Adrenal steroidogenesis following B lymphocyte depletion therapy in new onset Addison's disease

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Supplemental information

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PATIENTS and METHODS

Patient exclusions

Patients with active viral illness, including HIV, Hepatitis B or C, shingles/Zoster, recent or partially treated TB or other active, severe infections; unexplained radiographic abnormality on chest X-ray; diabetes mellitus, previous cytotoxic or immunosuppressive treatment other than glucocorticoids, significant cardio-respiratory, chronic renal or non-autoimmune liver disease, malignancy, pregnancy, breast feeding or plan for pregnancy within 24 months, known non-autoimmune cause for adrenal failure (haemorrhage, adrenoleukodystrophy etc.), or other existing contraindication to rituximab, were excluded.

Ongoing treatment regimen

At diagnosis, all patients had been treated with oral hydrocortisone in doses ranging from 15 to 30mg daily. Following the first rituximab infusion, oral prednisolone 20mg daily was taken by patients 1 to 4, instead of their oral hydrocortisone. Prednisolone doses were gradually weaned from 20mg daily to 3mg daily over the first 13 weeks of the study in patients 1 to 4. A protocol amendment was made in the light of the data from the first 4 patients, and lower prednisolone doses of 3mg and 5mg daily were used in patients 5 and 6, respectively. Patients 5 and 6 were maintained on this constant low dose of prednisolone throughout the first 13 weeks. Following the 13 week outcome assessment, all patients were changed to oral hydrocortisone in split

doses ranging from 12.5 to 25mg daily. All patients received fludrocortisone (50 to 200 μg daily) for at least 12 months following rituximab therapy.

Outcome measures and assessments

All cortisol measurements in the text and figures are the peak concentration of the 3 timepoints during each test. Serum cortisol was measured using a competitive chemoluminescent immunoassay on a Centaur platform (Siemens, UK) with a limit of detection (LD) of 25nmol/l (0.91μg/dl). Serum aldosterone measurements were made by solid-phase radioimmunoassay (DPC coat-a-count kit), with a limit of detection (LD) of 70 pmol/l. Serum DHEAS was assayed by solid-phase competitive chemoluminescence on an Immulite platform (Siemens) with a limit of detection (LD) of 0.4 μmol/l. Adrenal cell antibodies were assayed by indirect immunofluorescence of patient sera on monkey adrenal slices (Prodiagnostics). Serum 21-hydroxylase antibodies were measured in duplicate by immunoprecipitation of radiolabelled recombinant *in vitro* translated 21-hydroxylase protein (RSR ltd. Cardiff, UK).[18] For patient 5, the baseline sample measured >5,000 U/ml of 21-hydroxylase antibodies, so samples were diluted 100-fold to obtain titres. The concentration of serum immunoglobulin M (IgM) was measured by immunometric nephelometry (BN2, Dade Behring). Peripheral lymphocyte subsets were measured using a Becton Dickinson flow cytometer with 6-colour staining including CD45, CD3, CD4, CD8 and CD19 (BD reagents), and analysis of 10,000 events. HLA-DR genotyping was performed using allele-specific PCR amplification of genomic DNA according to standard UK National Blood Service protocols.

Supplemental figure: Participant recruitment flowchart

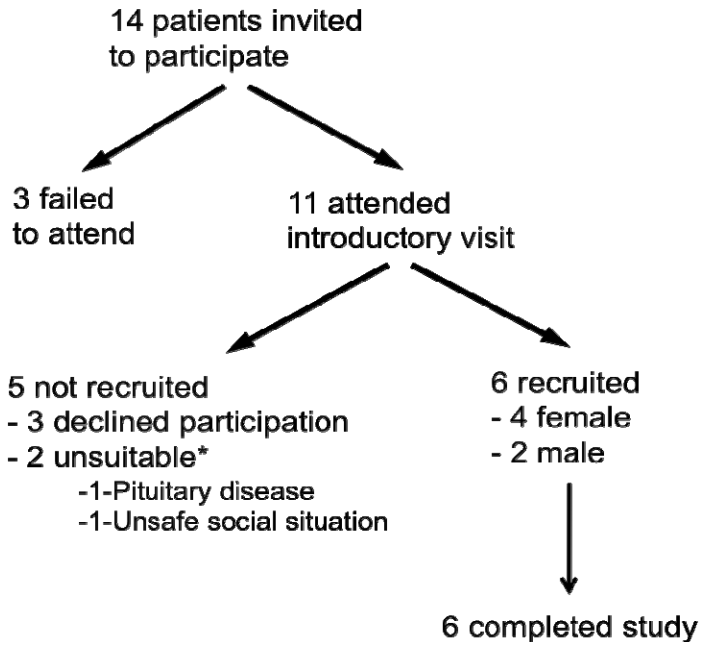

Figure legend: Participant recruitment flowchart.

Numbers of patients invited to attend, recruited and not recruited are documented according to CONSORT guidelines. *Two patients were deemed unsuitable for the study. One because the diagnosis was that of central hypoadrenalism. The other had a chaotic lifestyle and failed to attend several appointments.