of elevation of ACTH secretion and the ACTH-dependent Cushing's Syndrome, characterized by excessive ACTH secretion.

[0004] ACTH-dependent Cushing's Syndrome includes roughly 80% of patients having endogenous Cushing's Syndrome and consists of two major forms: Cushing's Disease and Ectopic Cushing's Syndrome. The former is caused by a pituitary tumor and the latter is caused by a tumor outside the pituitary. Correct differential diagnosis between the Cushing Disease and Ectopic Cushing's Syndrome is important for endocrinologists to recommend transphenoidal surgery or appropriate imaging to identify source of the ectopic ACTH secretion.

[0005] Current approaches of differentially diagnosing patients with ACTH-dependent Cushing's Syndrome involve measuring ACTH levels from samples obtained from both inferior petrosal venous sinus (IPS)– a procedure referred to as inferior petrosal venous sinus sampling (IPSS) – and from the internal jugular or another peripheral vein from the patient. Some of these approaches require collecting blood samples both before and after administration of an agent, such as CRH, DDAVP, or metyrapone. ACTH concentration in the samples are measured and a central to periphery ACTH ratio is determined and compared with a predetermined threshold to determine whether the patient has Cushing's Disease or Ectopic Cushing's Syndrome. Generally, a central-to-periphery ACTH ratio of >2 before and >3 after the administration of CRH or DDAVP is consistent with Cushing Disease while a lower ratio favors Ectopic Cushing's Syndrome. These procedures require not only prolonged catheterization with the likelihood of infection, thrombosis, or bleeding rising with the duration of catheterization, but also sophisticated handling that can only be conducted by trained professionals, *e.g.*, an interventional radiologist. Abnormal venous drainage of the pituitary could lead to false negative IPSS results that could delay transphenoidal surgery, lead to unnecessary imaging studies and possibly unnecessary bilateral adrenalectomy. In addition, CRH is expensive to produce and metyrapone is currently not available in the United States. These current diagnosis methods thus have serious limitations.

BRIEF SUMMARY

[0006] Provided herein is a method of differentially diagnosing Cushing's Disease from Ectopic Cushing's Syndrome in a patient with an established diagnosis of ACTH-dependent Cushing's Syndrome. The method comprises: (i) determining a baseline cortisol level

and a baseline adrenocorticotropic hormone (ACTH) level from one or more pretreatment samples taken from the patient; (ii) administering a glucocorticoid receptor antagonist (GRA) to the patient for a period of not less than 5 weeks and in an amount effective to raise ACTH and cortisol levels in a healthy individual by at least two fold; (iii) determining a second ACTH level and a second cortisol level from one or more second samples taken from the patient in step (ii); (iv) calculating a baseline ratio of cortisol to ACTH ("baseline C:A ratio") using the baseline levels of cortisol and ACTH, and calculating a GRA-exposed ratio of cortisol to ACTH ("GRA-exposed C:A ratio") using the second cortisol and ACTH levels; and, (v) diagnosing the patient as having Ectopic Cushing's Syndrome if the GRA-exposed C:A ratio of cortisol to ACTH has decreased by greater than 50% compared to the baseline C:A ratio, or diagnosing the patient as having Cushing's Disease if the GRA-exposed C:A ratio has increased by greater than 50% compared to the baseline C:A ratio.

[0006a] In one aspect of the present disclosure, there is provided a method of differentially diagnosing Cushing's Disease from Ectopic Cushing's Syndrome in a patient with an established diagnosis of ACTH-dependent Cushing's Syndrome, wherein:

(i) a baseline cortisol level and a baseline adrenocorticotropic hormone (ACTH) level is determined from one or more pretreatment samples obtained from the patient;

(ii) a glucocorticoid receptor antagonist (GRA) is to be administered to the patient for a period of not less than 5 weeks and in an amount effective to raise ACTH levels in a healthy individual by at least two-fold;

(iii) a second ACTH level and a second cortisol level from one or more second samples obtained from the patient in step (ii) is determined after 5 weeks of treatment with GRA;

(iv) a baseline ratio of cortisol to ACTH is calculated using the baseline levels of cortisol and ACTH, and a GRA-exposed ratio of cortisol to ACTH is calculated using the second cortisol and ACTH levels; and,

(v) a GRA-exposed ratio of cortisol to ACTH which has decreased by greater than 50% compared to the baseline ratio is diagnostic of Ectopic Cushing's Syndrome in the patient, or a GRA-exposed ratio of cortisol to ACTH which has increased by greater than 50% compared to the baseline ratio is diagnostic of Cushing's Syndrome in the patient.

[0006b] In another aspect of the present disclosure, there is provided a system for differentially diagnosing Cushing's Disease from Ectopic Cushing's Syndrome in a patient with an established diagnosis of ACTH-dependent Cushing's Syndrome, the system comprising:

a processor;

a memory coupled with the processor via an interconnect;

a communications interface coupled with the interconnect and adapted to receive a first set of values and a second set of values corresponding to analytical results from samples obtained from the patient;

a comparison engine communicatively coupled with the processor, wherein the comparison engine is configured to:

receive the first set of values, which represent a set of pre-treatment values corresponding to a baseline cortisol level and baseline adrenocorticotropic hormone (ACTH) or a baseline ratio of the baseline cortisol level to the baseline ACTH level;

receive the second set values, which represent a set of post-treatment values corresponding to a cortisol level and an ACTH level or a ratio of the cortisol to the ACTH level of the patient after a minimum of 5 weeks of treatment with a glucocorticoid receptor antagonist (GRA) in an amount effective to raise cortisol levels in a healthy individual by at least two-fold; and

compare the first set of values and the second set of values to determine a differential relationship between the pre-treatment values and the post-treatment values; and

an output module communicatively coupled with the processor and configured to output an indication, wherein the indication represents a positive diagnosis for Ectopic Cushing's Syndrome if the differential relationship represents a pre-determined decrease of the exposed-GRA levels as compared to the baseline levels and the indication represents a positive diagnosis for Cushing's Disease if the differential relationship represents a pre-determined increase of the exposed-GRA levels as compared to the baseline levels.

[0006c] In a further aspect of the present disclosure, there is provided a method of differentially diagnosing Cushing's Disease from Ectopic Cushing's Syndrome in a patient with an established diagnosis of ACTH-dependent Cushing's Syndrome, the method comprising:

obtaining, with a computing system associated with a treating physician, a first set of values representing a baseline cortisol level and a baseline ACTH level or a baseline ratio between the baseline cortisol and ACTH levels corresponding to one or more pre-treatment samples from the patient;

obtaining, with the computing system, a second set of values representing an exposed-GRA cortisol level and an exposed-GRA ACTH level or a ratio between the exposed-GRA cortisol and ACTH levels corresponding to one or more post-treatment samples from the patient obtained after undergoing a GRA treatment according to a predetermined protocol;

determining, with the computing system, a differential relationship between the first and second set of values; and

outputting to a user, with the computing system, an indication representing a positive diagnosis for Ectopic Cushing's Syndrome, if the differential relationship represents a pre-determined decrease of the exposed-GRA levels as compared to the baseline levels; and

outputting to a user, with the computing system, an indication representing a positive diagnosis for Cushing's Disease, if the differential relationship represents a pre-determined increase of the exposed-GRA levels as compared to the baseline levels.

[0007] In some embodiments, the GRA is a non-steroidal GRA. In some embodiments, GRA is mifepristone. In some embodiments, the GRA is selected from the group consisting of

- (R)-4a-(ethoxymethyl)-1-(4-fluorophenyl)-6-((4-(trifluoromethyl)phenyl)sulfonyl)-4,4a,5,6,7,8-hexahydro-1H-pyrazolo[3,4-g]isoquinoline;
- 6-((1r,4r)-4-phenylcyclohexyl)-5-(3-(trifluoromethyl)benzyl)pyrimidine-2,4(1H,3H)-dione;
- (R)-(1-(4-fluorophenyl)-6-((4-(trifluoromethyl)phenyl)sulfonyl)-4,4a,5,6,7,8-hexahydro-1H-pyrazolo[3,4-g]isoquinolin-4a-yl)(thiazol-2-yl)methanone;

- (R)-(1-(4-fluorophenyl)-6-((1-methyl-1H-pyrazol-4-yl)sulfonyl)-4,4a,5,6,7,8-hexahydro-1H-pyrazolo[3,4-g]isoquinolin-4a-yl)(4-(trifluoromethyl)pyridin-2-yl)methanone; and
- ((4aR,8aS)-1-(4-fluorophenyl)-6-((2-methyl-2H-1,2,3-triazol-4-yl)sulfonyl)-4,4a,5,6,7,8,8a,9-octahydro-1H-pyrazolo[3,4-g]isoquinolin-4a-yl)(4-(trifluoromethyl)pyridin-2-yl)methanone.

[0008] In some embodiments the one or more second samples are taken from the patients after mifepristone has been administered for a minimum of 6 weeks, e.g., between 6 and 10 weeks, or

between 10 and 24 weeks; and/or mifepristone is administered on a daily basis and is equal to 5-20 mg/kg of the patient.

[0009] In some embodiments, the pretreatment samples and second samples are from saliva. In some embodiments, the pretreatment samples and second samples are from plasma and measured in μg/dl. In some embodiments, the pretreatment samples and second samples are from 24-hour urine collections.

[0010] Provided herein is also a system configured for facilitating differential diagnosis between Ectopic Cushing's Syndrome and Cushing's Disease in a patient with an established diagnosis of ACTH-dependent Cushing's Syndrome. Such systems can encompass a computing system having a diagnostic module that includes a processor coupled with a memory via an interconnect, a communications module or input and an output module. It is appreciated that the interconnect can include an interconnect bus or can include any communication coupling, including wireless couplings and remotely access through a network, the internet or a cloud server. The memory can include a tangible, non-transitory storage medium having instructions recorded thereon for causing the processor to compare a first set of values corresponding to baseline levels of cortisol and ACTH in the patient before exposure to a GRA and a second set of values corresponding to exposed-GRA levels of cortisol and ACTH in the patient after a treatment with GRA. Typically, the GRA treatment is administration of a GRA for a minimum of 5 weeks amount effective to raise ACTH and cortisol levels in a health individual by at least two fold. The system is then configured to determine a differential relationship between the first and second set of values or associated ratios and output the differential relationship and/or a positive diagnosis of Ectopic Cushing's Syndrome or Cushing's Disease to a user.

[0011] There is also provided methods of differentially diagnosing Cushing's Disease from Ectopic Cushing's Syndrome in a patient with an established diagnosis of ACTH-dependent Cushing's Syndrome. Such methods can be facilitated by use of any of the systems described herein, or similar such systems. An example of such a method includes a step of obtaining, with a computing device associated with a treating physician, a first set of values and a second set of values. The first set of values represent a baseline cortisol level and a baseline ACTH level or a baseline ratio between the baseline cortisol and ACTH levels and correspond to

one or more pre-treatment samples from the patient. The second set of values represent an exposed-GRA cortisol level and an exposed-GRA ACTH level or a ratio between the exposed-GRA cortisol and ACTH levels corresponding to one or more post-treatment samples from the patient obtained after undergoing a GRA treatment according to a predetermined protocol. The methods can be designated as corresponding to baseline or post-treatment samples within a memory of the computing device. The ratio between a cortisol level and an ACTH level may be determined, termed herein a "C:A ratio". A baseline C:A ratio may be determined from levels measured prior to GRA treatment, and a GRA-exposed C:A level may be determined from levels measured during or following GRA treatment. The method further includes determining, with the computing device, a differential relationship between the first and second set of values and then outputting to a user an indication of the differential diagnosis. If the differential relationship represents a decrease of the ratio calculated from exposed-GRA level of cortisol divided by the exposed-GRA level of ACTH that is greater than a pre-determined decrease (e.g. the C:A ratio calculated from exposed-GRA levels is less than 50% of baseline C:A ratio calculated from baseline cortisol and ACTH levels), the differential relationship indicates a positive diagnosis for Ectopic Cushing's Syndrome. If the differential relationship represents a pre-determined increase (e.g. an increase of greater than 50% of baseline ratio) of the exposed-GRA levels (*e.g.*, the ratio calculated from exposed-GRA level of cortisol divided by the exposed-GRA level of ACTH that is greater than the baseline C:A ratio by at least 50 % of the baseline C:A ratio calculated from baseline cortisol and ACTH levels), the differential relationship indicates a positive diagnosis for Cushing's Disease. The output can be displayed on a user interface display of the computing device or can be output to an external computing device for display or printing to a user.

[0012] In one embodiment, the systems are configured to automatically perform the diagnosis methods described herein when baseline and post-treatment samples are obtained from a patient with an established diagnosis of ACTH-dependent Cushing's Syndrome. In another embodiment, the system is configured to perform the diagnostic methods described herein upon receiving a request for such a diagnosis from a treating physician or associated personnel.

[0013] There is also provided a method of concurrently treating Cushing's Syndrome and differentially diagnosing Cushing's Disease from Ectopic Cushing's Syndrome in

a patient with an established diagnosis of ACTH-dependent Cushing's Syndrome. The method comprises: (i) determining a baseline cortisol level and a baseline adrenocorticotropic hormone (ACTH) level from one or more pretreatment samples taken from the patient; (ii) administering a glucocorticoid receptor antagonist (GRA) effective to treat Cushing's Syndrome in the patient, thereby treating Cushing's Syndrome; (iii) continuing said treatment of Cushing's Syndrome by administering said GRA to the patient for a period of not less than 5 weeks and in an amount effective to raise ACTH and cortisol levels in a healthy individual by at least two fold; (iv) determining a second ACTH level and a second cortisol level from one or more second samples taken from the patient in step (iii); (v) calculating a baseline ratio of cortisol to ACTH ("baseline C:A ratio") using the baseline levels of cortisol and ACTH, and calculating a GRA-exposed ratio of cortisol to ACTH ("GRA-exposed C:A ratio") using the second cortisol and ACTH levels; and, (vi) diagnosing the patient as having Ectopic Cushing's Syndrome if the GRA-exposed C:A ratio of cortisol to ACTH has decreased by greater than 50% compared to the baseline C:A ratio, or diagnosing the patient as having Cushing's Disease if the GRA-exposed C:A ratio has increased by greater than 50% compared to the baseline C:A ratio.

[0014] There is also provided methods of concurrently treating Cushing's Syndrome and differentially diagnosing Cushing's Disease from Ectopic Cushing's Syndrome in a patient with an established diagnosis of ACTH-dependent Cushing's Syndrome. Such methods can be facilitated by use of any of the systems described herein, or similar such systems. An example of such a method includes a step of obtaining, with a computing device associated with a treating physician, a first set of values; then administering a glucocorticoid receptor antagonist (GRA) effective to treat Cushing's Syndrome in the patient, thereby treating Cushing's Syndrome; and then obtaining, with a computing device associated with a treating physician, a second set of values. The first set of values represent a baseline cortisol level and a baseline ACTH level or a baseline ratio between the baseline cortisol and ACTH levels and correspond to one or more pre-treatment samples from the patient. The second set of values represent an exposed-GRA cortisol level and an exposed-GRA ACTH level or a ratio between the exposed-GRA cortisol and ACTH levels corresponding to one or more post-treatment samples from the patient obtained after undergoing a GRA treatment according to a predetermined protocol. The predetermined protocol may comprise one, two, or more administrations of a GRA to treat Cushing's Syndrome in the patient. The methods can be

WHAT IS CLAIMED IS:

1. A method of differentially diagnosing Cushing's Disease from Ectopic Cushing's Syndrome in a patient with an established diagnosis of ACTH-dependent Cushing's Syndrome, wherein:

(i) a baseline cortisol level and a baseline adrenocorticotropic hormone (ACTH) level is determined from one or more pretreatment samples obtained from the patient;

(ii) a glucocorticoid receptor antagonist (GRA) is to be administered to the patient for a period of not less than 5 weeks and in an amount effective to raise ACTH levels in a healthy individual by at least two-fold;

(iii) a second ACTH level and a second cortisol level from one or more second samples obtained from the patient in step (ii) is determined after 5 weeks of treatment with GRA;

(iv) a baseline ratio of cortisol to ACTH is calculated using the baseline levels of cortisol and ACTH, and a GRA-exposed ratio of cortisol to ACTH is calculated using the second cortisol and ACTH levels; and,

(v) a GRA-exposed ratio of cortisol to ACTH which has decreased by greater than 50% compared to the baseline ratio is diagnostic of Ectopic Cushing's Syndrome in the patient, or a GRA-exposed ratio of cortisol to ACTH which has increased by greater than 50% compared to the baseline ratio is diagnostic of Cushing's Syndrome in the patient.

2. The method of claim 1 wherein the GRA is a non-steroidal GRA.

3. The method of claim 1 wherein the GRA is mifepristone, the one or more second samples are taken from the patient after mifepristone has been administered for a minimum of 6 weeks and on a daily basis equal to between 5-20 mg/kg of the patient.

4. The method of claim 1 wherein the GRA is selected from the group consisting of (R)-4a-(ethoxymethyl)-1-(4-fluorophenyl)-6-((4-(trifluoromethyl)phenyl)sulfonyl)-4,4a,5,6,7,8-hexahydro-1H-pyrazolo[3,4-g]isoquinoline; 6-((1r,4r)-4-phenylcyclohexyl)-5-(3-(trifluoromethyl)benzyl)pyrimidine-2,4(1H,3H)-dione; (R)-(1-(4-fluorophenyl)-6-((4-(trifluoromethyl)phenyl)sulfonyl)-4,4a,5,6,7,8-hexahydro-1H-pyrazolo[3,4-g]isoquinolin-4a-yl)(thiazol-2-yl)methanone; (R)-(1-(4-fluorophenyl)-6-((1-methyl-1H-pyrazol-4-yl)sulfonyl)-4,4a,5,6,7,8-hexahydro-1H-pyrazolo[3,4-g]isoquinolin-4a-yl)(4-(trifluoromethyl)pyridin-2-

receptor antagonist (GRA) in an amount effective to raise cortisol levels in a healthy individual by at least two-fold; and

compare the first set of values and the second set of values to determine a differential relationship between the pre-treatment values and the post-treatment values; and

an output module communicatively coupled with the processor and configured to output an indication, wherein the indication represents a positive diagnosis for Ectopic Cushing's Syndrome if the differential relationship represents a pre-determined decrease of the exposed-GRA levels as compared to the baseline levels and the indication represents a positive diagnosis for Cushing's Disease if the differential relationship represents a pre-determined increase of the exposed-GRA levels as compared to the baseline levels.

10. The system of claim 9, wherein the output module is configured such that the pre-determined decrease corresponding to a positive diagnosis for Ectopic Cushing's Syndrome is a decrease of the exposed-GRA ratio of greater than 50% as compared to the baseline ratio.

11. The system of claim 9, wherein the output module is configured such that the pre-determined increase corresponding to a positive diagnosis for Cushing's Disease is an increase of the exposed-GRA ratio of greater than 50% as compared to the baseline ratio.

12. The system of claim 9, wherein the comparison engine is further configured such that comparing the first and second set of values comprises determining which of the set of post-treatment values have changed from the set of pre-treatment values, and

wherein the output module is further configured output another indication to the user representing which of the set of post-treatment values have changed from the set of pre-treatment values, wherein the values are labeled in terms of post and pre-treatment values and wherein an increase in the ratio of cortisol to ACTH predicts a positive diagnosis for Cushing's Disease and wherein a decrease in the ratio of cortisol to ACTH predicts a positive diagnosis for Ectopic Cushing's Syndrome.

13. The system of claim 9, wherein the output module communicates said indication to the user in terms of a positive diagnosis of either Cushing's Disease or Ectopic Cushing's Syndrome.

14. The system of claim 9 further comprising:

an analytic device for measuring cortisol and ACTH levels in patient samples where the device is coupled to the processor in a manner permitting the output of the levels to the processor as one or more values.

15. A method of differentially diagnosing Cushing's Disease from Ectopic Cushing's Syndrome in a patient with an established diagnosis of ACTH-dependent Cushing's Syndrome, the method comprising:

obtaining, with a computing system associated with a treating physician, a first set of values representing a baseline cortisol level and a baseline ACTH level or a baseline ratio between the baseline cortisol and ACTH levels corresponding to one or more pre-treatment samples from the patient;

obtaining, with the computing system, a second set of values representing an exposed-GRA cortisol level and an exposed-GRA ACTH level or a ratio between the exposed-GRA cortisol and ACTH levels corresponding to one or more post-treatment samples from the patient obtained after undergoing a GRA treatment according to a predetermined protocol;

determining, with the computing system, a differential relationship between the first and second set of values; and

outputting to a user, with the computing system, an indication representing a positive diagnosis for Ectopic Cushing's Syndrome, if the differential relationship represents a pre-determined decrease of the exposed-GRA levels as compared to the baseline levels; and

outputting to a user, with the computing system, an indication representing a positive diagnosis for Cushing's Disease, if the differential relationship represents a pre-determined increase of the exposed-GRA levels as compared to the baseline levels.

16. The method of claim 15, wherein in the pre-determined treatment protocol, GRA is to be administered to the patient for a minimum of five weeks in an amount effective to raise ACTH and cortisol levels in a healthy individual by at least two-fold.