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Optimising prednisolone or prednisone replacement in adrenal insufficiency

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Abstract

Context:

Patients with adrenal insufficiency (AI) have a higher mortality than the general population, possibly because of excess glucocorticoid exposure at inappropriate times. The cortisol circadian rhythm is difficult to mimic with twice or thrice-daily hydrocortisone. Prednisolone is a once-daily alternative which may improve patient compliance through its convenience.

Objectives:

Prednisolone day curves can be used to accurately down-titrate patients to the minimum effective dose. This study aimed to review prednisolone day curves and determine therapeutic ranges at different timepoints after administration.

Methods:

Between August 2013 and May 2021, 108 prednisolone day curves from 76 individuals receiving prednisolone replacement were analysed. Prednisolone concentrations were determined by ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). Spearman's correlation coefficient was used to determine the relationship between 2-, 4- and 6-hour prednisolone levels compared to the validated standard 8-hour prednisolone level (15-25 µg/L).

Results:

The median dose was 4mg prednisolone once daily. There was strong correlation between the 4-hour and 8-hour (R=0.8829, p ≤0.0001), and 6-hour and 8-hour prednisolone levels (R=0.9530, p ≤ 0.0001). Target ranges for prednisolone were 37-62 µg/L at 4-hours, 24-39 µg/L at 6-hours and 15-25 µg/L at 8-hours. Prednisolone doses were successfully reduced in 21 individuals and of these, three were reduced to 2mg once daily. All patients were well upon follow-up.

Conclusion:

This is the largest evaluation of oral prednisolone pharmacokinetics in humans. Low dose prednisolone of 2-4mg is safe and effective in most patients with AI. Doses can be titrated with either 4-hour, 6-hour, or 8-hour single timepoint drug levels.

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Introduction

Patients with adrenal insufficiency (AI) have premature morbidity and mortality (1). Mildly elevated levels of glucocorticoids or non-circadian timing of therapy may contribute towards cardiovascular disease and increased mortality (2).

Replacement with glucocorticoid therapy is the mainstay of treatment, with an ultimate objective to mimic the circadian cortisol profile (3). Current guidelines recommend the use of hydrocortisone in divided doses or low-dose (3-5mg) prednisolone (4).

Over-replacement with glucocorticoids avoids adrenal crisis at the expense of an increased risk of developing multiple co-morbidities including obesity, diabetes, cardiovascular disease, and osteoporosis (5). It is crucial to achieve an optimum dose for symptomatic control and avoidance of adrenal crises, whilst minimising overexposure to glucocorticoids (6). This may be particularly challenging due to the inter-individual variability in physiological response and metabolic clearance of exogenous glucocorticoids (4).

Traditionally used hydrocortisone therapy has a short half-life of approximately 1.8 hours, and therefore requires multiple doses to maintain therapeutic levels (7). Use of multiple-dose glucocorticoids results in a non-physiological profile with supraphysiological levels in the evenings when individuals are more sensitive to the effects of cortisol (8).

In comparison, prednisolone has a longer half-life attributed to the presence of a double bond between C1-C2, thus allowing for once-daily regimens. In a study of

patients with congenital adrenal hyperplasia, prednisolone was six to eight times more potent than hydrocortisone (6, 9). Consequently, a hydrocortisone dose of 20 mg daily (e.g., 10mg + 5mg + 5mg) is equivalent to a prednisolone dose of 2–4mg once-daily. This may be because prednisolone has half the affinity for corticosteroid-binding globulin as compared to cortisol and therefore, higher concentrations of unbound prednisolone are present (10). Furthermore, prednisolone has a longer half-life and greater avidity for the glucocorticoid receptor and takes a longer time to dissociate. Once-daily prednisolone regimens are associated with greater patient satisfaction and compliance (5).

Concerns about adverse metabolic outcomes associated with prednisolone are based on evidence using higher doses of prednisolone. A study analysing 25 patients receiving either hydrocortisone (30mg/day) or prednisolone (7.5mg/day) concluded that a greater proportion of patients treated with prednisolone developed densitometric osteoporosis (11). Data from European Adrenal Insufficiency Registry (EU-AIR) compared cardio-metabolic profiles between individuals receiving prednisolone (average dose 5mg) vs. hydrocortisone (average dose 21.5mg) (10). In this cohort, those receiving prednisolone had significantly higher total cholesterol and low-density lipoprotein levels compared with those receiving hydrocortisone. A large population-based study that reviewed historical GP prescriptions for prednisolone and hydrocortisone found that patients who had been on prednisolone had a higher mortality than those who had been on hydrocortisone, although the doses used were not reported (12). The excess mortality may be the result of over-replacement with prednisolone, as higher doses such as 5mg in the morning and 2.5mg in the evening were commonly used. These findings have not been reproducible in subsequent

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studies with lower doses of prednisolone, where results have demonstrated no significant difference in parameters including bone density, HbA1C, blood pressure, body mass index and waist circumference, between prednisolone and hydrocortisone use (5, 10, 13, 14).

There remains a paucity of evidence on the optimum prednisolone dose to ensure adequate replacement whilst minimising adverse side effects. If 15-25 mg hydrocortisone is the correct replacement dose, then prednisolone doses of 1.8mg–4.2 mg daily may be more appropriate (13).

At Imperial College Healthcare NHS Trust (ICHNT), an eight-hour prednisolone level between 15 and 25µg/L indicates adequate replacement (13, 15). However, the 8-hour timepoint may be inconvenient for patients attending outpatient clinics, and therapeutic levels at other timepoints are important. Use of prednisolone levels may also have wider applicability in guiding prednisolone dosing in patients who may eventually be weaned off prednisolone completely.

To our knowledge, this is the largest evaluation of prednisolone pharmacokinetics in patients with AI. Previous studies have evaluated prednisolone pharmacokinetics in healthy volunteers at larger doses but have been limited by sample size (16, 17).

Materials and Methods

Design

We analysed individuals receiving established prednisolone therapy who had prednisolone day curves performed between August 2013 – May 2021 at ICHNT. Prednisolone day curves were performed as part of routine clinical care and dose optimisation. Between 2013 and 2015, prednisolone day curves were the standard of care, and the prednisolone assay was then optimised. Day curves were validated against 8-hour prednisolone levels by 2015 (13) and subsequently patients were routinely managed using 8-hour levels for their convenience. Thereafter, day curves were performed based upon clinical assessment and in challenging cases.

Demographic data, cause of adrenal failure (primary, secondary, or glucocorticoid-induced), laboratory values and clinical measures were obtained from interrogating electronic medical records. All patients with SAI were assessed 6 weeks following pituitary surgery with a dynamic pituitary function test to assess their requirement for replacement glucocorticoid therapy.

We included individuals in both outpatient and inpatient settings who were receiving prednisolone replacement for more than 6 months. The prednisolone dose was administered in the early morning, in the fasted state.

Pharmacokinetic data were derived using the values obtained from prednisolone day curves generated from a total of 76 individuals. One patient (patient D) who was on prednisone 10mg once daily from the United States for a chronic autoimmune

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disease wanted to convert to prednisolone as he moved to the UK and requested day curves to compare them.

As the samples in this study were collected as part of routine clinical care and were part of a subsequent audit *(Registration number: END_021)*, informed consent was not required.

Measurement of prednisolone levels

Serum samples were obtained to measure prednisolone levels up to 8 hours following administration of an individual's regular replacement prednisolone dose.

Blood samples were collected into SST BD Vacutainer tubes, containing serum clot activator and a serum separating gel. All specimens were spun and separated within 4 hours, and serum was stored at a temperature of −20 °C before analysis.

Analytical Methods

Prednisolone concentrations were determined by ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) (Waters Quattro Premier Mass Spectrometer). The assay is controlled using custom internal quality controls and is a UKAS accredited assay. This method has demonstrated intra-assay and inter-assay co-efficient variations of 2.7% and 4.1%, respectively, at a prednisolone concentration of 50 µg/L. In-depth methodology is detailed in a previous paper by Williams et al (13). Time-points within a single day curve for each individual were run on one assay.

Data Analysis and Statistics

The mean and standard deviation (±SD) were calculated for parametric data. The median and interquartile range (IQR) were used to describe non-parametric continuous data. Categorical variables were described using proportions and frequencies. The Spearman's rank correlation was used to determine strength and direction of relationship between 8-hour prednisolone levels vs. 2hour, 4-hour and 6-hour levels.

Day curve data from patients receiving 2-6mg was used to generate a semi-log plot. The 95% confidence intervals of the slope of the graph were determined. The modulus of these values was taken as the elimination constant (k). Using the first order elimination equation, where (t) is time, C_0 is the initial concentration, and $C_{(t)}$ is the concentration at (t):

$$C_{(t)} = C_0 \,.\, e^{-kt}$$

The existing 15-25 µg/L 8-hour target range was used to generate target ranges at 4-hours and 6hours.

Measured prednisolone concentrations were also plotted against time to produce prednisolone day curves. The peak level (C_{max}) and time to peak level (T_{max}) were determined using actual collection time points. Area under prednisolone level-time curve (AUC) was determined using the trapezoid method.

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Statistical significance was defined as a p value **<**0.05. All graphs, areas under the curve to 24-hours ($AUC_{0–24h}$), and pharmacokinetic data were created using GraphPad Prism 6 (GraphPad Software).

Results

Baseline characteristics

In total, 108 prednisolone day curves were analysed from 76 individuals. Baseline characteristics are summarised in Table 1. Figure 1 illustrates the distribution of daily replacement doses that individuals received in this cohort.

Within our patient cohort, 7.9% (n=6) had primary AI (PAI), 63.2% (n=48) had secondary AI (SAI) and 28.9% (n=22) had tertiary AI (glucocorticoid-induced AI) [Table 1]. The causes of SAI are summarised in Table 2.

Pharmacokinetics

In the total population, the median (IQR) dose of prednisolone was 4 (3 - 5) mg corresponding to a mean (±SD) maximal time to peak level (T_{max}) of 1.7 (± 1) hours after administration, with a median (IQR) 8-hour level of 29 (19 - 42.7) µg/L. The median (IQR) half-life was 3.1 (2.6 – 3.6) hours with AUC of 485 (366.5 – 703.9) µg · h/L. The median half-life of prednisolone remained similar at each prednisolone dose analysed.

Pharmacokinetic data are summarised in Table 3. Individuals are routinely commenced on a standard dose of 4mg, many of whom are yet to be downtitrated.

The individuals receiving 3mg or less (Table 3) have had their doses actively reduced using the prednisolone levels obtained from day curves. This accounts for the 8-hour concentrations within the target range for individuals receiving <2mg and 3mg but being above 25 µg/L in the other groups.

Prednisolone day curves

Figure 2 summarises prednisolone day curves for individuals on doses 3mg (n=21), 4mg (n=28) and 5mg (n=15) using average prednisolone levels. These levels were used to slowly down-titrate the individual doses.

Multiple dose profiles

Of the 108 prednisolone day curves analysed, 22 individuals had repeat day curves on different doses of prednisolone. Figure(s) 3a, 3b and 3c demonstrate the metabolism of varying dosages of prednisolone over a period of 8 hours in three individuals. Patient A (Figure 3a) had a bilateral adrenalectomy many years previously for recurrent pituitary dependent Cushing's disease. She was gradually weaned from a prednisolone dose of 10mg (8-hour level: 120.2 µg/L) to a dose of 3mg (8hour level: 37 µg/L) and finally to 2mg (8-hour level: 21 µg/L) which she has remained well on for several years. Patient B (Figure 3b) was weaned from a dose of 3mg to 1mg – however on a dose of 1mg, the 8-hour level was undetectable, and the patient felt unwell hence a maintenance dose of 2mg was established with an 8-hour level of 16 µg/L. Patient C (Figure 3c) was weaned from a prednisolone dose of 2mg to 1mg, with an 8-hour level of 19 µg/L and 6-hour level of 36 µg/L. After this dose

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reduction, the baseline cortisol increased to 194 mmol/L, and she was weaned off prednisolone and remains clinically well.

Prednisolone vs. Prednisone

For centres using prednisone, Table 4 summarises serum prednisolone levels after administration of prednisone (United States Pharmacopeia) and prednisolone (British Pharmacopeia). An oral dose of 10mg of both medications were taken on consecutive days by patient D. After oral intake, prednisone and prednisolone (10mg) were rapidly absorbed and identical serum concentrations of 161 µg/L were obtained at 90 minutes post ingestion. The 8-hour level was 37 µg/L following prednisone and 35 µg/L following prednisolone.

Correlation of prednisolone levels and time of measurement

Figure 4 demonstrates the correlation between 8-hour prednisolone level as compared to 6-hour, 4hour, 2-hour, and 1-hour prednisolone levels.

There was strong correlation between 8-hour and 6-hour prednisolone levels ($R=0.9530$, $p \leq 0.0001$) and 8-hour and 4-hour prednisolone levels ($R=0.8829$, $p \leq 0.0001$).

Determining target ranges

Target ranges were determined using a semi-log plot (Table 5; Figures 5 & 6). The calculated half-life was 3.1 hours (95% CI: 2.80-3.41) based on an elimination constant of 0.23 h^{-1}.

Clinical outcomes

Previous studies have stated that replacement is appropriate if the 8-hour level of prednisolone is between 15 µg/L and 25 µg/L. In our patient population, 55.2% (n=42/76) of individuals had prednisolone levels at 8-hours above the optimum range and could have their dose reduced.

To determine if patients reported adverse symptoms whilst on prednisolone replacement, electronic medical records from follow-up appointments were reviewed. The last day curve reviewed was completed in May 2021 and the final review of electronic patient records began in July 2022. This ensured a minimum of 1 year follow-up for all individuals in the dataset. The decision to wean prednisolone was made by a panel of Endocrinologists in a multi-disciplinary team meeting. Individuals were deemed 'well' if they remained asymptomatic, with an adequate blood pressure and normal electrolytes on replacement dose prednisolone. Of the 63 (82.9%) who were deemed well, 35 (55.6%) had an 8-hour prednisolone level greater than 25 µg/L.

Within the follow-up period, 21 individuals with 8-hour prednisolone levels greater than 25 µg/L successfully had their prednisolone dose decreased and remained well

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for at least 12 months following dose reduction. Early morning cortisol levels were also checked prior to prednisolone administration. Eight individuals were unexpectedly found to have recovery of the hypothalamic-pituitary-adrenal (HPA) axis during the follow up period after being switched from hydrocortisone to prednisolone. Endogenous cortisol production only became apparent when they were switched to prednisolone. These individuals had a diagnosis of SAI and showed evidence of recovery of cortisol production during prednisolone weaning. Individuals were successfully weaned off prednisolone completely during the observed follow-up period if they had baseline cortisol levels of over 200nM before prednisolone [Table 6] (18). Prior to their conversion to prednisolone, it is likely that five of these patients had supra-physiological glucocorticoid exposure. Four of these were iatrogenic from exogenous glucocorticoid exposure and one due to endogenous cortisol secretion due to Cushing's disease. The remaining three patients had pituitary adenomas, and recovery of their HPA axis may have been due to reduction in effective glucocorticoid exposure when they were switched from hydrocortisone to prednisolone. However, in PAI, prednisolone levels were used to optimise replacement prednisolone doses. Three patients required up-titration of their fludrocortisone dose. All three patients were clinically and biochemically stable on prednisolone for at least 1 year prior to acute up-titration of their fludrocortisone. In all three cases, fludrocortisone was increased based on electrolyte levels and clinical assessment during routine follow-up.

Discussion

This is the largest dataset of prednisolone pharmacokinetics in patients with hypoadrenalism and demonstrates that once-daily very low dose prednisolone is a safe and effective glucocorticoid replacement therapy. Dose titration remains a significant clinical challenge. Serum prednisolone levels can be used to optimise doses to ensure individuals receive the minimal effective dose and avoid excess steroid exposure. Dose decreases correlate well with clinical symptomatology. We have found a strong correlation between 4-hour and 6-hour prednisolone level measurements compared with 8-hour levels. This data supports single timepoint blood sampling at either 4-hour, 6-hour, or 8hours to accurately gauge adequate replacement status. This offers flexibility and greater convenience for patients and clinicians in the outpatient setting.

For many years, individuals with AI have received over-replacement with oral glucocorticoids (19, 20). With higher doses of prednisolone, symptoms may be controlled, and individuals have a lower risk of adrenal crises. With prolonged excess steroid exposure, however small the excess, there is an increased risk of detrimental metabolic and bone side effects (6). Historically, 5 – ≥7.5mg of prednisolone was a commonly used maintenance dose (21-23) . However, this has been shown to be associated with increased morbidity, mortality, and healthcare costs (23).
By using prednisolone day curves to gauge adequacy of replacement, maintenance doses can be reduced to a median replacement dose of 4mg, as demonstrated in our patient population.

Patients with AI are known to have very low cortisol levels upon awakening, possibly explaining early morning fatigue and nausea. We therefore recommend, patients

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take prednisolone as early as possible upon wakening. Other studies are trialling glucocorticoid pump replacement therapy to mimic circadian rhythms, however there is so far limited evidence for their use (24, 25). The importance of pulsatile therapies in patients with Addison's disease will be clearer once the Medical Research Council 'PULSES' trial is complete (MR/R010919/1).

Patient reported symptoms and the clinician's discretion are the main deciding factors on weaning regimens. Weaning may be challenging due to the adverse effects of glucocorticoid withdrawal (26). Prednisolone levels may offer an additional tool in this joint decision-making process and provide an evidence base to attempt prednisolone weaning in those who show signs of HPA axis recovery.

Our study is limited by the retrospective nature of data collection and accurate documentation. There is a potential for selection bias in the cohort of patients in whom a clinical decision was made to perform a day curve. However, prednisolone doses can be tailored using a reliable assay, with a recommended target 8-hour prednisolone trough value of 15 – 25µg/L. We are the first centre to be using low-dose prednisolone in conjunction with serum prednisolone levels. In the absence of end organ markers, these levels have been defined clinically (13, 15). Measuring prednisolone at 8-hours may be inconvenient in an outpatient setting. Therefore, we have derived 4-hour and 6-hour prednisolone target ranges. This will enable individuals to take their prednisolone dose in the morning to mimic the intrinsic circadian rhythm and enable earlier sampling. Prednisolone levels at 1- and 2-hours are dominated by variability in absorption, show poor correlation with 8-hour levels and therefore, should not be relied upon.

Prednisolone and prednisone are active and inactive glucocorticoids respectively and prednisone requires activation by hepatic first pass metabolism by 11β-hydroxysteroid dehydrogenase. The bioavailability profile of prednisone and prednisolone were similar and there was no difference as indicated by the area under the curve comparisons and serum concentration-time curves.

The median elimination half-life was 3.1 hours, which is comparable to data reported in other studies (17, 27). In countries where prednisolone is not available, an identical dose of prednisone can be used. This should not be confused with methylprednisolone, which is 20% more potent than both prednisolone and prednisone. It is important to note therefore that 4mg methylprednisolone is equivalent to 5mg of prednisolone and 5mg prednisone (28).

Patients with auto-immune diseases are frequently managed with anti-inflammatory high-dose prednisolone. Following remission of their primary disease, a large cohort are unable to stop glucocorticoids because of tertiary AI (glucocorticoid-induced adrenal suppression). The clinical implications of this reverberate across a wide range of specialties, including respiratory and rheumatology (23, 29, 30).

 Accepted Manuscript published as EC-23-0097.R2. Accepted for publication: 08-Jun-2023

Conclusion

Optimising prednisolone doses using prednisolone levels facilitates reduction in the dose required in patients with AI, thereby reducing unnecessary glucocorticoid exposure and the associated side effects. Previous replacement doses of 5 - ≥7.5mg are harmful. Patients should therefore be titrated to the lowest possible safe dose and the majority of patients require 2 - 4mg. Although glucocorticoids may have differential glucocorticoid receptor binding and downstream effects (24, 25), data from head-to-head trials including PRED-AID (NCT03936517) and HYPER-AID (NCT03608943) will provide further evidence for use of low-dose prednisolone in AI.

There is inter-individual variability in prednisolone metabolism on a single given dose. For the vast majority within our cohort, prednisolone dosages of 5mg may be supra-therapeutic. Use of prednisolone levels will enable dose reduction and avoid adverse effects associated with excess glucocorticoid use. We have demonstrated strong correlation between 8-hour vs. 6-hour and 4-hour prednisolone levels. Sampling at earlier time points allows for greater flexibility for patients and clinicians, enabling optimum prednisolone titration.

Declaration of interest

No conflict of interest.

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References

1. Erichsen MM, Lovas K, Fougner KJ, Svartberg J, Hauge ER, Bollerslev J, Berg JP, Mella B & Husebye ES. Normal overall mortality rate in Addison's disease, but young patients are at risk of premature death. *European journal of endocrinology* 2009 **160** 233-237.

2. Tomlinson JW, Holden N, Hills RK, Wheatley K, Clayton RN, Bates AS, Sheppard MC & Stewart PM. Association between premature mortality and hypopituitarism. West Midlands Prospective Hypopituitary Study Group. *Lancet (London, England)* 2001 **357** 425-431.

3. Hahner S, Ross RJ, Arlt W, Bancos I, Burger-Stritt S, Torpy DJ, Husebye ES & Quinkler M. Adrenal insufficiency. *Nature reviews. Disease primers* 2021 **7** 19.

4. Caetano CM & Malchoff CD. Daily Glucocorticoid Replacement Dose in Adrenal Insufficiency, a Mini Review. *Frontiers in endocrinology (Lausanne)* 2022 **13** 897211.

5. Smith DJF, Prabhudev H, Choudhury S & Meeran K. Prednisolone has the same cardiovascular risk profile as hydrocortisone in glucocorticoid replacement. *Endocrine connections* 2017 **6** 766-772.

6. Choudhury S, Tan T, Lazarus K & Meeran K. The Use of Prednisolone versus Dual-Release Hydrocortisone in the Treatment of Hypoadrenalism. *Endocrine connections* 2021.

7. Czock D, Keller F, Rasche FM & Haussler U. Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoids. *Clinical pharmacokinetics* 2005 **44** 61-98.

8. Muller L & Quinkler M. Adrenal disease: Imitating the cortisol profile improves the immune system. *Nature reviews. Endocrinology* 2018 **14** 137-139.

9. Caldato MC, Fernandes VT & Kater CE. One-year clinical evaluation of single morning dose prednisolone therapy for 21-hydroxylase deficiency. *Arquivos Brasileiros de Endocrinologia e Metabologia* 2004 **48** 705-712.

10. Quinkler M, Ekman B, Marelli C, Uddin S, Zelissen P, Murray RD & EU-AIR Investigators. Prednisolone is associated with a worse lipid profile than

 Accepted Manuscript published as EC-23-0097.R2. Accepted for publication: 08-Jun-2023

hydrocortisone in patients with adrenal insufficiency. *Endocrine connections* 2017 **6** 1-8.

11. Jodar E, Valdepenas MP, Martinez G, Jara A & Hawkins F. Long-term follow-up of bone mineral density in Addison's disease. *Clinical endocrinology* 2003 **58** 617-620.

12. Ngaosuwan K, Johnston DG, Godsland IF, Cox J, Majeed A, Quint JK, Oliver N & Robinson S. Mortality Risk in Patients With Adrenal Insufficiency Using Prednisolone or Hydrocortisone: A Retrospective Cohort Study. *The journal of clinical endocrinology and metabolism* 2021 **106** 22422251.

13. Williams EL, Choudhury S, Tan T & Meeran K. Prednisolone Replacement Therapy Mimics the Circadian Rhythm More Closely Than Other Glucocorticoids. *The Journal of Applied Laboratory Medicine* 2016 **1** 152-161.

14. Valero MA, Leon M, Ruiz Valdepenas MP, Larrodera L, Lopez MB, Papapietro K, Jara A & Hawkins F. Bone density and turnover in Addison's disease: effect of glucocorticoid treatment. *Bone and mineral* 1994 **26** 9-17.

15. Choudhury S, Machenahalli P, Tan T & Meeran K. Inadvertent treatment of hypoadrenalism with prednisolone in pemphigus: A case report. *Clinical Case Reports* 2019 **7** 987-989.

16. Magee MH, Blum RA, Lates CD & Jusko WJ. Prednisolone Pharmacokinetics and Pharmacodynamics in Relation to Sex and Race. *Journal of clinical pharmacology* 2001 **41** 1180-1194.

17. Bashar T, Apu MNH, Mostaid MS, Islam MS & Hasnat A. Pharmacokinetics and Bioavailability Study of a Prednisolone Tablet as a Single Oral Dose in Bangladeshi Healthy Volunteers. *DoseResponse* 2018 **16** 1559325818783932.

18. Pofi R, Feliciano C, Sbardella E, Argese N, Woods CP, Grossman AB, Jafar-Mohammadi B, Gleeson H, Lenzi A, Isidori AM & Tomlinson JW. The short Synacthen (corticotropin) test can be used to predict recovery of hypothalamo-pituitary-adrenal axis function. *The Journal of clinical endocrinology and metabolism* 2018.

19. Kiko N & Kalhan A. Comparison of Various Glucocorticoid Replacement Regimens Used in Chronic Adrenal Insufficiency: A Systematic Review. *Dubai Diabetes and Endocrinology Journal* 2020 **26** 50-68.

20. Dallman MF, Akana SF, Bhatnagar S, Bell ME & Strack AM. Bottomed out: metabolic significance of the circadian trough in glucocorticoid concentrations. *International Journal of Obesity* 2000 **24 Suppl 2** S40-S46.

 Accepted Manuscript published as EC-23-0097.R2. Accepted for publication: 08-Jun-2023

21. van Staa TP, Leufkens HG & Cooper C. The epidemiology of corticosteroid-induced osteoporosis: a meta-analysis. *Osteoporosis International: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 2002 **13** 777-787.

22. Liu D, Ahmet A, Ward L, Krishnamoorthy P, Mandelcorn ED, Leigh R, Brown JP, Cohen A & Kim H. A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. *Allergy, Asthma & Clinical Immunology* 2013 **9** 30.

23. Dalal AA, Duh MS, Gozalo L, Robitaille M, Albers F, Yancey S, Ortega H, Forshag M, Lin X & Lefebvre P. Dose-Response Relationship Between Long-Term Systemic Corticosteroid Use and Related Complications in Patients with Severe Asthma. *Journal of managed care & specialty pharmacy* 2016 **22** 833-847.

24. Choudhury S, Lightman S, Meeran K. Improving glucocorticoid replacement profiles in adrenal insufficiency. *Clin Endocrinol (Oxf).* 2019 **91(3)** 367-371.

25. Lightman SL, Birnie MT, Conway-Campbell BL. Dynamics of ACTH and Cortisol Secretion and Implications for Disease. Endocr Rev. 2020 1;41(3)

26. Baker E. Is there a safe and effective way to wean patients off long-term glucocorticoids? *British journal of clinical pharmacology* 2021 **87** 12-22.

27. Frey FJ, Amend WJ, Lozada F, Frey BM, Holford NH & Benet LZ. Pharmacokinetics of prednisolone and endogenous hydrocortisone levels in cushingoid and non-cushingoid patients. *European journal of clinical pharmacology* 1981 **21** 235-242.

28. British National Formulary. Glucocorticoid therapy: Treatment Summaries. 2022

29. Suehs CM, Menzies-Gow A, Price D, Bleecker ER, Canonica GW, Gurnell M & Bourdin A. Expert Consensus on the Tapering of Oral Corticosteroids for the Treatment of Asthma. A Delphi Study. *American journal of respiratory and critical care medicine* 2021 **203** 871-881.

30. Sagar R, Mackie S, W Morgan A, Stewart P & Abbas A. Evaluating tertiary adrenal insufficiency in rheumatology patients on long-term systemic glucocorticoid treatment. *Clinical endocrinology* 2020.

 Accepted Manuscript published as EC-23-0097.R2. Accepted for publication: 08-Jun-2023

Figures

Figure 1: Distribution of daily replacement doses that individuals (n=76) received.

Figure 2: *Combined prednisolone Day Curve profiles in individuals receiving 2mg (n=2), 3mg (n=21), 4mg (n=28) and 5mg (n=15) prednisolone doses. Error bars represent standard error of the mean (SEM).*

Figure 3a: *Prednisolone Day Curves for Patient A at 3mg, 4mg, 7.5mg and 10mg prednisolone doses.*

Figure 3b: *Prednisolone Day Curves for Patient B at 1mg, 2mg, 3mg prednisolone doses.*

Figure 3c: *Prednisolone Day Curves for Patient C at 1mg and 2mg prednisolone doses.*

Figure 4(a-d): *Correlations between a) 8-hour vs. 6-hour prednisolone levels; b) 8-hour vs. 4-hour prednisolone levels; c) 8-hours vs. 2-hours prednisolone levels and d) 8-hours vs. 1-hour prednisolone levels.*

Figure 5: *Semi-log plot.*

Figure 6: *Proposed target ranges of prednisolone levels and timing of measurement.*

 Accepted Manuscript published as EC-23-0097.R2. Accepted for publication: 08-Jun-2023

Tables

Table 1: Baseline characteristics.

Parameter	**Patient population (n=76)**
Age, years (Mean [SD])	61 (±13)
Gender (Female)	60.5% (n=46)
Reason for Prednisolone administration	
Primary adrenal insufficiency (PAI)	7.9% (n=6)
Secondary adrenal insufficiency (SAI)	63.2% (n=48)
Tertiary adrenal insufficiency (TAI) *(Glucocorticoid-induced AI)*	28.9% (n=22)
Median (IQR) dose of Prednisolone, mg	4.0 (3.0 - 5.0)
Median (IQR) dose of Fludrocortisone, µg *(PAI, n=6)*	100 (50 – 125)

Table 2: Causes of secondary adrenal insufficiency.

Cause of secondary adrenal insufficiency	Patient population (n=48)
Pathology requiring trans-sphenoidal pituitary surgery	
Cushing's disease	*16.6% (n=8)*
Pituitary adenoma	*37.5% (n=18)*
Craniopharyngioma	*12.5% (n=6)*
Pituitary apoplexy	*6.2% (n=3)*
Rathke cleft cyst	*2.1% (n=1)*
Unilateral adrenalectomy	
Cushing's syndrome	*10.4% (n=5)*
Pheochromocytoma (with ACTH co-secretion)	*4.2% (n=2)*
Empty sella syndrome	*2.1% (n=1)*
Sheehan's syndrome	*2.1% (n=1)*
Langerhans' cell histiocytosis	*2.1% (n=1)*
Lymphocytic hypophysitis	*2.1% (n=1)*
Hypoxic brain injury (cortisol deficiency)	*2.1% (n=1)*

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Table 3: Pharmacokinetic data of total population and by prednisolone dose.

Pharmacokinetics	***Total (n=76)***	***≤ 2mg (n=4)***	***3mg (n=21)***	***4mg (n=28)***	***5mg (n=15)***	**≥6mg (n=8)**
$C_{8\,h}$, µg/L (Median [IQR])	*29 (19 – 42.7)*	*22.2 (16.8 - 23.1)*	*21.4 (16 – 36)*	*29 (19.4 – 39.5)*	*37.1 (26.8 – 44.8)*	*48.5 (27.3 – 93.3)*
AUC, µg · h/L (Median [IQR])	*485 (366.5 – 703.9)*	*328 (229.1 – 387.7)*	*405 (361 – 525.5)*	*449.6 (424 – 681.9)*	*487 (329.3 – 775.2)*	*693.9 (458.6 – 1237.9)*
T_{max}, h (Mean [SD])	*1.7 (1)*	*1.6 (0.5)*	*1.7 (0.6)*	*2 (0.8)*	*2.3 (1.4)*	*1.9 (1.4)*
Half-life, h (Median [IQR])	*3.1 (2.6– 3.6)*	*3 (2.9 – 3.1)*	*3 (2.4 – 3.6)*	*3.1 (2.7 – 3.5)*	*3.1 (2.7 – 4)*	*3.4 (2.5 – 4.3)*

Table 4: Prednisolone levels in patient D after an oral dose of prednisone and prednisolone on consecutive days.

Time (mins)	10mg Prednisone	10mg Prednisolone
75	166	170
90	161	161
105	154	155
120	146	146
240	109	98
360	64	64
480	37	35
600	18	22

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Table 5: Prednisolone level target ranges (µg/L) at 4-hour and 6-hour time points.

Time from prednisolone dose (n=number of values)	**Target range (µg/L)**
6-hours (n=76)	24 - 39
4-hours (n=76)	37 - 62

Table 6: Pathology of individuals successfully weaned off prednisolone during follow-up.

Cause of AI	Pathology	Patients successfully weaned off Prednisolone (n=8)
SAI	Unilateral adrenalectomy	
	Cushing's syndrome	*12.5% (n=1)*
	Pheochromocytoma (ACTH co-secretion)	*12.5% (n=1)*
SAI	Cushing's disease	*12.5% (n=1)*
SAI	Pituitary adenoma	*37.5% (n=3)*
TAI	Inflammatory bowel disease (Glucocorticoid-induced adrenal insufficiency)	*25% (n=2)*

SAI – secondary adrenal insufficiency; TAI – tertiary adrenal insufficiency.

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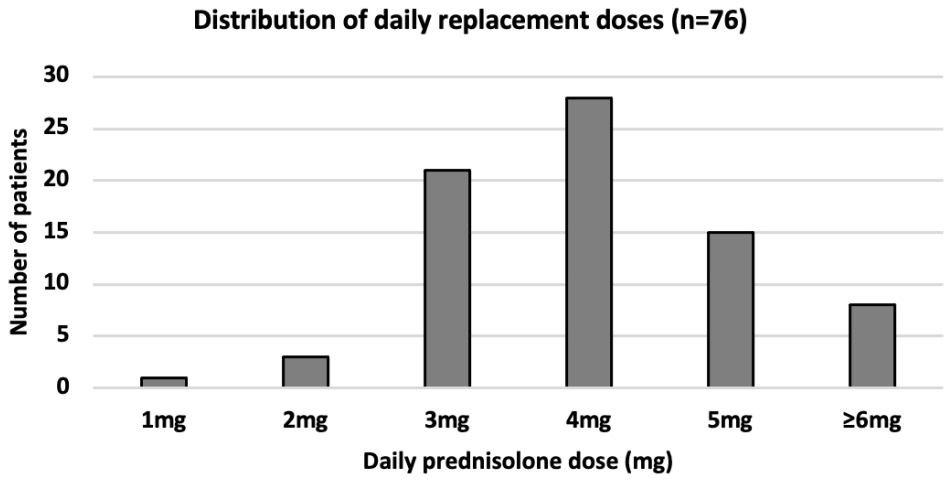

Figure 1: Distribution of daily replacement doses that individuals (n=76) received.

192x101mm (144 x 144 DPI)

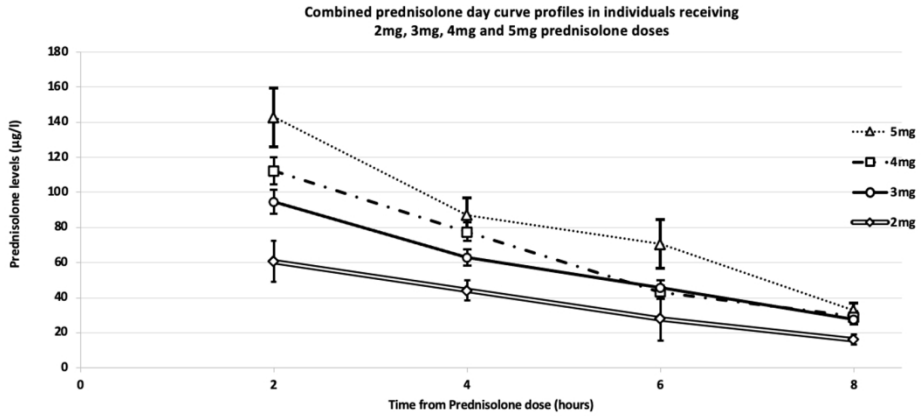

Figure 2: Combined prednisolone Day Curve profiles in individuals receiving 2mg (n=2), 3mg (n=21), 4mg (n=28) and 5mg (n=15) prednisolone doses. Error bars represent standard error of the mean (SEM).

320x140mm (144 x 144 DPI)

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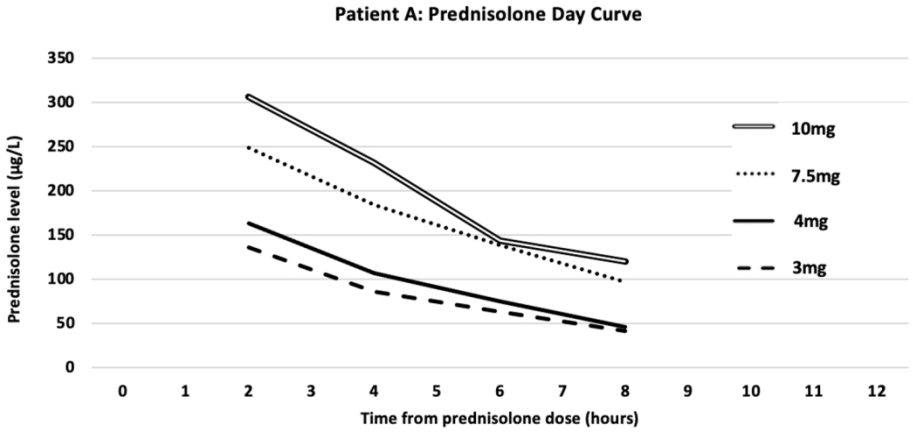

Figure 3a: Prednisolone Day Curves for Patient A at 3mg, 4mg, 7.5mg and 10mg prednisolone doses.

287x134mm (144 x 144 DPI)

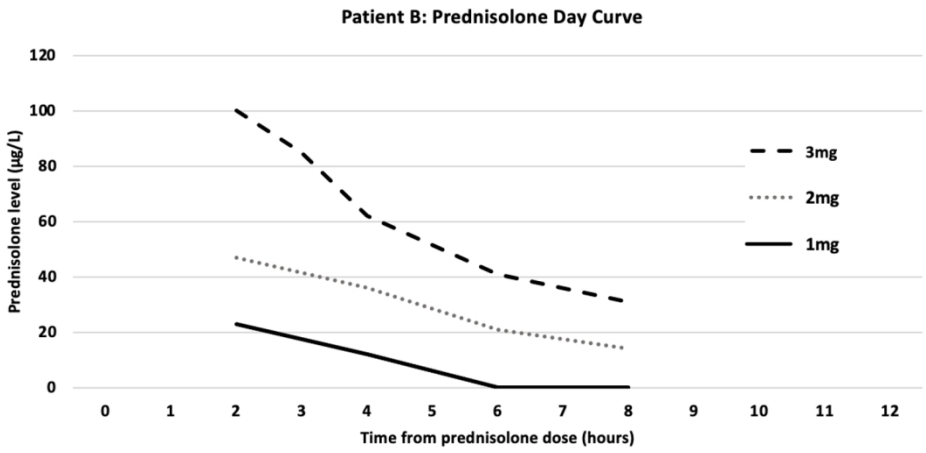

Figure 3b: Prednisolone Day Curves for Patient B at 1mg, 2mg, 3mg prednisolone doses.

292x136mm (144 x 144 DPI)

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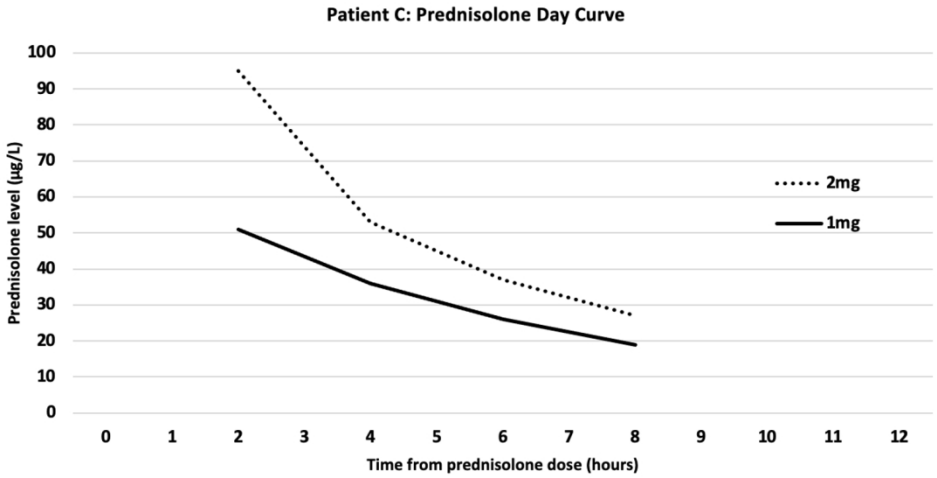

Figure 3c: Prednisolone Day Curves for Patient C at 1mg and 2mg prednisolone doses.

292x147mm (144 x 144 DPI)

Figure 4a:

R=0.9530, p value ≤0.0001

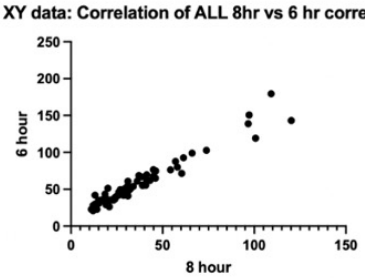

Figure 4b:

R=0.8829, p value ≤0.0001

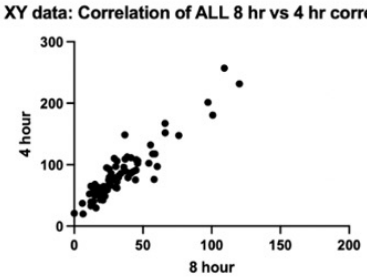

Figure 4c:

R=0.6759, p value ≤0.0001.

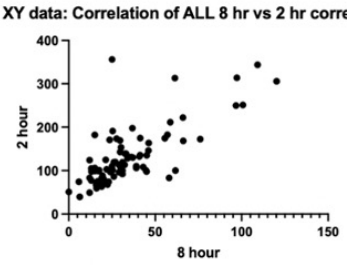

Figure 4d:

R=-0.2049, p value ≤0.1931

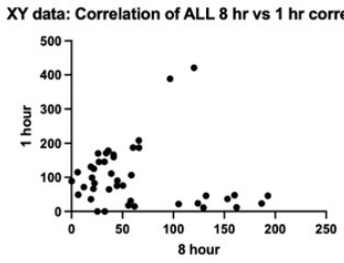

Figure 4(a-d): Correlations between a) 8-hour vs. 6-hour prednisolone levels; b) 8-hour vs. 4-hour prednisolone levels; c) 8-hours vs. 2-hours prednisolone levels and d) 8-hours vs. 1-hour prednisolone levels.

159x142mm (144 x 144 DPI)

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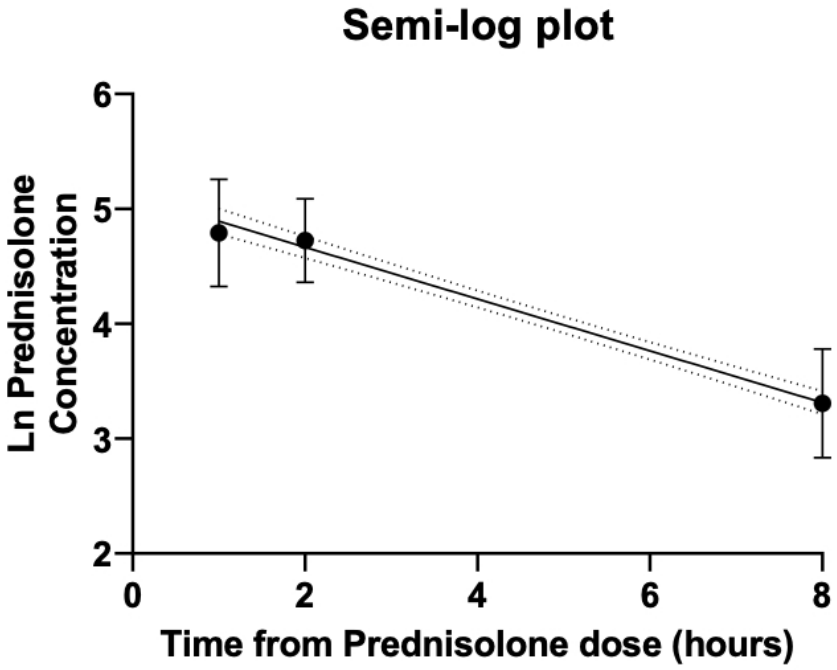

Figure 5: Semi-log plot.

133x100mm (144 x 144 DPI)

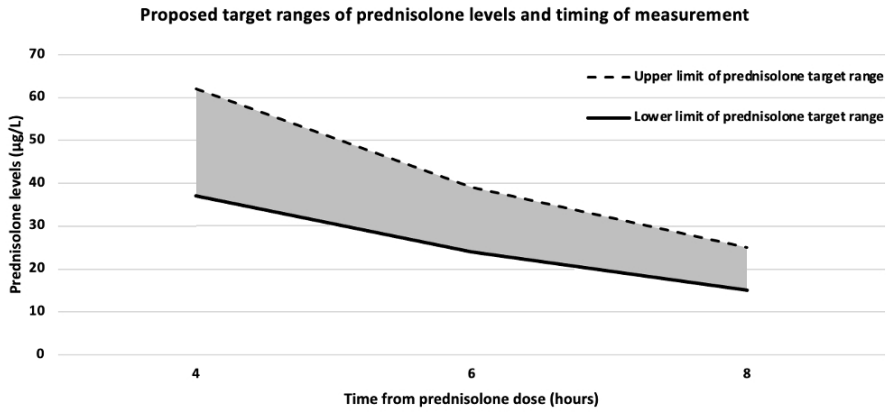

Figure 6: Proposed target ranges of prednisolone levels and timing of measurement.

195x94mm (144 x 144 DPI)