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MORBIDITY AND MORTALITY IN RHEUMATOID ARTHRITIS PATIENTS WITH PROLONGED THERAPY-INDUCED LYMPHOPENIA. 12 YEAR OUTCOMES

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ABSTRACT

Objective. To assess immunologically relevant outcomes in a cohort of RA patients with long-term therapy-induced lymphopenia.

Methods. Fifty three RA patients who received the lymphocytotoxic monoclonal antibody CAMPATH-1H (alemtuzumab, MabCampath®) between 1991 and 1994 were assessed for mortality, and infectious and malignant morbidity using interviews, case note review and NHS Central Registry mortality monitoring. Lymphocyte subsets were enumerated by flow cytometry. A retrospective, matched-cohort study of mortality was performed with 102 control subjects selected from the EULAR database of patients with rheumatic disorders.

Results. Patients remained lymphopenic: median $CD3^+CD4^+$, $CD3^+CD8^+$, $CD19^+$ and $CD56^+$ lymphocyte counts measured at a mean follow-up of 11.8 years from first dose of CAMPATH-1H were 0.59, 0.275, 0.13 and 0.105 x 10^9 / L respectively. 27 of 51 cases and 46 of 101 controls had died, providing a mortality rate ratio of 1.20 (95% CI 0.72-1.98). Causes of death were similar to those expected for a hospital-based RA cohort. No opportunistic infections were noted and only 3 infections documented following 36 elective orthopaedic procedures.

Conclusions. Despite continued lymphopenia 11.8 years after therapy, our patient cohort does not display excess mortality or unusual infectious morbidity and surgery is well tolerated. These data are reassuring for clinicians and patients considering lymphocytotoxic or other immunomodulatory therapy for RA.

CAMPATH-1H (Alemtuzumab, MabCampath®) was the first humanised therapeutic monoclonal antibody (mAb). It recognises CD52, a highly glycosylated, GPI-anchored, peptide of unknown function that is present on T and B lymphocytes, monocytes and NK cells(1). In the early 1990s a number of clinical trials studied the response to CAMPATH-1H in patients with refractory rheumatoid arthritis (RA) (summarised in (2)). The rationale was that lymphocyte depletion and reconstitution would result in autoreactive lymphocytes being replaced by a tolerant immune system. Trials were curtailed largely as a consequence of delayed reconstitution, particularly of T-lymphocytes, resulting in a cohort of profoundly lymphopenic patients (3).

In a previous study we demonstrated that, despite continued lymphopenia for a median of 6 years from therapy, there was no detectable consequence on mortality or infectious morbidity in RA recipients of CAMPATH-1H (3). The progress of this cohort remains relevant for several reasons: CAMPATH-1H continues to be developed for other autoimmune indications; alternative lymphocytotoxic regimes, such as high-dose cyclophosphamide with stem cell rescue, may carry similar long-term implications; and Rituximab, a B-cell depleting therapy, has recently gained a licence for use in RA. In the current study we extend follow-up of our cohort to a median of 12 years.

PATIENTS AND METHODS

Patients and controls

Patients treated with CAMPATH -1H between 1991 and 1994 in Cambridge (UK) were identified from the study database. Follow up information was obtained for patients who were alive on 31st December 1998 (conclusion of our previous study).

Morbidity data was collected up to 31st December 2004 and mortality data to 9th March 2006. Information was obtained as outlined previously (3). Particular attention was paid to episodes of infection and/or treatment with antibiotics, surgical procedures, cancers and other co-morbidities. For a retrospective, matched cohort study of mortality, the survival experience of our cohort was matched to patients entered into the European League Against Rheumatism (EULAR) database for patients with rheumatic disorders who have received immunosuppressive agents (6), as previously described (3).

Of the original 53 patients, 13 deaths were described previously (3). There were 14 additional deaths in the current follow-up period. 5 patients could not be contacted, including 3 previously reported lost to follow-up. Only one of these patients is known to have emigrated, and therefore we will be informed if any of the other 4 die (as a consequence of 'flagging' with the National Health Service Central Registry (3)). 20 patients were interviewed for the current study (including 3 that subsequently died) and hospital notes were reviewed for 5 patients (including one that died after consenting to study inclusion).

Lymphocyte subset analysis

Lymphocyte subset analysis ($CD3^+CD4^+$ and $CD3^+CD8^+$ T cells, $CD19^+$ B cells and $CD56^+$ Natural Killer (NK) cells) was performed on peripheral blood from each patient that was interviewed.

Statistical Analysis

For mortality rate statistics, duration of follow-up was calculated to 9th March 2006 for the patients assumed to have remained in the UK (and therefore 'flagged'), and to the last clinic visit for the patient known to have emigrated. For the retrospective,

matched cohort study, the mortality rates in cases and controls were obtained by dividing the number of deaths in each group by the number of person-years of follow-up. The mortality rate ratio was obtained by dividing case mortality rate by control mortality rate. Kaplan-Meier survival curves were plotted using STATA software (Stata Corporation, College Station, TX). The log-rank test was used to compare survival between cases and controls and to compare survival in different CAMPATH-1H dosing cohorts.

Underlying cause of death was transcribed from death certificates using ICD9 or ICD10 codes. These were subsequently classified by ICD chapter, allowing a comparison of cause of death between cases and controls. Comparisons of mortality were examined using either chi-squared or Fisher's exact tests, where groups contained a sufficient number of deaths. Deaths from neoplasms were analysed further utilising Poisson regression.

RESULTS

Demographics

The pre-treatment cohort demographics were described previously (3). In brief, this was a cohort of refractory RA patients with mean age of 54 (range 25.5-70) years at the time of first treatment and median disease duration 9 (range 2-35) years. They had received a median of 4 DMARDs prior to CAMPATH-1H (range 1-8). 38 were female and 15 male. Details of the 25 surviving patients for whom information was available are documented in Table 1. The median (inter-quartile range [IQR]) time since first treatment with CAMPATH-1H to fail date was 12.7 (7.5 – 13.3) years for all study participants, 12.1 (6.0 – 13.2) years for cases and 12.8 (8.0 – 13.3) years for

controls. The total duration of follow-up for CAMPATH-1H treated patients was 540.08 patient-years.

Mortality

Two cases could not be matched with appropriate controls and are excluded from the analysis (3). One control was excluded from the current analysis for a technical reason. Until 9th March 2006 there were 73 deaths - 27 of 51 cases and 46 of 101 controls. The mortality rate for cases was 5 deaths per 100 person years and for controls was 4 per 100 person years, giving an incidence rate ratio of 1.20 (95% CI 0.72-1.98). There was no difference in all-cause mortality rate between cases and controls (*P*=0.3894, log-rank test, Figure 1).

The 14 previously unreported deaths are listed in Table 2A. Six were primarily cardiovascular (5 ischemic heart disease, one bowel infarction) and three due to infection (bronchopneumonia in each case), typical of an RA population. There were 2 malignancies, 2 deaths from G-I causes and a death secondary to primary sclerosing cholangitis. Table 2B classifies all deaths reported since baseline, according to ICD chapter. Deaths from neoplasms and diseases of the digestive system were more common in cases than controls but this did not reach statistical significance. Deaths from neoplasms were analysed further utilising Poisson regression giving a mortality rate ratio of 3.33 (95% CI 0.85, 13.09), which was not statistically significant.

Infections

During the current morbidity follow-up period there were 3 major, definite infections where the causative pathogen was identified. Patient 1 sustained a left hip fracture. in 2002. This was fixed with a dynamic hip screw (DHS) which became infected with

methicillin-resistant staphylococcus aureus (MRSA) necessitating its removal. In 2003 the same patient developed septic arthritis of the right shoulder (pathogen not identified) which was surgically irrigated. This patient has since developed chronic ulceration of the left lower limb and an infected left 2nd metacarpophalangeal joint. Patient 10 developed cardiovascular collapse and a right knee aspirate grew beta haemolytic streptococcus. The patient made a full recovery with antimicrobial and supportive therapy. Patient 37 developed infection of the right first metatarso-phalangeal joint from which staphylococcus, pseudomonas and diptheroids were cultured. The infection responded fully to treatment with clarithromycin and metronidazole. In November 1999 the same patient developed a chest infection which was treated with augmentin and in September 2002 disseminated herpes zoster infection which was treated with famciclovir.

Patient 44 developed a number of soft tissue and joint infections, although the infecting organisms could not be identified from the case records. In June 2000 antibiotics were prescribed for a wound infection following bilateral forefoot surgery. In 2003, whilst taking etanercept, antibiotics were prescribed for cellulitis of the lower leg and in June 2004 for a possible septic arthritis. Between October and December 2004 intravenous antibiotics were administered for a septic arthritis of the elbow and an infected shoulder prosthesis, which was subsequently removed. Other patients have reported minor infections of the chest (patients 3,5,18,26,38) urinary tract (patient 26), ear (patient 32) and skin (patient 41), each of which was successfully treated in the community with oral antimicrobial agents.

Orthopaedic surgical procedures

There were 36 elective orthopaedic interventions in 14 patients between December 31st 1998 and December 31st 2004. Three post-operative infections were recorded:

an infection of the hand following 5th extensor tendon repair, plus DHS and forefoot infections documented above.

Non fatal malignancies

Patient 14 was diagnosed with carcinoma of the breast in October 1999 and treated with lumpectomy and axillary lymph node excision. Patient 5 had a squamous cell carcinoma (SCC) removed from the right temple in 2003. This patient, described previously (3), was receiving immunosuppression following liver transplantation. Patients 16 and 37 had basal cell carcinomas removed from the face and patient 37 also had a SCC removed from the face.

Lymphocyte subsets

The median $CD3^+CD4^+$, $CD3^+CD8^+$ and $CD19^+$ lymphocyte counts measured between 157 and 180 months since the first dose of CAMPATH-1H were 0.59, 0.275, and 0.13 x 10^9 / L respectively (Table 1). In 2001 we reported equivalent values of 0.185, 0.095 and 0.115 x 10^9 / L, 73 to 84 months post-therapy. This comparison demonstrates strong recovery of CD4+ T-cells in the intervening years, less marked recovery of CD8+ T-cells and surprisingly little movement in B-cell counts. It is particularly notable that most patients now have a CD3+CD4+ lymphocyte count within the reference range. CD3+CD8+ T-cell counts and NK cell counts remain sub-normal in approximately 50% of subjects, and B-cell counts are low in the majority. CD56+ NK cell counts were not previously reported.

DISCUSSION

The development of CAMPATH-1H for RA was curtailed largely secondary to anxieties surrounding prolonged therapy-induced lymphopenia. At the time of the first CAMPATH-1H trials in RA, the prevailing dogma was that full reconstitution should

be expected following immune ablation. Subsequent studies, encompassing a variety of conditions, have emphasised factors which challenge that assumption (7) and our own work in RA has highlighted potential disease-specific factors such as impaired thymic function and defective immune homeostasis (8, 9). Ours is the longest reported follow-up of autoimmune patients following CAMPATH-1H and it is not yet possible to state with certainty whether the extreme delay in lymphocyte reconstitution is disease-specific (7, 9, 10). In this context it is reassuring to see CD4+ T-cell counts have now entered the normal range for the majority of surviving patients. Our previous data did not suggest an association between depth of lymphopenia and mortality or morbidity, so this is unlikely to represent 'survival of the immunologically fittest'. There remain a significant number of patients with subnormal numbers of circulating CD8+ T-cells, B-cells and NK cells (Table 1).

Our mortality statistics emphasise the poor outlook for RA patients with refractory disease at the end of the last century (11). Overall 48% of patients (combined cases and controls) had died at a median of 12.65 years since initial treatment, when the median age was approximately 54 years. There was no significant difference in mortality between cases and controls, who were selected from the EULAR database of RA patients treated with immunosuppressive drugs, mainly azathioprine or cyclophosphamide (Figure 1). The mortality rates in both cases and controls are at the higher end of published statistics for RA cohorts, consistent with the fact that these patients fall at the severe end of the disease spectrum. As per our previous publication, there was no difference in mortality according to the total dose of CAMPATH-1H received or the number of courses, some patients receiving up to 3 courses of treatment (data not shown). The leading causes of death were those commonly seen in a cohort of RA patients: diseases of the circulatory, respiratory

and digestive systems. Diseases of the musculoskeletal system (RA in 5, septic arthritis in 2) featured prominently as a cause of death amongst controls but not cases. Deaths from neoplasms were more common in cases than in controls, although this did not reach statistical significance. One CAMPATH-1H recipient developed non-Hodgkin's lymphoma, a known complication of severe RA, but the other tumours witnessed are not specifically associated with immunosuppressive conditions. Although these data might suggest a mild and selective immunodeficiency, mortality rates secondary to diseases of the digestive system had a similar incidence to neoplasms in cases and controls. These are more difficult to reconcile with an immunodeficiency state, suggesting that both statistics could represent chance findings in a small cohort of patients. It should also be noted that most CAMPATH-1H recipients subsequently received additional DMARDs.

As with mortality, parameters of morbidity did not distinguish CAMPATH-1H recipients from other RA cohorts with refractory disease. Apart from a single case of disseminated Herpes zoster, no opportunistic infections were reported and the commonest sites for infection requiring intervention were the lungs, the skin (particularly lower extremities) and the joints. A large number of orthopaedic procedures were performed, the vast majority without infectious complications. Morbidity data are not available for our control cohort but in a recent study from the British Society for Rheumatology Biologics Registry, anti-TNF therapies were also associated with an increased incidence of serious infections affecting the skin and soft tissues (12). Three of our cases were using TNFα blockade at the time of follow-up and several others had received these treatments in the interval since CAMPATH-1H treatment (data not shown)

Our approaches to immunomodulation of autoimmune disease are becoming increasingly sophisticated. TNFα blockade, costimulation blockade and B-cell depletion are widely practised in RA and, increasingly, in other autoimmune conditions. As well as providing valuable new treatment opportunities, these therapies offer novel insights into immune physiology. One of these is that the human immune system has a high level of in-built redundancy - despite the potency of these therapies, infectious complications have been uncommon. Although our own experience is necessarily limited, it is one of the most remarkable: CAMPATH-1H rendered our patients pan-lymphopenic and it has taken up to12 years for them to regain normal lymphocyte counts; yet they have not appeared overtly immunosuppressed beyond the first few weeks after therapy. We have previously rehearsed the possible explanations for their immune sufficiency (3).

In conclusion, we report long-term follow-up of patients that received one of the first biological therapies for RA. Despite profound biological effects of therapy, our patients have continued to behave like a cohort of patients with refractory RA. They have not displayed increased mortality or unusual morbidities. Notwithstanding the limited size of the original cohort, our data are reassuring in an era when potent biological therapies are being increasingly introduced for the management of RA (2).

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TABLE 1. Duration of follow-up, current therapy and lymphocyte counts of patients included in current analysis (20 interviews, 5 case note analyses; patients 1, 24, 44 and 50 died during the follow-up period).

Patient reference number and sex	Age at first treatment (y)	Duration of follow-up (y)	Current DMARDs	Current* CD4+ count (x 10^9/L)	Current CD8+ count (x 10^9/L)	Current B-cell count (x 10^9/L)	Current NK cell count (x 10^9/L)
1F	69.00	13.25	Nil	0.68	0.59	0.16	0.12
3F	54.08	12.00	Nil	0.26	0.31	0.02	0.23
4F	60.50	11.92	MTX, INF	0.52	0.16	0.18	0.09
5F	34.25	11.83	Aza	0.77	0.79	0.11	0.01
6F	49.42	11.93	MTX	N/A	N/A	N/A	N/A
10M	45.75	10.50	MTX, IM gold	0.31	0.26	0.03	0.12
14F	45.83	12.67	sc MTX	0.37	0.20	0.19	0.18
15M	47.75	11.92	N/A	N/A	N/A	N/A	N/A
16F	53.92	11.58	MTX	0.41	0.50	0.03	0.33
17F	43.17	11.42	Nil	0.76	0.57	0.22	0.08
18M	58.67	11.92	Nil	0.42	0.24	0.02	0.19
21F	25.50	10.50	MTX	0.49	0.11	0.07	0.09
24F	61.33	12.67	Aza	0.66	0.23	0.19	0.01
26F	48.00	12.00	MTX, HQ	0.13	0.02	0.03	0.09
27M	34.50	12.42	ETN	0.38	0.12	0.36	0.25
29F	59.75	12.83	Nil	0.72	0.20	0.15	0.08

31F	43.75	12.02	MTX, Cic	N/A	N/A	N/A	N/A
32F	53.58	11.50	Cic	0.96	0.63	0.23	0.21
37F	64.50	11.58	Aza	0.79	0.33	0.06	0.02
38F	64.42	11.83	ETN	0.68	0.51	0.11	0.27
41F	52.75	10.83	Nil	0.46	0.14	0.05	0.09
43F	71.67	12.67	N/A	N/A	N/A	N/A	N/A
44F	53.08	11.00	Nil	N/A	N/A	N/A	N/A
46F	47.50	11.75	MTX	1.68	0.58	0.40	0.08
50M	41.08	11.83	Aza, HQ	0.29	0.26	0.09	0.02

N/A, data not available; MTX, methotrexate; Aza, azathioprine; HQ, hydroxychloroquine; cic, ciclosporin; INF, infliximab; ETN, etanercept

* Normal ranges for lymphocyte counts:

CD3+CD4+, 0.3 - 1.4 x 10^9/L

CD3+CD8+, 0.2 - 0.9 x 10^9/L

CD19+, 0.1 - 0.5 x 10^9/L

CD56+, 0.12 - 0.88 x 10^9/L

TABLE 2. Mortality data for CAMPATH-1H recipients

A. Mortality from 01/01/1999 to 09/03/2006

Patient reference number	Age at 1st dose (years)	Time from 1st dose to death (years)	Cause of death
1	68.9	13.5	Ischemic heart disease, RA
2	48.8	5.9	Primary sclerosing cholangitis, RA, Crohns Disease
7	57.1	9.5	Metastatic leiomyosarcoma of stomach
11	54.2	10.1	Bowel infarction secondary to atrial fibrillation
20	64.3	6.2	Ischemic heart disease
22	62.3	8.8	Upper gastro-intestinal bleed
24	61.3	13.4	Ischemic heart disease
30	55.4	8.8	Bronchopneumonia, multi infarct dementia
39	66.5	4.8	Sigmoid bowel perforation (non-malignant)
40	50.1	11.6	Bronchopneumonia
44	53.1	11.0	Ischemic heart disease
45	71.2	7.4	Bronchopneumonia, pulmonary fibrosis
49	68.3	12.6	Ischemic heart disease
50	41.1	12.7	Small cell carcinoma of lung

B. Causes of death according to ICD code for entire cohort.

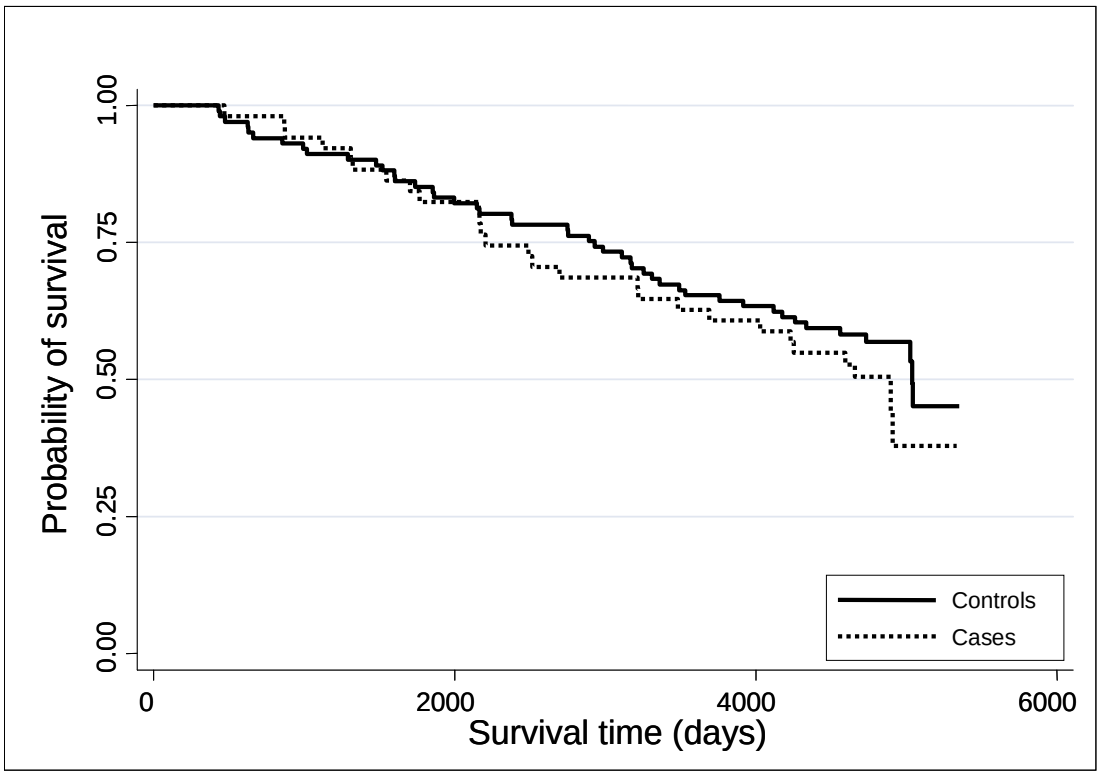
ICD label	controls (n=101)	cases (n=51)
Infectious & parasitic	1	1
Neoplasms*	3	5
Blood	1	0
Endocrine, nutritional & metabolic	4	0
Circulatory	16	10
Respiratory	7	5
Digestive	3	5
Musculoskeletal	7	1
Genito-urinary	1	0
Injury & Poisoning	1	0
External causes	1	0
Unknown causes	1	0
Total	46	27

* One case each of non-Hodgkin's lymphoma, lung squamous cell carcinoma, breast adenocarcinoma, colon adenocarcinoma and stomach leiomyosarcoma

FIGURE LEGENDS

Figure 1. Kaplan-Meier survival plots for 51 rheumatoid arthritis patients who received CAMPATH-1H (cases) and 101 matched controls from the EULAR database.

FIGURE 1.


Probability of survival
0.00
0.25
0.50
0.75
1.00
0
2000
4000
6000
Survival time (days)
Controls
Cases