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Mortality in Cushing's disease more than 10 yrs after remission – a multicentre study

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Background Despite several recent studies no agreement has been reached on the long-term survival in Cushing's disease (CD). We studied life expectancy in patients with CD whose hypercortisolism remained in remission for more than 10 yrs. Secondly we aimed to identify factors determining survival.

Methods Individual case records from specialist referral centres in UK, Denmark, Netherlands, and New Zealand were reviewed. Mortality rates were compared between CD and the general population and expressed as standardised mortality ratio (SMR). There were no significant differences in demographic data, duration of follow-up, comorbidities, treatment number or type, between women and men, so sexes were pooled for survival analysis.

Findings 51 (16%) of the cohort had died. Median survival from study entry (10 yrs after cure) was similar for women (31 yrs) and men (28 yrs). The overall SMR was 1·61 (95% CI: 1·23-2·12) (P< 0·01). The SMR for circulatory disease was increased at 2·72 (95% CI: 1·88-3·95) (P<0.01), but deaths from cancer were not higher than expected (SMR=0·79, 95% CI: 0·41-1·51). Presence of diabetes, but not hypertension, was an independent risk factor for mortality (hazard ratio=2·82, 95% CI 1·29-6·17). A step-wise reduction in survival with increasing number of treatments was observed. *Patients cured by pituitary surgery alone had normal long-term survival (SMR = 0.95 (95% CI : 0.58-1.55)*

Interpretation Cushing's disease patients who have been in remission for more than 10 yrs are still at increased risk of overall mortality compared to the general population, particularly due to circulatory disease. Notwithstanding this median survival from cure is excellent at about 40 yrs. Treatment complexity, reflecting more difficult to control disease, appears to impact negatively on survival.

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Introduction

The predominant cause of Cushing's syndrome, after exclusion of exogenous glucocorticoid usage, is *pituitary ACTH dependent form also defined as Cushing's disease (CD) general references (1-4)* which accounts for around *70-85% of cases (3).* Although rare, with an incidence reported to 2-3/million/yr – the variation partly or wholly depending on the stringency of diagnostic criteria. *(3).* Some studies suggest CD is becoming more common due to greater awareness of its impact on vascular disease, diabetes mellitus, hypertension, opportunistic infections, and thromboembolic disease leading to more frequent screening. CD is most often insidious in onset and if undiagnosed and untreated leads to vastly increased morbidity and premature mortality. Further, population-based studies in older normal persons reported a significantly higher risk of death from cardiovascular disease for those with urinary free cortisol excretion (hazard ratio=5·0)[5] or salivary cortisol concentration (hazard ratio 1·6-1·8[6] in the highest versus those in the lowest tertile. Furthermore, in a large study of UK civil servants followed prospectively the hazard ratio for cardiovascular death was 1·9 for those with the slowest rate of fall in salivary cortisol throughout the day.[7] Moreover, increased all cause and cardiovascular mortality has been demonstrated in apparently non-Cushingoid subjects with adrenal incidentalomas whose cortisol levels fail to completely suppress with dexamethasone.[8,9] The above evidence points to mild subclinical and chronic elevations in cortisol production being deleterious with respect to longevity. Several cohort studies[10-18] have examined whether mortality in CD is influenced by restoration of eucortisolism or not with variable results. The first metaanalysis of the early studies showed an overall standardised mortality ratio (SMR) of 2·2.[13] Pooled SMR (1·2) for patients in remission showed no significant increase in mortality which, however, was markedly increased for patients with persistent disease (SMR=5·5). Persistent disease, older age at diagnosis, treatment of hypertension and diabetes mellitus were the main determinants of increased mortality. *Subsequent to that metaanalysis(13) there have been four further studies, from New Zealand (14), Birmingham (15),Oxford (17) and Bulgaria (18), all confirming that patients in initial remission have better mortality outcome than those who do not achieve remission initially. When these were included in a recent metaanalysis(19) pooled SMR for patients in initial remission was 2·5 but there was considerable heterogeneity reflected by a wide range of SMR's (0·3-10).* The individual studies contributing to the metaanalysis varied considerably in their definitions of remission and relapse and more importantly at what time after initial treatment remission was deemed to have occurred. In some instances remission was defined within a few weeks and in others up to 3 yrs dependent on the type of treatment (see refs. 13 & 19 for details). *Moreover, a major limitation of all these individual studies was that the division of the cohorts was based on remission immediately after initial treatment with no information provided on relapse/recurrence of hypercortisolism during follow-up to census date, which could clearly drive mortality especialy from vascular disease.* Therefore, *in an attempt to remove these issues, minimise subject heterogeneity,* and determine the very long-term survival for CD in remission the present multicentre study included only those patients who survived 10 yrs or more from the time of curative treatment and were in remission up until the census date or their death.

Materials and Methods

We performed a multicentre cohort study, the primary endpoint being mortality. The present study used an individual patient data approach combining data into a single database. Contributing centres were Birmingham UK; Oxford UK, Stoke-on-Trent UK; Leiden, Netherlands; Aalborg, Denmark; Auckland, New Zealand. Census dates at which the data were frozen were, 31/12/2013 (Birmingham), 31/12/2009 (Oxford), 1/12/2014 (Stoke-on-Trent), 1/1/2014 (Leiden), 1/1/2012 (Aalborg), 1/1/2012 (Auckland) or

earlier, respectively. *The patients included were all part of previous peer-reviewed publications (10,11,13,14,15,17) and managed at tertiary referral centres employing extant 'best practice' management to national and international standards of the time, so are likely to reflect national data. Unlike for acromegaly, there are no national CD registries/datasets.* Inclusion criteria were CD, cured of hypercortisolism for a minimum of 10 yrs at entry and continued cure *with no relapses of hypercortisolism* until database was frozen or death. *Definitions of 'cure' from each centre are provided in supplementary Table 1).* Follow-up for the present analyses started after 10 yrs of cure *and no patients were lost to follow-up*. The number or type of treatment to achieve cure was identified from the files with cure being defined after the last treatment. In the case of radiotherapy if this was given prophylactically to reduce the risk of Nelson's syndrome after bilateral adrenalectomy, the date of adrenalectomy was taken as cure date. The diagnostic criteria for CD and definition of remission could not be standardised and relied on those used by the individual centres which have all been published in their respective publications.[10,11,13,14,15,17] *(and see supplementary Table 1)* Similarly, treatment modalities varied between centres and with time. This was a retrospective study and initial diagnosis and treatment was from as early as 1958 (Stoke) through to 1997 (Leiden), see individual studies for details. In addition to demographic data and primary endpoint status of alive/dead and cause of death (from National Registry Offices or patient records) information was collected on initial treatment, subsequent treatments and dates thereof, radiotherapy usage, glucocorticoid and thyroxine replacement, and on treatment for hypertension and/or diabetes mellitus.

Statistical analysis

Continuous data are summarised as medians and ranges and discrete data as proportions. Survival data were graphically displayed using Kaplan-Meier plots and analysed using Cox regression. Factors associated with survival were examined individually with Cox regression analysis which included age *at baseline* as a covariate. Significance is taken as $P<0{\cdot}05$ and confidence intervals, *with no correction for multiple testing*, are presented. *Only age at study entry is included in the models, the other age variables given for information. There was no significant departure from proportional hazards assumptions for any of the variables.* Standardised mortality ratios (SMRs) were calculated as the sum of the observed over the sum of the expected number of deaths in the entire cohort. *Reference mortality rates were obtained via the Human Mortality Database for the relevant countries. Expected numbers of deaths used in the calculation of SMRs were calculated using standard cohort techniques described in detail in Breslow & Day (20). Briefly, expected numbers were derived by aggregating the person-years in each sex, age and calendar year strata and then multiplying the person-years by the corresponding mortality rate for the general population of the relevant country. Expected numbers were then summed across the strata and SMRs calculated as the sum of observed number of deaths over the sum of the expected number of deaths. When calculating SMRs the underlying cohort was stratified into 1-year age and 1-year calendar bands (both considered time dependent variables). All cause population mortality rates were available for the years: Denmark (1901-2011), New Zealand (1948-2008), Netherlands (1901-2012) and UK (1922-2013).* All analyses were carried out using Stata 13 (Stata v 13.1, Stata Corp, College Station, Texas).

Results

Baseline characteristics

There were 320 patients with 3790 person years of follow-up from 10 yrs after cure with a female:male ratio of 3:1. There were no significant differences between women and men with respect to any of the parameters shown in Table 1, so data from the sexes were pooled for survival analysis and predictors

thereof. The proportions with diabetes, hypertension, and on glucocorticoid replacement were similar in women and men. Treatment modalities are shown in Table 2, with no difference between women and men. Over 80% of the cohort were initially treated with transsphenoidal pituitary surgery with 65% requiring only this modality to achieve long-term cure. However, 35% required additional treatment. Less than 10% had bilateral adrenalectomy or external radiotherapy as first treatment and these were those who entered the study in the early yrs (pre 1975). Only 13% of the cohort required three or more treatments to achieve cure. *The average parient follow-up was 11.8 years from study entry and was no different between countries. (see Table 4).*

Mortality

In total 51 (16%) subjects died during follow-up from study entry (10 yrs post cure). The proportion was the same for women and men.(NS). Median (50%) survival for women was 31 yrs from study entry and 28 yrs for men (ns) (Fig 1A). The median age at death was 69 yrs for women and 65 yrs for men and average time to death from 10 yrs post cure was 14 yrs. *Among those patients who died there was no gender difference in age at cure; age at study entry, age at death; or duration of follow-up (Table 3). However, when the sexes were combined and the deceased group compared with the alive group there were differences: age at cure (medians and ranges): alive=34 (8-73) yrs vs dead= 42 (14-68) yrs P <0.001 (Wilcoxon rank sum test); age at study entry: alive= 44 (18-83) yrs 52 (24-78)yrs P<0.005; duration of follow-up from study entry: alive = 11 (1-47) yrs vs dead = 14 (1-38) yrs P <0.005.* The causes of death were predominantly circulatory (N=30) *(included cardiovascular and cerebrovascular disease, ruptured aortic aneurysm, plus pulmonary embolism)* in both women and men but all cancer deaths (N=9) occurred in women. There were only two recorded deaths from sepsis despite the fact that 68% of the cohort was on glucocorticoid replacement.

The standardised mortality ratio (SMR) for all cause mortality was 1·61 (95% CI: 1·23-2·12) indicating a 61% increase in mortality risk (Table 4). In absolute terms this translates to five excess number of deaths per 1000 persons per yr beyond that what would be expected based on general population mortality rates. There was evidence for heterogeneity ($P_{heterogeneity}$=0·03) between countries with Netherlands showing a low and New Zealand a high absolute observed risk (Table 4). *This heterogeneity disappeared after adjustment for pituitary surgery only (see supplementary Table 2) with proportionately fewer patients from New Zealand having pituitary surgery. Although men did not have an increased SMR (1.1; P=0.75) and women did (SMR = 1.81 p= <0.01) this gender difference was not significant ($P_{heterogeneity} = 0.14$).* The SMR for circulatory disease was 2·72 (95% CI: 1·88-3·95; P<0.01) *with no heterogeneity between countries (P=0.22). There was no significant variation in circulatory SMR by time since study entry ie. duration of follow-up. Mortality from cancer SMR = 0·79 (95% CI: 0·41- 1·51); P=0·41) was not increased. There was no significant variation in circulatory SMR stratified by time since study entry, ie. no evidence for increased risk of circulatory deaths in first 10 years after study entry than in subsequent years. There was a tendency for increased SMR with longer follow-up (>20yrs) although this did not reach significance. We examined SMR by decade of treatment but no significant trend was observed. We examined SMR by year of diagnosis pre 1985 and post 1985, this year being chosen as about midway from the earliest patient entry (1957) and the close of databases (average 2012). Results were pre-1985 obs/exp 28/16.2 SMR = 1.73 (95% CI 1.15-2.5; P = 0.004); sfter 1985 obs/exp 23/14.5 SMR = 1.59 (95% CI 1.01-2.39; P <0.03); $P_{heterogeneity} = 0.77$ (NS) between groups.*

Factors influencing mortality

Overall survival is depicted in the Kaplan-Meier plot in Fig 1A. Half the patients survived for 25 yrs or more from study entry (i.e. 35 yrs from cure). Factors associated with survival were examined with

multivariate Cox regression analysis, including age at baseline as a covariate. Presence of diabetes had a hazard ratio of 2·82 (95% CI 1·29-6·17) (P<0·005), hypertension 1·59 (95% CI 0·77-3·31 NS). *Patients not taking glucocorticoid treatment had an SMR =1.01 (95% CI 0.54-1.89) no different from that of the general population compared to patients taking glucocorticoids SMR= 1.99 (95%CI = 1.45-2.72) P = 0.04 (Table 4). 57% of patients having pituitary as only treatment were on steroids, while the proportion who received other treatments was 79%, a highly significant difference (p<0.001, Chisq = 13.5). When multivariate analysis (Cox regression) was applied the effect of being on glucocorticoids was no longer significant, either alone or with pituitary surgery in the model. Thyroxine was not a risk for mortality. Being on sex hormones was not a risk factor, but data were only available for 50% of the cohort.* There was an association between mortality and number of treatments, with higher risk with multimodal treatment (Fig 1B). Hazard ratios were 1·77 (95% CI 0·93-3·38) for two vs one treatments (P=0·08), and 2·6 (1·15-5·87) for three vs one treatments (P=0·02). Median survival times from study entry were: one treatment=33 yrs (95% CI 26-38); two treatments=27 yrs (19-28); three treatments 21 yrs (17-21). *When considered by centre Stoke, Oxford, and Auckland had the lowest proportion of patients receiving one treatment and the highest receiving three treatments (supplementary Table 2). There was a significant difference (P <0.001 Anova) in the number of treatments between centres. Patients treated by pituitary surgery as first and only treatment had a normal SMR (0.95) vs those who did not (2.53) the difference being highly significant (P <0.001). There was no heterogeneity between countries (supplementary Table 3). Moreover* patients cured by pituitary surgery only had a longer survival of 31 yrs vs 24 yrs if radiotherapy had been required at any time (P=0·03). Median survival for patients requiring bilateral adrenalectomy at any time was not reached 50% though for 75% survival it was 17 yrs vs 26 yrs for pituitary surgery only (P=0·1).

Discussion

The very long-term outcome of successfully treated CD is until now uncertain. Short-term studies[10-18]and metaanalyses have produced conflicting results[13,19], not least because these studies were very heterogeneous with respect to the timing after initial treatment and how cure/remission was defined, and the duration of follow-up was relatively short. The first meta-analysis by Clayton et al.[13] suggested that SMR was not significantly increased, provided that patients achieved *initial remission*. But we were cautious in our conclusion stating 'patients in remission do not appear to have worse mortality but this should be regarded as preliminary'. *Indeed, this caution was justified since two later studies one from New Zealand (14), and one from Oxford (17) showed significantly increased SMRs for patients with CD in remission, although a large study from Bulgaria (18) did not. It is not clear why there is such a discrepancy between these later reports. The latest meta analysis with these three studies included (19) shows that the overall SMR for CD initially in remission was 2.5 (95% CI = 1.4-4.2) and there was evidence of significant heterogeneity. In an attempt to clarify* this issue patients in the present study were deliberately selected whose hypercortisolism had been cured for at least 10 yrs *before* study entry and remained cured for the duration of follow-up until census date or death. With this selected cohort of over 300 subjects and over three and a half thousand person years of follow-up since 10 years after cure we show that overall excess mortality is *60% (SMR = 1.6) higher than* that of the general population from which they derive. *However, what we show here is that within cohorts of CD patients treated with different and several modalities over the years the mortality for patients treated with pituitary surgery alone have normal life expectancy. (SMR = 0.95). This is similar to the reports from purely surgical series from the USA with much shorter follow-up (Swearingen et al, Hammer et al). Overall 16% of the cohort died and the median age at death was >65 yrs, similar to that from earlier studies.The duration of follow-*

up for those who died was slightly longer(3yrs) than for those still alive, although there was no sex difference. The significance of this observation is uncertain. Interestingly, the SMR for men was not increased while it was for women. However, this SMR difference was not significant and should be interpreted with caution as there were only a quarter of deaths in men and the proportion of men that died was the same as for women. This indicates that more data are required for men. There were no differences between women and men with respect to demographic data, treatment modalities or number of treatments, substitution therapy with glucocorticoid, or comorbidities of diabetes or hypertension, so the sexes were well-matched and so were combined for survival analysis and predictors of survival.
Compared to our published metaanalysis[13] and that of van Haalan et al.[19] the upper confidence limit for SMR *in the present* study was reduced from 3 to 4 to 2·3, and in absolute terms the excess mortality is small amounting to 5/1000 patient years of follow up. Moreover, duration of survival after cure in this highly selected cohort was long at about 40 yrs.

Cause of death was predominantly circulatory which includes deaths from ischaemic heart disease, stroke, ruptured aortic aneurysms, and pulmonary emboli. There were too few deaths to analyse these seperately within this broad category or to compare meaningfully between the sexes. This confirms previous studies wherein cardiovascular disease was the main cause of the excess mortality. Cancer was not a contributor to excess mortality, which is in keeping with data from studies of patients with other forms of pituitary tumours wherein cancer deaths are not increased (reviewed in 25). As regards the cancer deaths in women these were from a variety of primary sources with none predominating.

Perhaps surprisingly given that two thirds of the cohort was receiving glucocorticoid replacement therapy there were only two deaths directly attributed to disseminated sepsis. When considering what drives the excess overall and circulatory mortality the total duration and degree of exposure to hypercortisolism may well be important. This is determined by several factors such as, but not exclusively, (1)time taken to reach a diagnosis which may be several years for a rare and insidious condition. There is no literature on this; (2) time taken from treatment to 'cure' which will depend on treatment number and modality; (3) post-treatment requirement for glucocorticoids replacement. In respect to the last point there is one published paper in acromegaly patients that higher dose of glucocorticoid treatment was an independent predictor of mortality (23). In our study data were not available on type, dose, frequency, or duration of glucocorticoid treatment. Were it available the variability thereof between centres and over an extended time period is unlikely to result in a large enough subgroup for meaningful analysis. And although we show an increase in SMR in patients treated with glucocorticoids this is not an independent risk factor in Cox regression analysis. Accordingly, at present we do not have a definitve answer to this important issue. Fewer patients who had pituitary surgery as only treatment were on steroids compared to other treament modalities. There is also evidence that hypogonadism and growth hormone deficiency are independent risk factors for mortality in hypopituitary patients (23,24), although in these studies patients with CD were excluded. In our study data were not available on growth hormone treatment, although for many GH was not available. Data were only available on gonadal hormone replacement in half the cohort so no meaningful conclusion can be drawn..
As in previous studies and summarised in our metaanalysis(13) treated diabetes conferred an independent risk for death, though hypertension did not. There is no obvious explanation for this difference given that half the cohort was receiving treatment for hypertension. The most interesting observation with regards to mortality risk was the clear trend to reduction in survival with increasing number of treatments required to achieve cure. Half those requiring only one treatment survived for 33 yrs from 10 yrs post cure while for those requiring three treatments the equivalent 50% survival was reduced by a third (21 yrs). This is

perhaps not surprising as the number of treatments reflects the difficulty controlling hypercortisolaemia, and perhaps also the severity thereof. In 60% of patients the only treatment modality was pituitary surgery and these patients had the best outcome.

There was significant heterogeneity between countries with respect to all cause SMR. This heterogeneity disappeared when pituitary surgery was entered into the multivariate analysis model and may largely be accounted for by patients from New Zealand having the lowest proportion of ptients treated by pituitary surgery. There is a hint that SMR is increased in patients with longest follow-up (>20 yrs). These are the patients treated in the early decades of the study, and largely came from, Stoke-on-Trent, Oxford and New Zealand. These centres had the highest proportion of subjects requiring three treatments to achieve cure, which we show impacts negatively on survival. This may also be part of the explanation for the heterogeneity. However we were not able to demonstrate an effect of decade of treatment on SMR. Because of the small numbers separate analyses of number of treatments by individual centre was not feasible.

The study limitations are: (1) Cohort study of (deliberately) highly selected patients who would be expected to be the healthiest and do well having already survived 10 yrs after cure before study entry which limits generalisability of the study, (2) Variable criteria for cure between centres which were highlighted in the metaanalyses.[13,19], (3) We did not consider shorter remission duration of say 5 yrs before study entry which would include more patients and may have produced different outcomes. (4) Significant heterogeneity in SMRs from individual centres *may limit the strength of the study, though this was eliminated when pituitary surgery was accounted for*. (5) It was decided to exclude patients with a recurrence after 10 yrs of cure. From a methodological point of view this can be considered a form of selection bias (by selection on future events related to the outcome, but this selection was applied to assess the effect on mortality unrelated to Cushing recurrence. Importantly, the exclusion of patients with such a late recurrence is likely to have underestimated the increased mortality risk in the whole cohort.

Advantages: (1) Large numbers of patients with prolonged follow-up of a rare disorder accurately defined and meticulously followed at referral centres experienced in dealing with this condition, (2) Rather than a meta-analysis of pooled patient data we obtained individual patient data from each participating centre, and (3) By obtaining results from geographically diverse regions these results are likely generalizable, at least to a *Western European* population.

What this study adds to the literature is a cohort of CD patients, treated by a variety of modalities, who have been 'permanently cured' with no recurrence, and no subjects lost to follow-up, for a very long time, and still shows such subjects at increased risk for all cause and circulatory mortality. This is in a setting of 'best' clinical practice available at the time. However, within this overall cohort patients treated by pituitary surgery alone have normal long-term survival. We believe this to be a defining study on this topic, as a prospective study on this scale and for this duration is unlikely to be available for several years.

What can we tell our patients with CD disease about their long-term survival prospects? From this study we can say that if you are cured for 10 yrs and remain without recurrence of hypercortisolism you will have a 60% increased risk of dying sooner than your peers, though in absolute terms this risk is small. Your survival after cure is 'on average' 40 yrs. However, should you be 'cured' by pituitary surgery alone your outcome *appears to be excellent*. You will, however, require life-long follow-up at an experienced centre having regular checks for diabetes, hypertension, and other cardiovascular risk factors.

Table 1.

Basic demographics of the cohort N=320
Values are medians and range; number and proportion

	Females (246-77%)	Males (74-23%)
Age at study entry	46 (18-84)	43 (20-80)
Age at cure	36 (8-74)	33 (10-70)
Age at census date	58 (24-86)	53 (25-90)
Number with diabetes*	24 (9·8%)	7 (9·5%)
Number with hypertension*	125 (50·8%)	39 (52·7%)
Number on steroid treatment•	158 (64%)	52 (72%)
Number on thyroxine treatment◆	95 (39%)	28 (38%)

*Defined as being on treatment at census date or death
•Information missing in 7 females and 2 males
◆Information missing in 28 females and 10 males
There were no significant differences between the sexes in any variable

TABLE 2. Treatments by gender (N = 320)

	Females (246-77%)	Males (74-23%)
Pituitary surgery only	144 (58.6%)	45 (60·1%)
Pituitary surgery + bilateral adrenalectomy	40 (16%)	12 (16·2%)
Pituitary surgery + radiotherapy*	20 (8%)	5 (6·8%)
Bilateral adrenalectomy only	7 (2·8%)	2 (2·7%)
Bilateral adrenalectomy + radiotherapy	15 (6%)	5 (6·8%)
Radiotherapy only*	16 (6·5%)	5 (6·8%)
Radiotherapy + bilat adrenalectomy + pituitary surgery	4 (1·6%)	0 (0%)
Pituitary surgery as first treatment	206 (83%)	62 (83·8%)
Bilateral adrenalectomy as first treatment	22 (8·9%)	7 (9·5%)
Radiotherapy as first treatment	20 (8·1%)	5 (6·7%)
Number of treatment modalities		
One	160 (65%)	47 (63%)
Two	54 (22%)	17 (23%)
Three or more	32 (13%)	10 13%)

**Footnote . In these patients medical therapy with metyrapone +/- aminoglutethamide was used for variable time periods whilst awaiting effect of radiotherapy.*

TABLE 3. Summary statistics for deaths by gender

	Females (246)	**Males (74)**
Number of deaths	41 (16·7%)	10 (13·5%)
Age at cure yrs (deceased)	42 (14-68)	43 (14-60)
Age at entry yrs (deceased)	52 (24-78)	53 (24-70)
Age at death yrs	69 (48-91)	66 (45-79)
Duration F-U to death* (yrs)	14 (1-37)	16 (3-28)
Duration F-U alive pts* (yrs)	11 (1-37)	11 (1-47)
Causes of death		
Cardiovascular$	18 (44%)	8 (80%)
Cancer^	9 (22%)	0 (0%)
Pulmonary embolism	2 (5%)	2 (20%)
Disseminated Sepsis	2 (5%)	0 (0%)
Other/unknown°	10(24%)	0 (0%)

All values are median and range

* from study entry ie 10 yrs after remission of hypercortisolism, add 10 yrs to each for TOTAL follow-up from time of remission

° Incudes: acute asthma (1), fibrosing alveolitis (1), chronic obstructive pulmonary disease/ bronchopneumonia (2), acute bronchitis (1), 'old age' (1), unknown (2)

^ *Cancer types were: lung (2): nasopharyngeal (1); bladder (1); metastatic uterine (1); malignant small bowel (1); pancreatic (1); meningioma (1); metyastatic primary unknown (1).*

$ includes 6 cerebrovascular related deaths, and 3 from ruptured aortic aneurysm

Table 4. Standardised mortality ratios (SMRs) with date of entry as date of 'cure' plus 10 years

	Subjects (%)	Obs/Exp	SMR (95%CI)	P_{value}	AER*
Overall	320 (100%)	51/31.7	1.61 (1.23, 2.12)	<0.01	5
Country					
England	135 (42.2%)	15/9.3	1.62 (0.98, 2.69)	0.06	4
New-Zealand	75 (23.4%)	20/7.3	2.73 (1.76, 4.23)	<0.01	14
Netherlands	36 (11.3%)	4/6.0	0.66 (0.25, 1.77)	0.41	0
Denmark	74 (23.1%)	12/9.1	1.33 (0.75, 2.33)	0.33	4
$P_{heterogeneity}$				0.02	
Sex					
male	74 (23.1%)	10/9.0	1.11 (0.60, 2.06)	0.75	1
female	246 (76.9%)	41/22.6	1.81 (1.33, 2.46)	<0.01	6
$P_{heterogeneity}$				0.14	
Year of cure					
<1985		28/15.9	1.76 (1.17, 2.54)	0.003	7
>=1985		23/14.7	1.57 (0.99, 2.35)	0.032	4
				0.68	
Follow-up					
10-14 yrs		8/7.8	1.03 (0.51, 2.06)	0.94	0
15-19 yrs		13/8.4	1.55 (0.90, 2.67)	0.12	4
20-24 yrs		15/7.1	2.12 (1.28, 3.51)	<0.01	12
25+ yrs		15/8.4	1.79 (1.08, 2.96)	0.03	12
P_{trend}				0.17	
Pituitary surgery					
No		35/13.8	2.53 (1.82, 3.53)	<0.001	11
Yes		16/16.8	0.95 (0.58, 1.55)	0.84	0
$P_{heterogeneity}$				<0.001	

Steroids				
No	10/9.9	1.01 (0.54, 1.89)	0.96	0
Yes	39/19.6	1.99 (1.45, 2.72)	<0.001	8
$P_{heterogeneity}$			0.04	

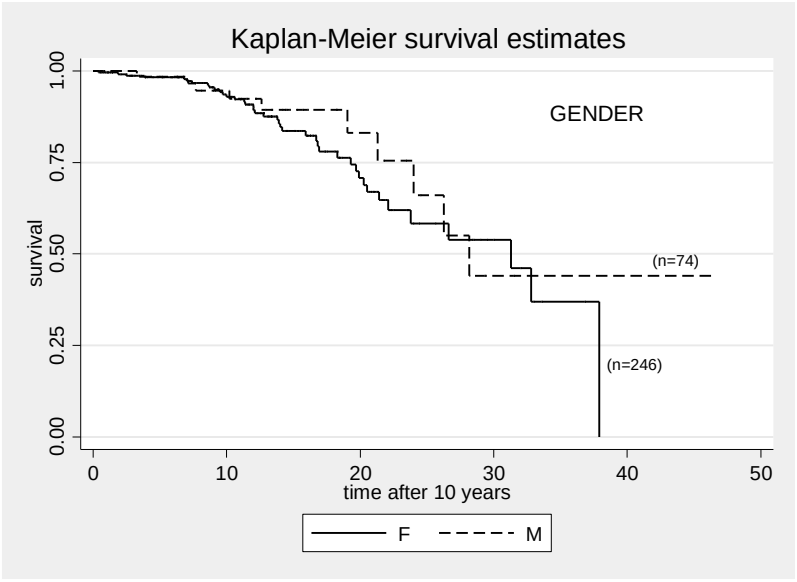
pyrs: accumulated number of person-years; obs: observed number of deaths; exp: expected number of deaths;

SMR: standardised mortality ratio; AER: absolute excess risk, i.e. excess number of deaths

*per 1,000 person-years

The average follow-up after 10 yrs is derived by dividing person/yrs by number contributing to this and was 11.8 overall. There was no difference between countries : England = 11.7yrs; New Zealand = 12 yrs; Netherlands = 12.8 yrs; Denmark = 11.2 yrs

A



B

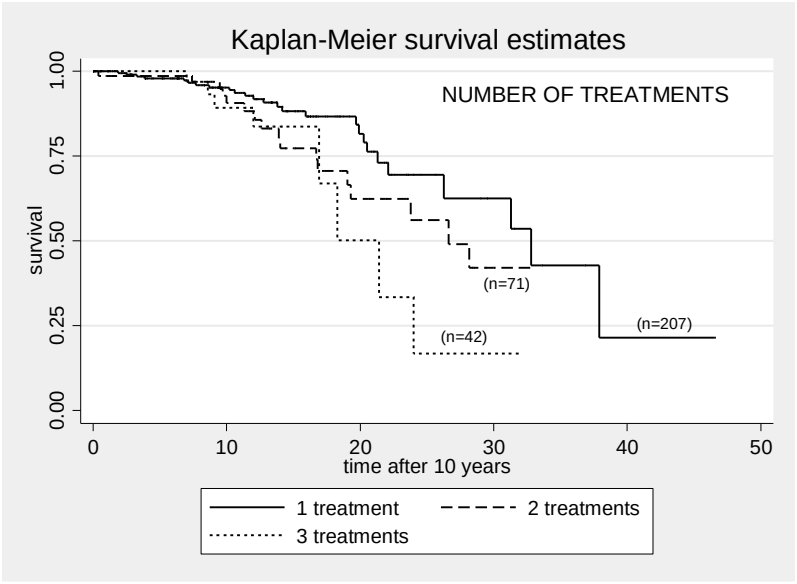


Figure 1: Kaplan-Meier survival curves by gender (A), and by number of treatments (B).

References

1. PivonelloR, DeLoo M, Cozzolino A, Colao A-M. The treatment of Cushing's Disease Endocrine Reviews 2015;36:385-486
2. Lacroix A, Feelders RA, Stratkis CA, Nieman LK. Cushing's Syndrome Lancet 2015;386:913-27
3. Newell-Price, Bertagna X, Grossman A, Nieman LK. Cushing's syndrome. Lancet 2006; 367:1605-17
4. Sharma ST, Nieman LK, Feelders RA. Cushing's syndrome:epidemiology and developments in disease management. Clinical Epidemiology 2015; 7:281-93
5. Vogelzangs N, Beekman ATF, Milaneschi Y, Bandinelli S, Ferrucci L, Penninx BWJH. Urinary cortisol and six-year risk of all-cause and cardiovascular mortality. *J Clin Endocrinol Metab* 2010;95:4959-64
6. Schoorlemmer RMM, Peeters GMEE, van Schoor NM, Lips P. Relationship between cortisol level, mortality, and chronic diseases in older persons. *Clin Endocrinol* 2009;71:779-86
7. Kumari M, Shipley M, Stafford M, Kivimaki M. Association of diurnal patterns in salivary cortisol with all cause and cardiovascular mortality: findings from Whitehall 2 study. *J Clin Endocrinol Metab* 2011;96:1478-85
8. Di Dalmazi G, Vicennati V, Garelli S, Casadio E, Rinaldi E, Giampalma E, Mosconi C, Golfieri R, Paccapelo A, Pagotto U, Pasquali R. Cardiovascular events and mortality in patients with adrenal incidentalomas that are either non-secreting or associated with intermediate phenotype or subclinical Cushing's syndrome: a 15 year retrospective study. *Lancet Diabetes Endocrinol* 2014;2:396-405
9. Debono M, Bradburn M, Bull M, Harrison B, Ross RJ, Newell-Price J. Cortisol as a marker for increased mortality in patients with incidental adrenocortical adenomas. *J Clin Endocrinol Metab* 2014;99:4462-70
10. Lindholm J, Juul S, Jorgensen JOL, Astrup J, Bjerre P, Feldt-Rasmussen U, Hagen C, Jorgensen J, Kosteljanetz M, Kristensen LO, Laurberg P, Schmidt K, Weeke J. Incidence and late prognosis of Cushing's syndrome: a population-based study. *J Clin Endocrinol Metab* 2001;86:117-23
11. Dekkers OM, Biermasz NR, Peirera AM, Roelfsema F, van Aken MO, Voormolen JHC, Romijn JA. Mortality in patients treated for Cushing's disease is increased compared with patients treated for non-functioning pituitary macroadenoma. *J Clin Endocrinol Metab* 2007; 92:976-81
12. Hammer GD, Tyrell JB, Lamborn KR, Applebury CB, Hannegan ET, Bell S, Rahl R, Lu A, Wilson CB. Transsphenoidal microsurgery for Cushing's disease: initial outcomes and long-term results. *J Clin Endocrinol Metab* 2004;89:6348-57
13. Clayton RN, Raskauskiene D, Reulen RC, Jones PW. Mortality and morbidity in Cushing's disease over 50 yrs in Stoke-on-Trent UK: Audit and metaanalysis of literature. *J Clin Endocrinol Metab* 2011;96:632-42
14. Bolland MJ, Holdaway IM, Berkeley JE, Lim S, Dransfield WJ, Conaglen JV, Croxson MS, Gamble GD, Hunt PJ, Toomath RJ. Mortality and morbidity in Cushing's syndrome in New Zealand. *Clin Endocrinol* 2011;75:436-42
15. Hassan-Smith ZK, Sherlock M, Reulen RC, Arlt W, Ayuk J, Toogood AA, Cooper M, Johnson AP, Stewart PM. Outcome of Cushing's disease following transsphenoidal surgery in a single center over 20 yrs. *J Clin Endocrinol Metab* 2012;97:1194-1201
16. Pikkarainen L, Sane T, Reuenan A. The survival and well-being of patients treated for Cushing's syndrome. *J Intern Med* 1999;245:463-68

17. Ntali G, Asimakopoulou A, Siamatras T, Komninos J, Vassiliadi D. Tzanela M, Tsagarakis S, Grossman AB, Wass JAH, Karavitaki N. Mortality in Cushing's syndrome: systematic analysis of a large series with prolonged follow-up. *Eur J Endocrinol* 2013;169:715-23

18. Yaneva M, Kalinov K, Zacharieva S. Mortality in Cushing's syndrome: data from 386 patients from a single tertiary referral center. *Eur J Endocrinol* 2013;169:621-27

19. van Haalan FM, Broersen LHA, Jorgensen JO, Pereira A, Dekkers OM. Mortality remains increased in Cushing's disease despite biochemical remission: a systematic review and meta-analysis. *Eur J Endocrinol* 2015;172:R143-R49

20. Breslow NE, Day NE. Statistical methods in cancer research . Vol 11. The design and analysis of cohort studies. 1987; Publication no. 82 . Lyon *IARC Scientific Publications*

21. Swearingen B, Biller BM, Barker 2nd FG, Katznelson L, Grinspoon S, Klibanski A, Zervas NT. Long-term mortality after trans-sphenoidal surgery for Cushing's disease. 1999; *Ann. Int. Med*. 130:821-24

22. Hammer GD, Tyrell JB, Lamborn KR, Applebury CB, Hannegan ET, Bell S, Rahl R, Lu A, Wilson CB. Trans-sphenoidal microsurgery for Cushing's disease: initial outcomes and long-term results. 2004: *J. Clin Endocrinol. Metab* 89:6348-57.

23. Sherlock M, Reulen RC, Alonso AA, Ayuk J, Clayton RN, Sheppard MC, Hawkins MM, Bates AS, Stewart PM, ACTH deficiency, high doses of hydrocortisone replacement, and radiotherapy are independent predictors of mortality in patients with acromegaly. *J. Clin. Endocrinol. Metab*. 2009; 94:4216-23

24. Tomlinson JW, Holden N, Hills RK, WheatleyK, Clayton RN, Bates AS, Shappard MC, Stewart PM. Association between premature mortality and hypopituitarism. Werst Midlands Prospective Hypopituitary Study Group. *Lancet* . 2001; 357:425-31

25. Sherlock M, Ayuk J, Tomlinson JW, Toogood AA,Aragon-Alonso A, Sheppard MC, Bates AS, Stewart PM. Mortality in patients with pituitary disease. *Endocrine Reviews* 2010; 31:301-42

SUPPLEMENTARY TABLE 1-DEFINITION OF 'CURE'/REMISSION IN CUSHINGS DISEASE

CENTRE	CRITERIA
Birmingham UK (15)	Initial = morning pl.cortisol <50 nmol/l 4d-6wks post surgery
	F-Up = normal UFC or suppression of pl. cortisol to < 50nmol/l after o/n dex
Stoke on Trent UK (13)	Initial = normal UFC + pl. cortisol <100 nmol/l after o/n dex
	F-Up = normal UFC x 2 or pl. cortisol <50nmol/l after o/n dex
Oxford UK (17)	Initial = 'undectable' morning pl. cortisol within days after surgery
	F-Up = one or more of following: normal pl. cortisol after o/n dex;

normal UFC; normal mean pl. cortisol from 5 point day curve

Auckland (14) Initial – not given

F-Up = normal UFC or normal pl. cortisol after o/n dex;

Leiden (11) Initial = not given

F- Up = pl. cortisol <100 nmol/l after o/n dex and normal UFC (<220 nmol/day)

Aalborg (10) Initial = subnormal pl. cortisol after synacthen +/- UFC ,< 50 nmol/day

F-Up = UFC < 250 nmol/day

Notes; 1) presence of adrenal insufficiency requiring glucocorticoid treatment = cure

2) 'normal' refers to the local reference range for the centre which varied by centre according to the assays used, and which themselves changed during the course of the follow-up

3) pl. = plasma; UFC = 24hr urine free cortisol; synacthen = synthetic ACTH 250 ug ; F-Up = follow up measured at variable regular intervals according to local protocols

4) o/n dex = plasma cortisol measured between 0800-1000 hrs following 1 or 2 mgs of dexamethasone administered at 2300 hrs the previous evening.

SUPPLEMENTARY TABLE 2.

NUMBER OF TREATMENTS PER PATIENT BY CENTRE (PROPORTION OF SUBJECTS %)

Number of treatments	1	2	3
Centre			
Birmingham (UK)	80	6.7	13.3
Stoke on Trent (UK)	64.1	28.2	7.7
Oxford (UK)	61.7	17.3	21.0
Aalborg (DK)	81.1	17.6	1.4
Leiden (NL)	77.8	19.4	2.8
Auckland (NZ)	42.7	33.3	24

The difference between numbers of treatments between centres was highly significant P <0.01 by Anova

Supplementary Table 3.

. Cause specific standardised mortality ratios by pituitary surgery

	Pituitary surgery			No pituitary surgery		
	N (%)	Obs/Exp	SMR (95%CI)	N (%)	Obs/Exp	SMR (95%CI)
Overall	170 (53%)	16/16.8	0.95 (0.58, 1.55)	150 (47%)	35/13.8	2.53 (1.82, 3.53)
Country						
England	71 (53%)	4/4.7	0.86 (0.23, 2.20)	64 (47%)	11/4.6	2.40 (1.20, 4.30)
New-Zealand	17 (223%)	3/1.3	2.30 (0.47, 6.71)	58 (77%)	17/5.9	2.87 (1.67, 4.59)
Netherlands	28 (78%)	2/3.7	0.55 (0.07, 1.97)	8 (22%)	2/1.7	1.16 (0.14, 4.19)
Denmark	54 (73%)	7/7.2	0.98 (0.39, 2.01)	20 (27%)	5/1.6	3.18 (1.03, 7.42)
$P_{heterogeneity}$			0.367			0.607

obs: observed number of deaths; exp: expected number of deaths; SMR: standardised mortality ratio

Supplementary Figure

Cumulative all cause mortality with duration of follow-up in patients treated pre 1985 vs after 1985

The apparent difference was not significant.