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Characterization of Adrenocortical Function in Patients with Severe Infections Using Corticotropin-stimulated Serum Steroid Profiles

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Adrenocortical Monitoring with MS

Objective

To better understand the need for fully automated LC-MS/MS solutions

LMU

University Hospital, Ludwig-Maximilians University (LMU), Munich

LMU founded 1472 - best ranked university in the EU – 14 Nobel Prize winners

Hospital >10.000 employees, > 1.700 physicians, > 4.000 medical students

Institute of Laboratory Medicine: approx. 9 Mio analyses per year

LC-MS/MS in routine diagnostic use since 1999

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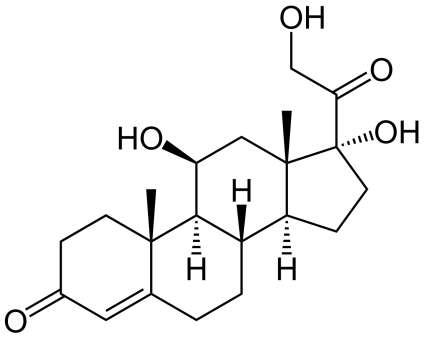
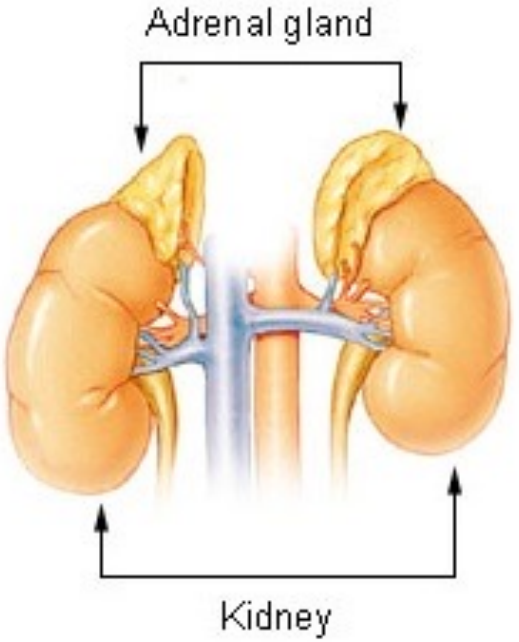
What is the role of cortisol ?

Controls the body's medium- and longer-term response to physiological stress
(e.g., infection, starvation, tissue damage, surgery)

Metabolic, immunological, circulatory and psychological effects, etc.

Secreted from the cortex of the adrenal glands





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What can go wrong?

Adrenocortical dysfunctions

- Inborn – congenital adrenal hyperplasia (CAH) – defective enzymes
- Destruction of the adrenal glands in tuberculosis
- Atrophy of the adrenal cortex under long-term steroid therapy
- Addison‘s Disease - autoimmune destruction of the adrenal cortex

Body is unable to react adequately to stress e.g. surgery →

Addison‘s crisis (hypotension) → Addison‘s coma → shock → death

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How is the adrenocortial function tested today?

One-point measurement of serum cortisol useless

Dynamic studies required: ACTH-stimulation test

- ACTH is injected i.v.
  = endogenous regulator of cortisol synthesis, secreted from the pituitary gland (syn. Corticotropin, Synacthen ®)
- Serum is sampled before and 60 min after injection
- Measurement of cortisol in the serum samples
  so far by immunoassays
- Normal: increase of cortisol to > 18 µg/dL

= biochemical stress simulation test
typically done in patient with suspected Addison's disease

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Can steroids help patients with severe infections?

- Favourable effects of steroid administration observed on gas exchange and circulation of patients with sepsis – case reports, single studies

- variable effects in individual patients

→ Concept of **relative adrenal failure** in sepsis - **Prof. Briegel** and others

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Identifying patients who benefit from steroids in sepsis ?

Several studies on steroid administration with unclear evidence

Main problem probably lack of personalization

Corticosteroids for treating sepsis in children and adults (Review)

Annane D, Bellissant E, Bollaert PE, Briegel J, Keh D, Kupfer Y, Pirracchio R, Rochwerg B

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Novel approaches to adrenal gland diagnostics

Multi-analyte steroid profiling of the synthetic pathways with LC-MS/MS („*steroidomics*“)

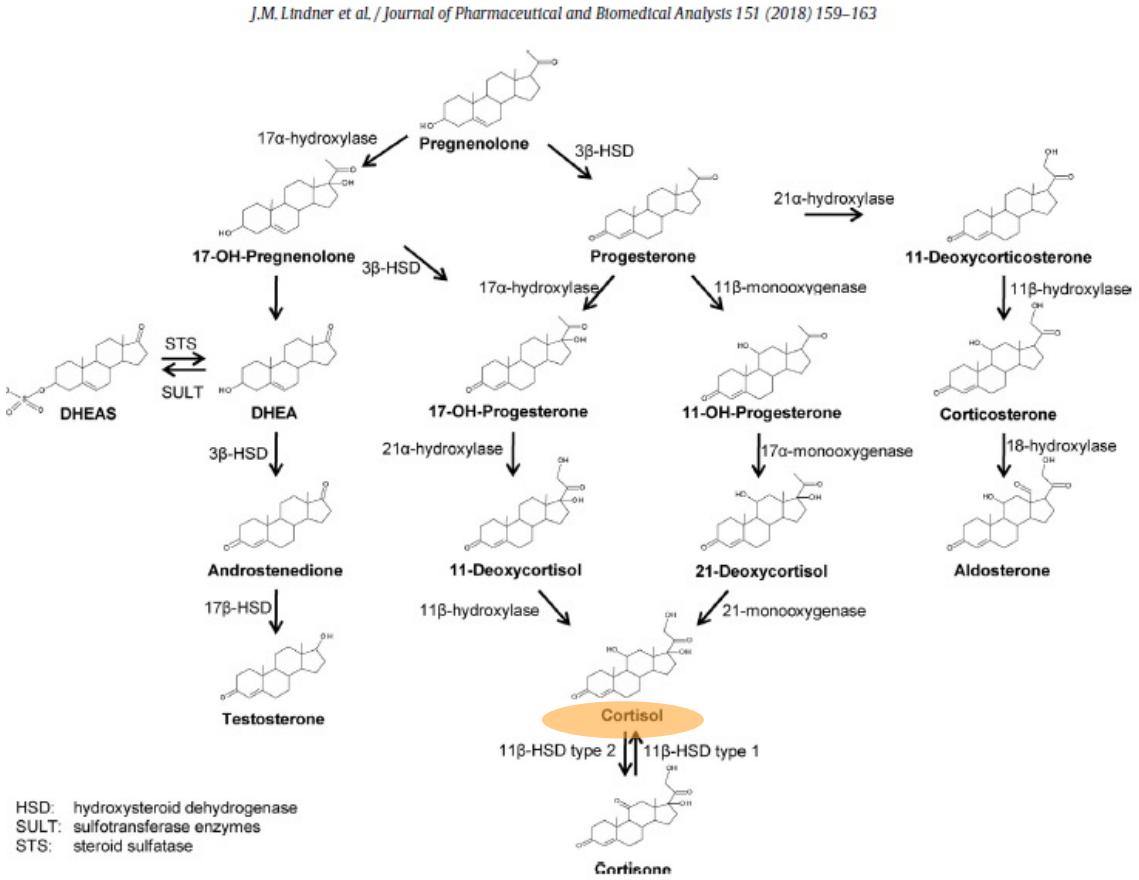

Fig. 1. Metabolic pathway of corticosteroids.

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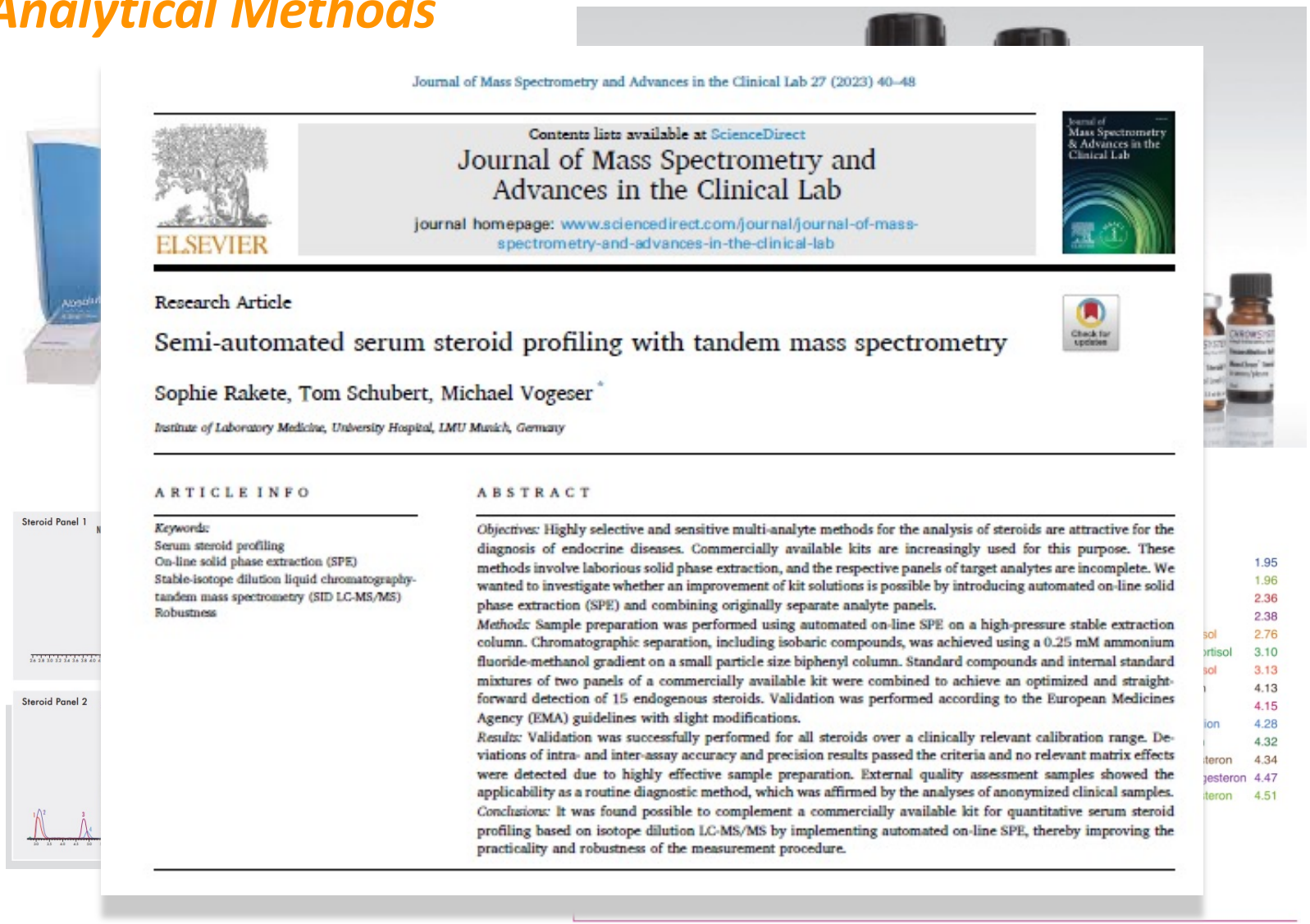
Analytical Methods



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Research Article

Semi-automated serum steroid profiling with tandem mass spectrometry

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ARTICLE INFO

Keywords:
Serum steroid profiling
On-line solid phase extraction (SPE)
Stable-isotope dilution liquid chromatography-tandem mass spectrometry (SID LC-MS/MS)
Robustness

ABSTRACT

Objectives: Highly selective and sensitive multi-analyte methods for the analysis of steroids are attractive for the diagnosis of endocrine diseases. Commercially available kits are increasingly used for this purpose. These methods involve laborious solid phase extraction, and the respective panels of target analytes are incomplete. We wanted to investigate whether an improvement of kit solutions is possible by introducing automated on-line solid phase extraction (SPE) and combining originally separate analyte panels.

Methods: Sample preparation was performed using automated on-line SPE on a high-pressure stable extraction column. Chromatographic separation, including isobaric compounds, was achieved using a 0.25 mM ammonium fluoride-methanol gradient on a small particle size biphenyl column. Standard compounds and internal standard mixtures of two panels of a commercially available kit were combined to achieve an optimized and straightforward detection of 15 endogenous steroids. Validation was performed according to the European Medicines Agency (EMA) guidelines with slight modifications.

Results: Validation was successfully performed for all steroids over a clinically relevant calibration range. Deviations of intra- and inter-assay accuracy and precision results passed the criteria and no relevant matrix effects were detected due to highly effective sample preparation. External quality assessment samples showed the applicability as a routine diagnostic method, which was affirmed by the analyses of anonymized clinical samples.

Conclusions: It was found possible to complement a commercially available kit for quantitative serum steroid profiling based on isotope dilution LC-MS/MS by implementing automated on-line SPE, thereby improving the practicality and robustness of the measurement procedure.

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MS/MS-steroid profiling

+

ACTH-stimulation testing

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First Reference range study for steroid profiling after ACTH

- 36 healthy individuals
- Cortisol, Cortisone, Corticosterone, 11-Deoxycortisol, 11-Deoxycorticosterone, 17-OH-Progesterone
- Baseline and 60 after 250 µg ACTH i.v.

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The dynamics of a serum steroid profile after stimulation with intravenous ACTH

Johanna M. Lindner[a,*,1], Anna Catharina Suhr[a,1], Stefanie H. Grimm[a], Patrick Möhnle[b], Michael Vogeser[a], Josef Briegel[b]

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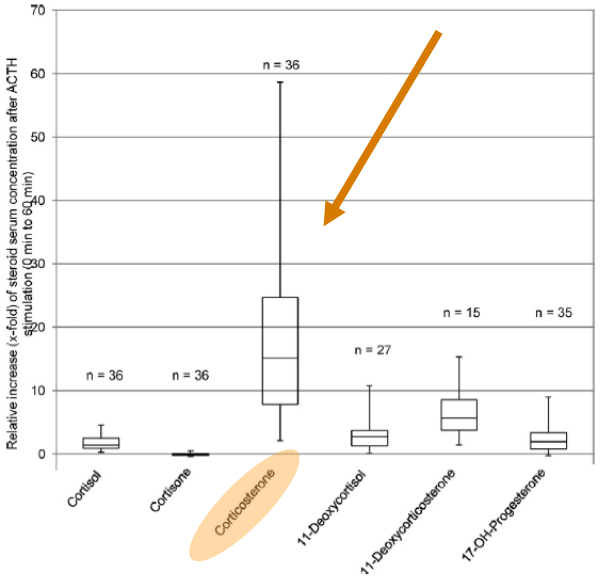

Fig. 2. Relative increase (x-fold) of steroids in serum after iv ACTH administration (250 µg) (0 min to 60 min). The bars display the minimum and the maximum of the respective increases. The different number of cases results from the lacking basal concentration of some samples due to their low concentrations (<LLOQ).

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Translation > *HYPRESS trial*

- **HY**drocortison for **PR**evention of **S**eptic **S**hock

  JAMA 2016;316:1775-85
- Multicentre, placebo-controlled, double-blind trial
- Funded by the German Federal Ministry of Education and Research
- 180 adult patients with sepsis - not in shock
- i.v. Cortisol vs. i.v. Placebo,
- ACTH-stimulation testing prior to randomization
- 360 samples for MS-Steroid profiling (Johanna Lindner)

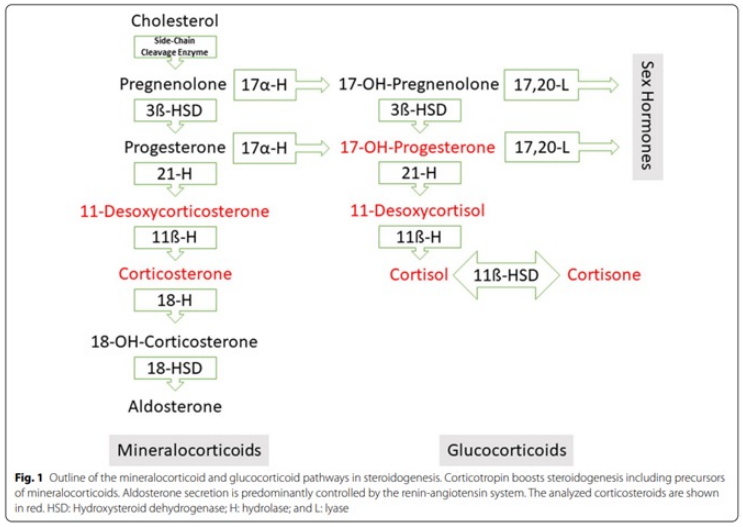

Fig. 1 Outline of the mineralocorticoid and glucocorticoid pathways in steroidogenesis. Corticotropin boosts steroidogenesis including precursors of mineralocorticoids. Aldosterone secretion is predominantly controlled by the renin-angiotensin system. The analyzed corticosteroids are shown in red. HSD: Hydroxysteroid dehydrogenase; H: hydrolase; and L: lyase

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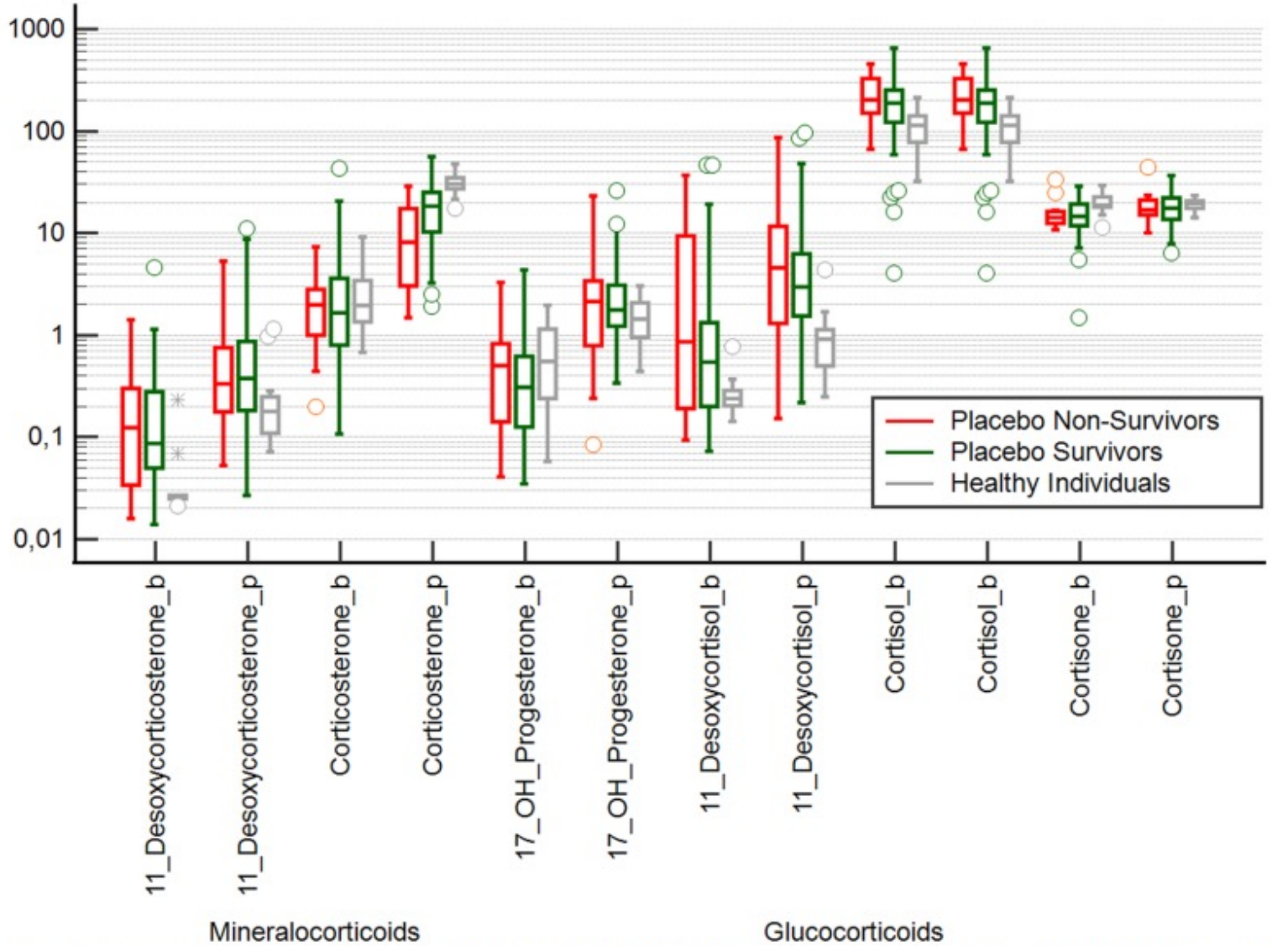
Results



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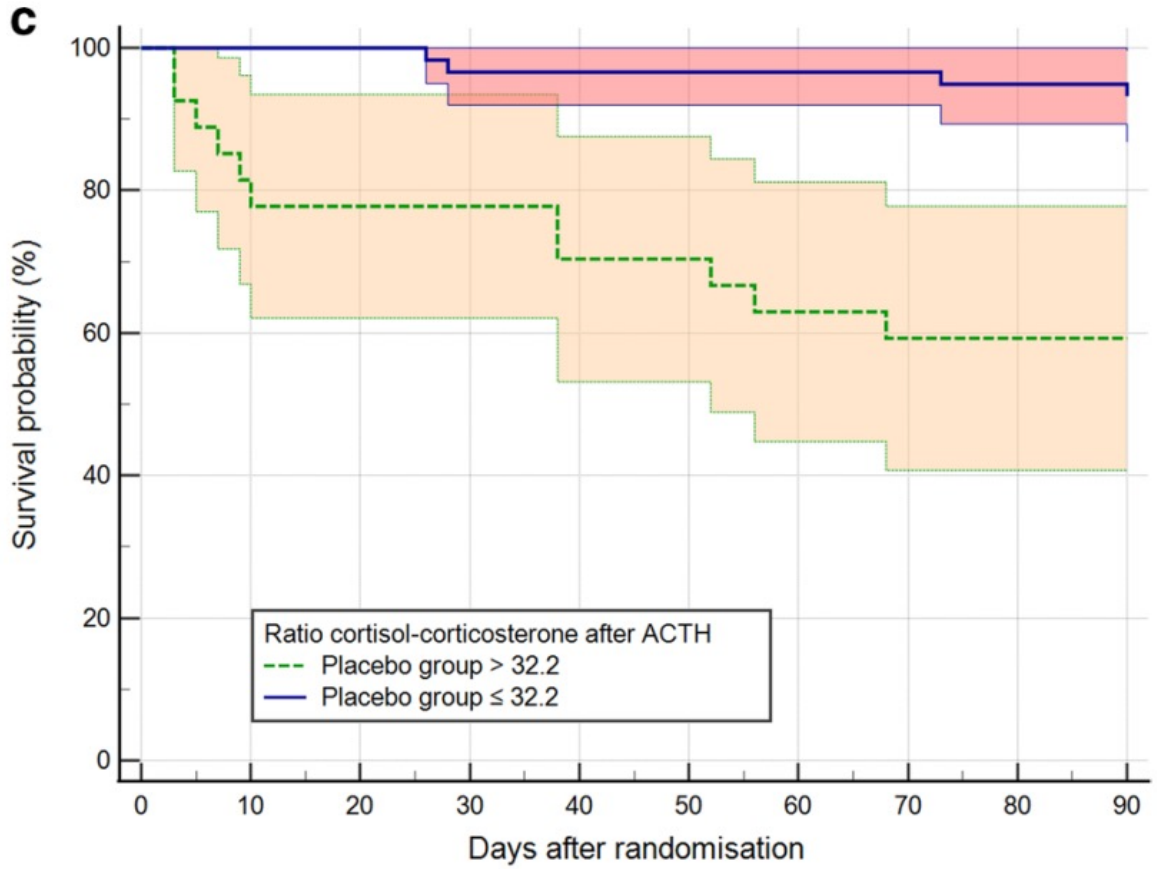
- Data from the placebo group were evaluated for their power to predict shock development and death
- Powerful marker:
  cortisol-to-corticosterone ratio after ACTH



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Briegel *et al. Critical Care* (2022) 26:343
https://doi.org/10.1186/s13054-022-04224-5

Critical Care

RESEARCH Open Access

Corticotropin-stimulated steroid profiles to predict shock development and mortality in sepsis: From the HYPRESS study

Josef Briegel[1,8*], Patrick Möhnle[2], Didier Keh[3], Johanna M. Lindner[4], Anna C. Vetter[4], Holger Bogatsch[5], Dorothea Lange[1], Sandra Frank[1], Ludwig C. Hinske[1,6], Djillali Annane[7], Michael Vogeser[4] and SepNet Critical Care Trials Group

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Specific conclusion

LC-MS/MS based steroid profiling allows Identification of a high-risk population of patients with sepsis who might benefit most from administration of steroids

→ ratio of stimulated cortisol-to-corticosterone as a novel biomarker

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General conclusion - 1

Patterns derived from MS/MS multianalyte analyses have additional diagnostic value compared to single compound evaluation by immunoassay

multi-dimensional approach vs. **one-dimensional** diagnostic approach

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General conclusion - 2

Steroid treatment of patients with sepsis:

example of many specific diagnostic challenges where novel multi-dimensional testing strategies could improve patient management through ***personalization***

Unmet diagnostic needs may not be very obvious to the medical public.

Medicine has many facets and is complex

“rare diseases / conditions are frequent” – taken together - mosaic

Close collaboration between clinicians and analysts required to address needs

Clinical pathologist at the interface

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General conclusion – 3

The HYPRESS publication was a ***retrospective*** analysis –

For prospective studies and to have an impact on patient management in the ICU, serum steroid profiles need to be available ***24/7*** from **automated, fully standardized MS-based clinical laboratory analysis systems with random access**

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Outlook

- i-RECORDS study – International Rapid rEcognition of COrticoseroiD sensitivity or resistance in Sepsis
- Multicenter study funded by the European Commission
  → MS steroid profiling in a multi-omics approach.
- Application of artificial intelligence to predict corticosteroid response in sepsis

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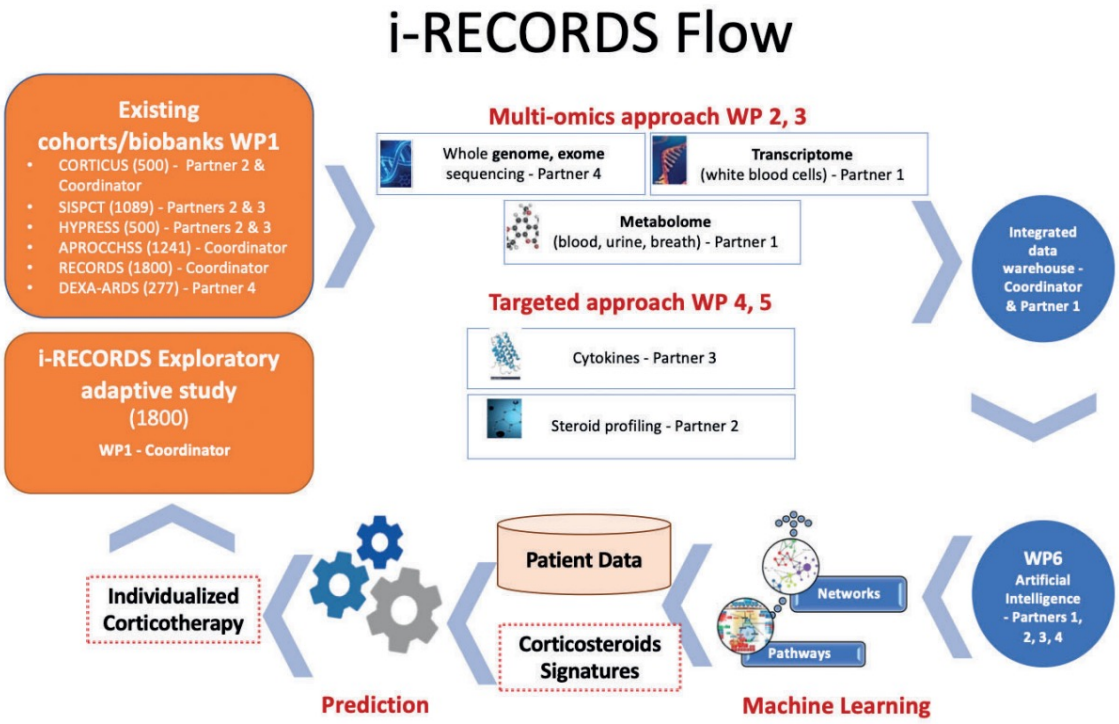

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Thank you very much

for your attention !

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