; in addition, in the absence of a  
282 common treatment algorithm, the influence of differences in the management of GD cannot be  
283 completely discounted. Nevertheless, our work represents first documentation of alemtuzumab-  
284 induced thyroid dysfunction in a large patient cohort, including the course, management and outcome  
285 of Graves' with prolonged duration of follow up.

286

## 287 CONCLUSION

288 Alemtuzumab is highly effective therapy for relapsing-remitting MS (number need to treat to benefit:  
289 5; number needed to treat for a serious adverse event: 148) (25). However, the development of thyroid  
290 autoimmunity months or years after treatment is a frequent complication, requiring ongoing  
291 biochemical surveillance for at least 4 years after alemtuzumab therapy, to detect and treat TD  
292 promptly. This study suggests that alemtuzumab-induced TD, and GD in particular, can present  
293 unique challenges: in this context, GD may develop several years after alemtuzumab treatment,  
294 exhibit a fluctuating course (likely related to changing repertoire of stimulating versus blocking  
295 TRAb), with a need for definitive (RAI, surgery) or long-term ATD treatment which exceeds that in

296 conventional GD. Following recent regulatory approval of alemtuzumab for treatment of MS,  
297 endocrinologists will be required to manage this form of TD more often. Based on our experience, we  
298 suggest close monitoring of thyroid function in alemtuzumab-treated MS patients, particularly if they  
299 develop GD, offering early definitive treatment in drug-refractory or fluctuating cases.

300

301

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305

306

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380 **Figure Legends**

381 **Figure 1**

382 Overview of patients included in the study.

383 **Figure 2**

384 Course of thyroid dysfunction in a patient (patient 6 in Table 3) with fluctuating Graves' disease.

**385 Figure 3**

- 386 (a) Initial treatment modality in 52 patients with Graves' disease and follow up data. Three  
387 patients underwent radioiodine treatment due to intolerance of anti-thyroid drugs.
- 388 (b) **Longer term management** in 47 patients with Graves' disease following completion of initial  
389 course of anti-thyroid drug therapy.

**Table 1 (a).** Demographics and nature of thyroid dysfunction in all patients (n=102)

Gender	Age	No. treatment courses of alemtuzumab	Interval to Thyroid Dysfunction onset after last dose alemtuzumab	Type of Thyroid Dysfunction
Female 78% (n=80)	Mean 37.6 ± SD 9.2 years (range 20-60 years)	1 treatment (n=10)	Mean 22.9 ± SD 18.2 months (range 2-107 months)	Graves' Disease 71.6% (n=73)
Male 22% (n=22)		2 treatments (n=55)		Hypothyroidism with positive TRAb 11.7% (n=12)
		3 treatments (n=25)		Hashimoto's thyroiditis 5.8% (n=6)
		4 treatments (n=10)		Thyroiditis 4.9% (n=5)
		5 treatments (n=2)		Hypothyroidism, unspecified 2% (n=2)
		Hyperthyroidism, unspecified 2% (n=2)		
			Unknown 2% (n=2)	

**Table 1 (b).** Demographics and nature of thyroid dysfunction in cases with followup data (n=71)

Gender	Age	No. doses alemtuzumab	Interval to Thyroid Dysfunction onset after last dose alemtuzumab	Type of Thyroid Dysfunction
Female 75% (n=53)	Mean 37.8 ± SD 9.8 years (range 20-60 years)	1 treatment (n=6)	Mean 23.1 ± SD 20.2 months (range 2-107 months)	Graves' Disease 73.2% (n=52)
Male 25% (n=18)		2 treatments (n=38)		Hypothyroidism with positive TRAb 14.1% (n=10)
		3 treatments (n=19)		Hashimoto's thyroiditis 7.0% (n=5)
		4 treatments (n=7)		Thyroiditis 4.2% (n=3)
		5 treatments (n=1)		Hypothyroidism, unspecified 1.4% (n=1)

**Table 2.** Subset of patients with fluctuating Graves' disease (n=12)

Patient No.	Alemtuzumab cycles*	Episodes of thyroid dysfunction					
		Onset ±	Type	TFT results	TRAb (IU/L)	TSH Receptor Antibody Bioactivity	Outcome
1	0 / 12 / - / -	5 months	Subclinical Hypothyroidism	TSH 5.7 mU/L, FT4 10.9 pmol/L**	ND	TSAb +, TBAb +/-	Remission
		29 months later	Hyperthyroidism	TSH <0.03 mU/L, FT4 25 pmol/L**	3.4**	TSAb ++, TBAb -	
2	0 / 20 / - / -	18 months	Hypothyroidism	TSH 19.30 mU/L, FT4 10.3 pmol/L***	>40**	TSAb +/-, TBAb ++	Poor control on ATD necessitated RA!
		22 months later	Hyperthyroidism	TSH <0.03 mU/L, FT4 42.8 pmol/L***	>40**	TSAb -, TBAb +	
3	0 / 12 / - / -	6 months	Hyperthyroidism	TSH <0.03 mU/L, FT4 20.9 pmol/L**	ND	TSAb +/-, TBAb -	Hypothyroidism (5 months after stopping ATD)
		3 months later	Hypothyroidism	N/A	ND	TSAb ++, TBAb ++	
		18 months later	Hyperthyroidism	TSH <0.03 mU/L, FT4 28 pmol/L***	5.5**	TSAb +, TBAb -	
		19 months later	Hypothyroidism	TSH 27.1 mU/L, FT4 10.2 pmol/L**	ND	ND	
4	0 / 14 / 82 / -	12 months	Hyperthyroidism	TSH <0.03 mU/L, FT4 28 pmol/L**	ND	TSAb -, TBAb -	Remission
		3 months later	Hypothyroidism	TSH 13.40 mU/L, FT4 10.7 pmol/L***	ND	TSAb -, TBAb +/-	
		3 months later	Hyperthyroidism	N/A	N/A (7.0**, 5 months later)	TSAb ++, TBAb +/-	
5	0 / 12 / - / -	12 months	Hypothyroidism	TSH 30.10 mU/L, FT4 10.5 pmol/L***	ND	TSAb ++, TBAb ++	Relapse (4 months after stopping ATD)
		48 months later	Hyperthyroidism	TSH <0.03 pmol/L, FT4 41.6 pmol/L**	ND (8.1**, 7months earlier)	TSAb -, TBAb +/-	
6	0 / 12 / 53 / 99	25 months	Subclinical Hyperthyroidism	TSH <0.03 mU/L, FT4 19.5 pmol/L**	ND	TSAb +/-, TBAb +	Poor control on ATD
		3 months later	Hypothyroidism	TSH 18 mU/L, FT4 10 pmol/L**	ND	TSAb +, TBAb ++	
		20 months later	Hyperthyroidism	TSH <0.03 mU/L, FT4 31.9 pmol/L**	8.1**	TSAb -, TBAb +/-	
7	0 / 12 / - / -	12 months	Hypothyroidism	TSH 98 mU/L, FT4 5.2 pmol/L±±	>40±±	ND	Poor control on ATD necessitated RA!
		12 months later	Hyperthyroidism	TSH <0.01 mU/L, FT4 32.6 pmol/L±±	ND	ND	

**Table 2.** Subset of patients with fluctuating Graves' disease (n=12)

Patient No.	Alemtuzumab cycles*	Episodes of thyroid dysfunction					
		Onset ±	Type	TFT results	TRAb (IU/L)	TSH Receptor Antibody Bioactivity	Outcome
8	0 / 12 / 39 / -	11 months	Subclinical Hyperthyroidism	TSH <b>0.02</b> mU/L, FT4 14.9 pmol/L, FT3 <b>7.0</b> pmol/L $\pm\pm$	ND	ND	Poor control on ATD necessitated RAI
		17 months	Hypothyroidism	TSH <b>72</b> mU/L, FT4 <b>5.8</b> pmol/L $\pm\pm$	>40 $\pm\pm$	ND	
		10 months later	Hyperthyroidism	TSH <b>0.01</b> mU/L, FT4 <b>19.7</b> pmol/L, FT3 <b>8.3</b> pmol/L $\pm\pm$	ND	ND	
9	0 / 12 / - / -	20 months	Hyperthyroidism	TSH < <b>0.01</b> mU/L, FT4 <b>26</b> pmol/L $\pm\pm$	ND	ND	Poor control on ATD (awaiting 2 <sup>nd</sup> RAI treatment)
		4 months later	Hypothyroidism	TSH <b>69.2</b> mU/L, FT4 <b>5.2</b> pmol/L $\pm\pm$	>40 $\pm\pm$	ND	
		18 months later	Hyperthyroidism	TSH <b>0.01</b> mU/L, FT4 <b>46</b> pmol/L, FT3 <b>24.3</b> pmol/L $\pm\pm$	ND	ND	
10	0 / 12 / - / -	9 months	Hypothyroidism	TSH > <b>100</b> mU/L, FT4 <b>5.2</b> pmol/L $^{***}$	ND	TSAb ++, TBAb ++	Remission
		18 months later	Hyperthyroidism	TSH < <b>0.03</b> mU/L, FT4 <b>36.4</b> pmol/L $^{**}$	>40 $^{**}$ (date unknown)	TSAb ++, TBAb +	
11	0 / 12 / - / -	37 months	Hypothyroidism	TSH <b>6.9</b> mU/L, FT4 <b>9.7</b> pmol/L $^{**}$	ND	TSAb +/-, TBAb -	Relapse (7 months after stopping ATD)
		5 months later	Hyperthyroidism	TSH < <b>0.03</b> mU/L, FT4 <b>44.9</b> pmol/L $\pm\pm\pm$	Positive $\pm\pm\pm$	ND	
12	0 / 12 / - / -	12 months	Subclinical Hyperthyroidism	TSH <b>0.18</b> mU/L, FT4 <b>18.9</b> pmol/L, FT3 <b>6</b> pmol/L $^{**}$	ND	ND	Continuing trial of medical therapy (month 9 of a titration regimen)
		3 months later	Hypothyroidism	TSH <b>23</b> mU/L, FT4 <b>10.4</b> pmol/L $^{**}$	>40 $^{**}$	ND	
		16 months later	Hyperthyroidism	TSH < <b>0.03</b> mU/L, FT4 <b>28.6</b> pmol/L $^{**}$	>40 $^{**}$	ND	

\* Months of administration of alemtuzumab, with 0 denoting the first cycle and subsequent cycles timed in months from administration of first cycle

± Months from the **previous** dose of alemtuzumab at time of initial finding of thyroid dysfunction

\*\*Reference Ranges (RR): TSH 0.35-5.5 mU/L, FT4 10-19.8 pmol/L, FT3 3.5-6.5 pmol/L, TRAb > 1 IU/L positive. Results in **bold** are outside the

\*\*\*Reference Ranges (RR): TSH mU/L, FT4 11.5-22.7 pmol/L, FT3 pmol/L. Results in **bold** are outside the RR

±± Reference Ranges (RR): TSH 0.35-5.0 mU/L, FT4 9-19 pmol/L, FT3 2.6-5.7 pmol/L, TRAb > 1.5 mU/L positive. Results in **bold** are outside the RR

±±± Reference Range unknown

TSH Receptor Antibody Bioactivity results: - negative, +/- borderline, + positive (low signal), ++ positive (high signal)

Abbreviations: **TFT, thyroid function test**; TRAb, TSH Receptor antibody; TSAb, thyroid stimulating antibody; TBAb, thyroid blocking antibody; ND, not done; ATD, Anti-thyroid drugs

**Table 3.** Antibody profiling in patients with hypothyroidism and positive anti-TSH receptor antibody status

Patient No.	Alemtuzumab cycles*	Onset of Hypothyroidism±	TFT Results**	TPO Antibody RR 0-100 IU/ml	TRAb RR 0-1 IU/L	TSH Receptor Antibody Bioactivity
1	0 / 11 / 26 / -	Month 14	TSH <b>34</b> mU/L, FT4 <b>8.7</b> pmol/L	Negative	<b>9.4</b>	TSAb +/-, TBAb +
2	0 / 12 / 123 / -	Month 31	TSH <b>50.5</b> mU/L, FT4 <b>9.4</b> pmol/L	<b>1498</b>	<b>&gt;40</b>	TSAb ++, TBAb +
3	0 / 12 / - / -	Month 20	TSH <b>&gt;100</b> mU/L, FT4 <b>5</b> pmol/L, FT3 <b>3</b> pmol/L	ND	<b>11.4</b>	TSAb +, TBAb ++
4	0 / 12 / - / -	Month 11	TSH <b>17.6</b> mU/L, FT4 <b>4.0</b> pmol/L, FT3 <b>1.52</b> pmol/L	43	<b>&gt;40</b>	TSAb +, TBAb -

\* Months of administration of alemtuzumab, with 0 denoting the first cycle and subsequent cycles timed in months from administration of first cycle

± Months from the last dose of alemtuzumab

\*\*Reference Ranges (RR): TSH 0.35-5.5 mU/L, FT4 10-19.8 pmol/L, FT3 3.5-6.5 pmol/L, Results in **bold** are outside the RR

TSH Receptor Antibody Bioactivity results: - negative, +/- borderline, + positive (low signal), ++ positive (high signal)

Abbreviations: **TFT, thyroid function test**; TRAb, TSH Receptor antibody; TSAb, thyroid stimulating antibody; TBAb, thyroid blocking antibody; TPO, anti-thyroid peroxidase; ND, not done

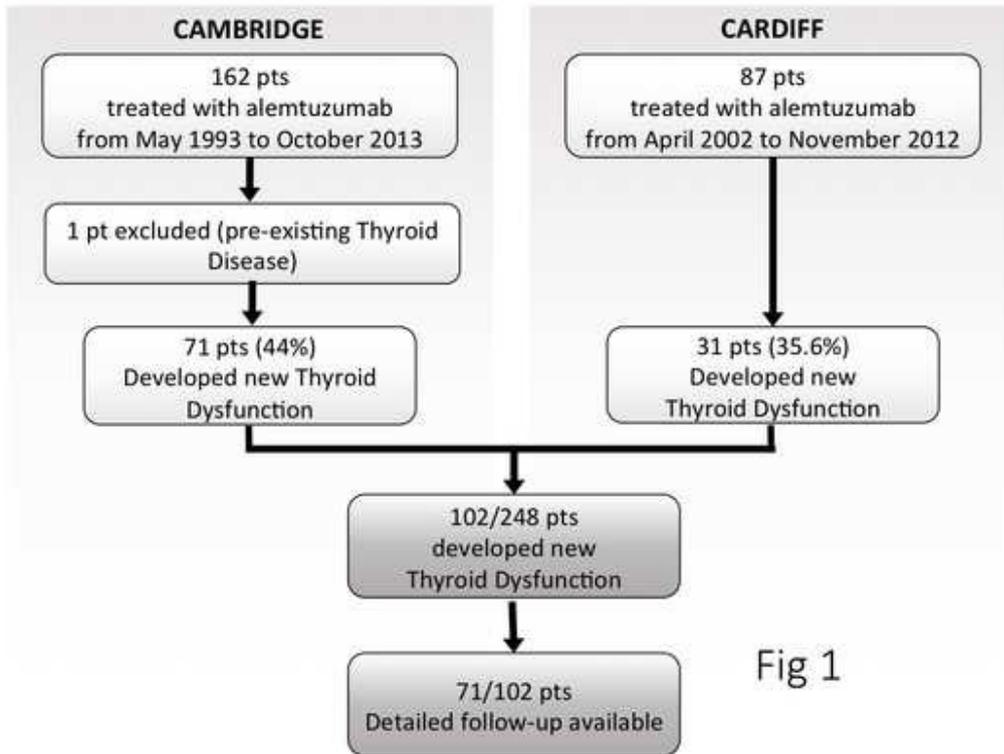


Fig 1

Fig 2

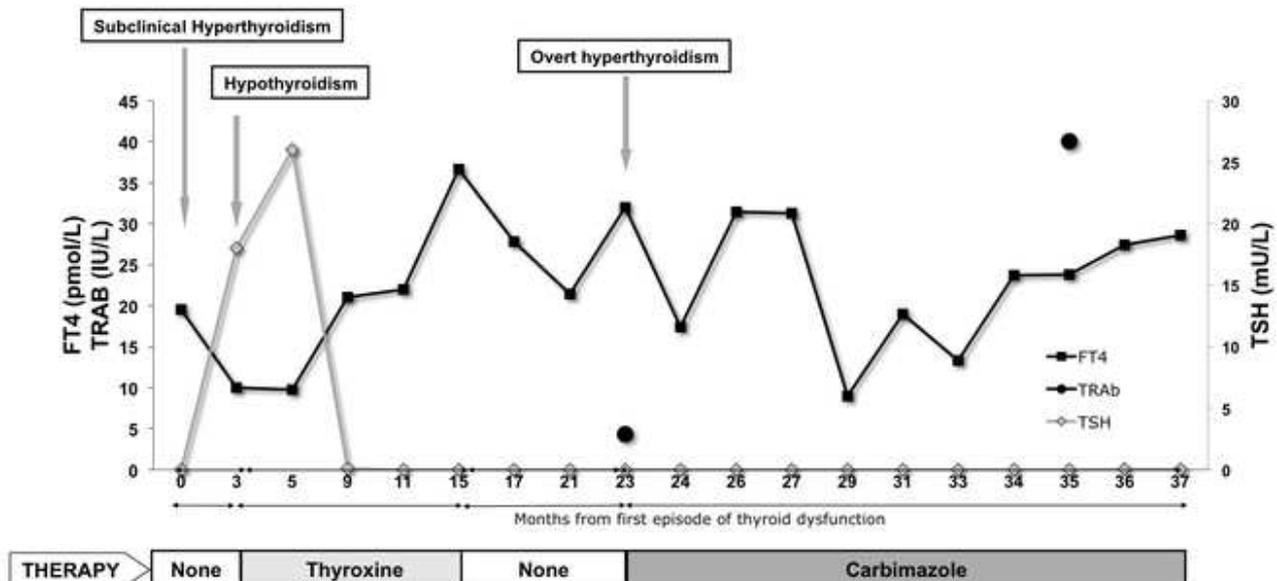


Fig 3

