Alemtuzumab-induced thyroid dysfunction exhibits distinctive clinical and immunological features

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Précis: In a retrospective analysis of alemtuzumab-induced thyroid dysfunction, Graves' disease with fluctuating status that was relatively refractory to treatment and anti-TSH receptor antibody positive hypothyroidism was recorded.

Abbreviated title: Alemtuzumab-induced Thyroid Dysfunction

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ABSTRACT

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

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

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

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

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

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

INTRODUCTION

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

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

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

required in 26%, compared to ~50% in conventional GD (8). Detailed analysis of TD in a large, phase 2 clinical trial showed that 23% of alemtuzumab-induced GD patients became euthyroid spontaneously, 15% developed hypothyroidism, with only 36% requiring radioiodine (RAI) or surgery (7, 9). In a subsequent phase 3 trial, only 2 out of 28 hyperthyroid patients required RAI or surgery (15); in another observational study 17 of the 22 patients with GD responded to drug therapy with only 3 cases requiring RAI (5). In contrast, anecdotal case reports suggest a poor response to anti-thyroid drug therapy (16) and GD with a fluctuating and unpredictable course has been noted (9).

Here, in one of the largest case series of alemtuzumab-induced TD, followed for over 20 years, we have documented the type, frequency and course of alemtuzumab-induced thyroid dysfunction and determined whether response to treatment differs compared to that reported in conventional thyroid disease.

SUBJECTS AND METHODS

We undertook detailed analysis of all MS patients who developed TD after treatment with alemtuzumab in clinical trials prior to its licensing at Addenbrooke's Hospital in Cambridge and University Hospital Wales in Cardiff over a 20 year period (1993 to 2013).

Alemtuzumab was administered intravenously on consecutive days for one or more cycles (five consecutive days for the first cycle; three consecutive days for subsequent cycles). The initial dose (20mg/day) was increased to 24mg/day in 2003 following a change in supplier. From 2006, the dose was reduced to 12mg/day to conform with the phase III study protocol. All patients receiving alemtuzumab at either centre had baseline thyroid function tests (TSH, FT4) prior to commencement of the drug, with TSH, FT4 and anti-thyroid peroxidase (TPO) antibody measurement 3 monthly for 2 years, 6 monthly for 2 years and then annually, or sooner if symptoms of TD developed. At each time-point patients also underwent clinical review including directed enquiry for thyroid-related symptoms. One patient with pre-existing thyroid disease (in her past medical history), was excluded from this analysis.

All patients who developed thyroid dysfunction (defined below) underwent evaluation by an

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

Laboratory measurements

Serum free T4, free T3 and TSH were measured using automated immunoassay systems (Advia Centaur, Siemens in Cambridge throughout and in Cardiff until 2010, with Abbott Architect thereafter). In Cambridge, TRAb was measured initially using a first generation ELISA assay, then Brahms Lumitest TRAK assay (RR 1-2 IU/L equivocal, >2 positive) from 2002. Cardiff also used the Brahms Lumitest TRAK assay (1-1.5 IU/L borderline, >1.5IU/L positive), changing to the Roche Cobas assay (RR 0.9-1.6 IU/L borderline, >1.6 IU/L positive) in 2014. Note that, as the upper, accurately quantifiable, limit of these assays is 40 IU/L (with levels greater than this being reported as ">40" IU/L), in calculations for this study we have used 40 whenever TRAb levels of >40 were reported. Information from Thermoscientific confirms that human TSH does not interfere with TRAb measurement in the lumitest TRAK assay, up to TSH values of at least 500mU/L.

Anti-TPO antibody was measured using various assays over the study period: in Cambridge Serodia agglutination assay (positive or negative) up to 2002, then Phadia ELISA (RR <100 iu/ml) until 2007 and Phadia immunocap (RR <100 iu/ml) till 2014 and then Siemens Centaur (RR <60 iu/ml)] until the present; in Cardiff the Advia Centaur assay (RR <60 kU/L) until 2010 then the Abbott Architect (RR <6 U/ml) until the present.

The bioactivity of anti-TSH receptor antibodies (TRAb) was measured using a Chinese Hamster Ovary (CHO) cell line stably transfected with the human TSH receptor and a cAMP responsive luciferase reporter, classifying them into receptor stimulating (TSAb) or blocking (TBAb) activities, as described previously (17, 18).

Definitions of Thyroid dysfunction

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

RESULTS

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

Patient Characteristics

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

Characteristics of Thyroid Dysfunction

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

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

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

Fluctuating Graves' Disease

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

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

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

TRAb positive Hypothyroidism

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

Outcome of Thyroid Dysfunction

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

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

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

treatments), 5 underwent thyroidectomy and 8 opted to remain on ATDs long-term (average duration 45 months, range 15-90 months). Reasons for RAI included relapsed (n=10), fluctuating (n=4) or difficult to control (n=3) GD; thyroidectomy was undertaken in either difficult to control (n=2) cases or patients requiring prolonged ATD treatment (n=3; 3, 4, 6 years on ATDs).

16 patients being followed after discontinuation of antithyroid drug treatment (average duration 82.6 months, range 28 to 137 months), remain in remission. One patient with fluctuating disease (patient 3 in Table 2) developed hypothyroidism spontaneously. Prior to discontinuation of anti-thyroid drug treatment TRAb levels were only checked in 7 patients (6 TRAb negative - 1 relapsed; 1 TRAb positive – relapsed).

DISCUSSION

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

The onset of TD was highly variable, but 89% occurred within 36 months of last administration of alemtuzumab, and 91% within four years, the period recommended for regular thyroid surveillance. In a previous clinical trial, risk of autoimmune dysfunction peaked at 12-18 months after last alemtuzumab treatment, with no recorded autoimmunity beyond 5 years after therapy (8). In contrast, 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) following the last dose of alemtuzumab. Whilst such dysfunction might be unrelated to alemtuzumab treatment, it may be prudent to consider surveillance for thyroid dysfunction (e.g. annual TSH measurement) for a longer period following alemtuzumab therapy.

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

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

determine whether lower remission rates correlate with differences in change of TRAb levels or activity following treatment.

13.4% of patients exhibited Graves' orbitopathy (GO), but this could be an underestimate as patients did not undergo routine ophthalmological assessment or MRI imaging, such that mild or subclinical dysthyroid eye disease might not have been recorded. Development of GO following alemtuzumab therapy is documented infrequently with occurrence of 6.25% of patients in one study (9). In the published literature of over 1000 alemtuzumab-treated patients 6 cases of GO have been recorded, but this incidence (0.6%) is also likely to be an underestimate as ophthalmopathy was not screened for routinely. Nevertheless, sight-threatening ophthalmopathy seems to be a rare complication of alemtuzumab treatment.

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

CONCLUSION

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

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

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Figure Legends

Figure 1

Overview of patients included in the study.

Figure 2

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

Figure 3

(a) Initial treatment modality in 52 patients with Graves' disease and follow up data. Three patients underwent radioiodine treatment due to intolerance of anti-thyroid drugs.

(b) Longer term management in 47 patients with Graves' disease following completion of initial 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) Male 22% (n=22)	Mean 37.6 ± SD 9.2 years (range 20-60 years)	1 treatment (n=10) 2 treatments (n=55) 3 treatments (n=25) 4 treatments (n=10) 5 treatments (n=2)	Mean 22.9 ± SD 18.2 months (range 2-107 months)	Graves' Disease 71.6% (n=73) Hypothyroidism with positive TRAb 11.7% (n=12) Hashimoto's thyroiditis 5.8% (n=6) Thyroiditis 4.9% (n=5) 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) Male 25% (n=18)	Mean 37.8 ± SD 9.8 years (range 20-60 years)	1 treatment (n=6) 2 treatments (n=38) 3 treatments (n=19) 4 treatments (n=7) 5 treatments (n=1)	Mean 23.1 ± SD 20.2 months (range 2-107 months)	Graves' Disease 73.2% (n=52) Hypothyroidism with positive TRAb 14.1% (n=10) Hashimoto's thyroiditis 7.0% (n=5) Thyroiditis 4.2% (n=3) 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 RAI
		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 RAI
		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 **0.02** mU/l, FT4 14.9 pmol/L FT3 **7.0** pmol/L**±±**	ND	ND	Poor control on ATD necessitated RAI
		17 months	Hypothyroidism	TSH **72** mU/L, FT4 **5.8** pmol/L**±±**	**>40±±**	ND	
		10 months later	Hyperthyroidism	TSH **0.01** mU/L, FT4 **19.7** pmol/L FT3 **8.3** pmol/L**±±**	ND	ND	
9	0 / 12 / - / -	20 months	Hyperthyroidism	TSH **<0.01** mU/L, FT4 **26** pmol/L**±±**	ND	ND	Poor control on ATD (awaiting 2nd RAI treatment)
		4 months later	Hypothyroidism	TSH **69.2** mU/L, FT4 **5.2** pmol/L**±±**	**>40±±**	ND	
		18 months later	Hyperthyroidism	TSH **0.01** mU/L, FT4 **46** pmol/L, FT3 **24.3** pmol/L**±±**	ND	ND	
10	0 / 12 / - / -	9 months	Hypothyroidism	TSH **>100** mU/L FT4 **5.2** pmol/L***	ND	TSAb ++, TBAb ++	Remission
		18 months later	Hyperthyroidism	TSH **<0.03** mU/L FT4 **36.4** pmol/L**	**>40**** (date unknown)	TSAb ++, TBAb +	
11	0 / 12 / - / -	37 months	Hypothyroidism	TSH **6.9** mU/L FT4 **9.7** pmol/L**	ND	TSAb +/-, TBAb -	Relapse (7 months after stopping ATD)
		5 months later	Hyperthyroidism	TSH **<0.03** mU/L FT4 **44.9** pmol/L**±±±**	**Positive ±±±**	ND	
12	0 / 12 / - / -	12 months	Subclinical Hyperthyroidism	TSH **0.18** mU/L, FT4 **18.9** pmol/L, FT3 6 pmol/L**	ND	ND	Continuing trial of medical therapy (month 9 of a titration regimen)
		3 months later	Hypothyroidism	TSH **23** mU/L, FT4 10.4 pmol/L**	>40**	ND	
		16 months later	Hyperthyroidism	TSH **<0.03** mU/L FT4 **28.6** 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 **34** mU/L, FT4 **8.7** pmol/L	Negative	**9.4**	TSAb +/-, TBAb +
2	0 / 12 / 123 / -	Month 31	TSH **50.5** mU/L, FT4 **9.4** pmol/L	**1498**	**>40**	TSAb ++, TBAb +
3	0 / 12 / - / -	Month 20	TSH **>100** mU/L, FT4 **5** pmol/L, FT3 **3** pmol/L	ND	**11.4**	TSAb +, TBAb ++
4	0 / 12 / - / -	Month 11	TSH **17.6** mU/L, FT4 **4.0** pmol/L, FT3 **1.52** pmol/L	43	**>40**	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

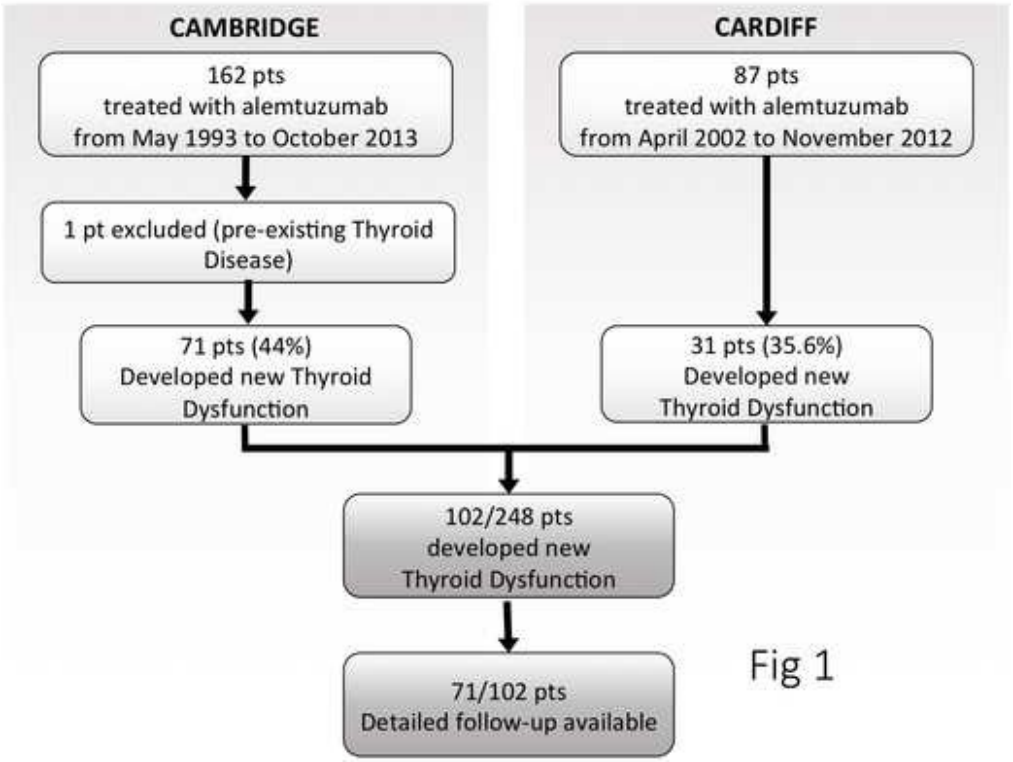

CAMBRIDGE
162 pts
treated with alemtuzumab
from May 1993 to October 2013
1 pt excluded (pre-existing Thyroid Disease)
71 pts (44%)
Developed new Thyroid Dysfunction
CARDIFF
87 pts
treated with alemtuzumab
from April 2002 to November 2012
31 pts (35.6%)
Developed new
Thyroid Dysfunction
102/248 pts
developed new
Thyroid Dysfunction
71/102 pts
Detailed follow-up available

Fig 1

Fig 2

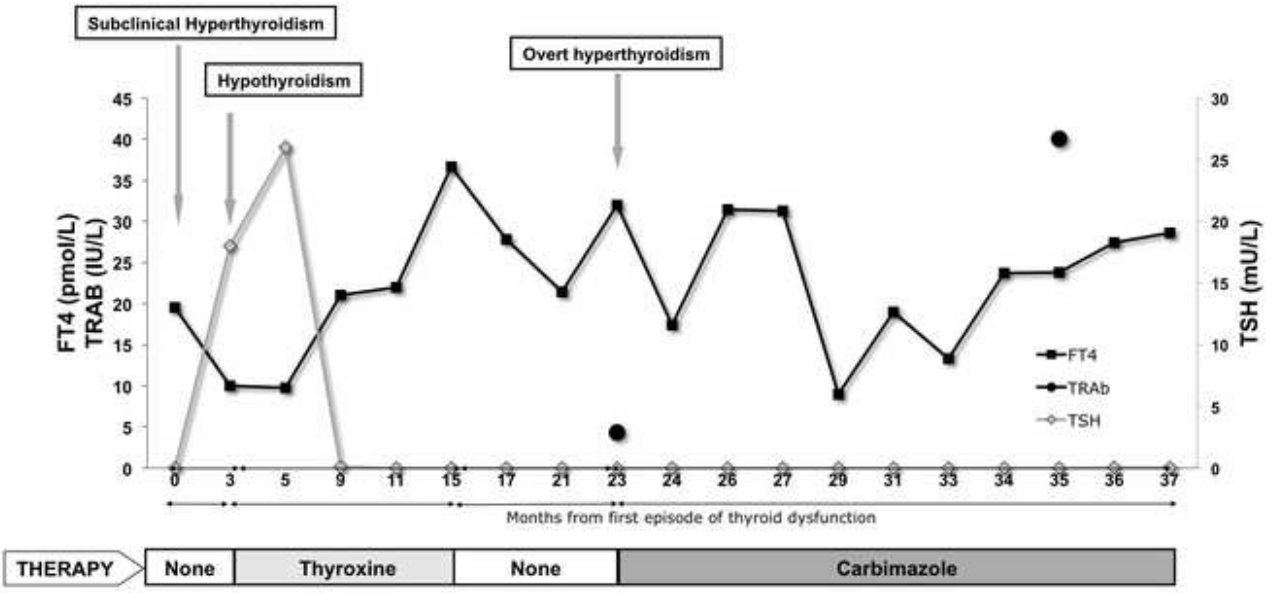
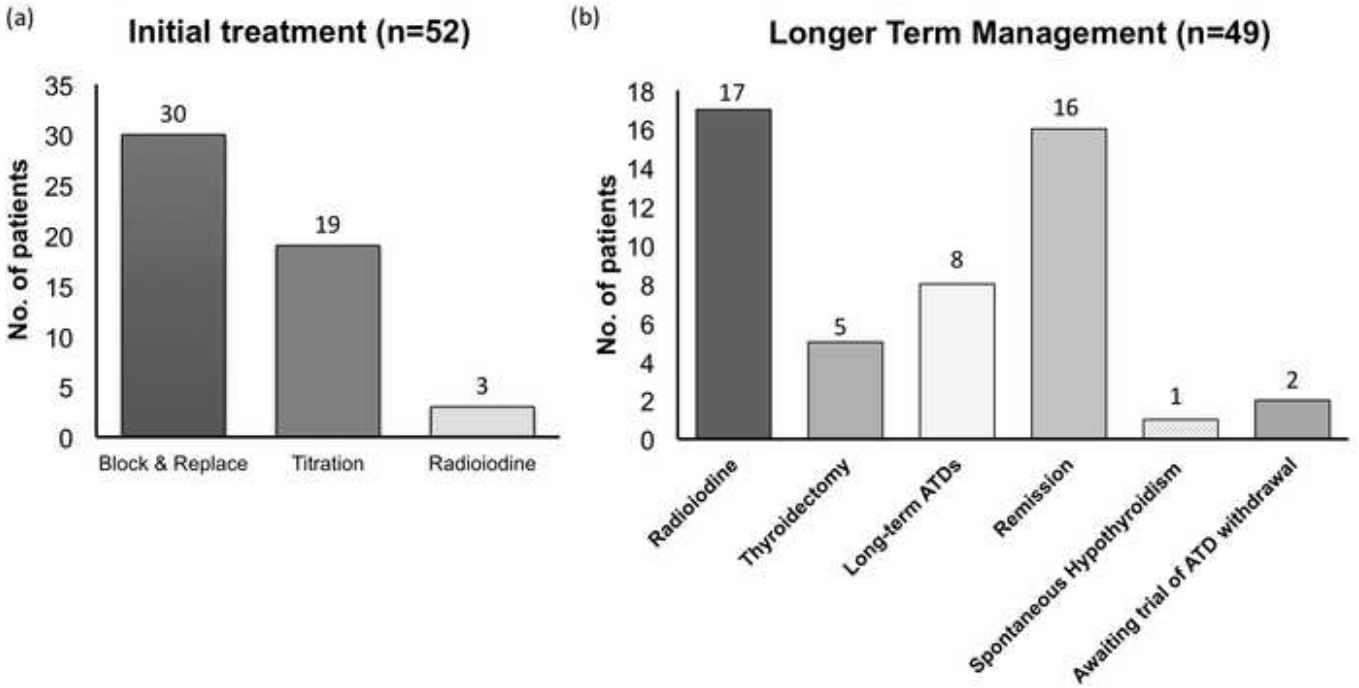

Subclinical Hyperthyroidism
Hypothyroidism
Overt hyperthyroidism
FT4 (pmol/L)
TRAB (IU/L)
TSH (mU/L)
FT4
TRAb
TSH
Months from first episode of thyroid dysfunction
THERAPY
None
Thyroxine
None
Carbimazole

Fig 3


(a)
Initial treatment (n=52)
No. of patients
35
30
25
20
15
10
5
0
30
19
3
Block & Replace
Titration
Radioiodine
(b)
Longer Term Management (n=49)
No. of patients
18
16
14
12
10
8
6
4
2
0
17
5
8
16
1
2
Radioiodine
Thyroidectomy
Long-term ATDs
Remission
Spontaneous Hypothyroidism
Awaiting trial of ATD withdrawal