Letter to the Editor

Establishment of revised diagnostic cut-offs for adrenal laboratory investigation using the new Roche Diagnostics Elecsys® Cortisol II assay

Révision des seuils diagnostiques à utiliser avec le nouveau réactif de dosage du cortisol plasmatique de Roche Diagnostics

Keywords: Cortisol; Synacthen test; Insulin tolerance test; Dexamethasone test

Mots clés : Cortisol ; Test au Synacthène ; Hypoglycémie insulinique ; Test à la dexaméthasone

Measurement of plasma cortisol levels plays a key role in the diagnosis and/or follow-up of patients with suspected dysfunction of the adrenocortical axis, such as Cushing syndrome or corticotroph deficiency. Accurate diagnosis requires measurement, not only in the basal state, but also after stimulation or suppression tests [1–3], the interpretation of which is based on consensus cut-off values. Thus, for a stimulation test, a maximum response of 500 nmol/L or more is considered normal [4,5], while for a suppression test a minimum of 50 nmol/L or less is usually considered as an adequate response [6–8].

These consensus values have been derived however using existing cortisol assays, notably the widely-used Elecsys® Cortisol immunoassay from Roche Diagnostics. A new Elecsys® Cortisol assay is now available (Elecsys® Cortisol II), which is also a competitive immunoassay, but employs a different antibody (monoclonal rather than polyclonal) and is standardised differently (IRMM/IFCC-451 Panel ID-GC-MS, in place of Enzymun-Test® Cortisol). One advantage claimed for this new assay is much lower cross-reactivity with other steroids (for example 12% against 6-α-Methylprenisolone at 0.1 μg/mL compared to 389% for the older assay, and 7.98% against prednisolone at 0.1 μg/mL compared to 171%). There is however a potential pitfall with the new assay, since preliminary data from our laboratory, as well those generated by Roche Diagnostics, indicated that it gives plasma cortisol results which are approximately 20% lower than with the earlier assay. It follows that if the existing cut-off was used for a stimulation test employing the new assay, a significant number of patients could be wrongly diagnosed as having an insufficient response.

To investigate this issue in more detail, we carried out a direct comparison of the existing Elecsys® Cortisol assay with the new Elecsys® Cortisol II assay to accurately determine the magnitude of any systematic bias and to derive revised diagnostic cut-offs for use with the new assay.

For comparison of the two assays, we analysed 863 consecutive samples received in our laboratory. Most were left-over plasma samples from patients undergoing regular endocrine follow-up of Cushing disease or hypothalamic-pituitary dysfunction, the remainder were from patients from other clinics being checked for a possible corticotroph or adrenal abnormality. Among these samples, 28 were from patients who had undergone a suppression (dexamethasone) test, and 109 from patients who had undergone a stimulation test (a Synacthen test in 88 cases, an Insulin Tolerance test [ITT] in 21). Cortisol concentrations were measured on a Cobas® e601 analyser with reagents from Roche Diagnostics GmbH (Mannheim, Germany). The measurements were performed on the same day with the same analyser calibrated for the two sets of reagents. Statistical analyses were performed using GraphPad Prism version 5.01. A two-tailed P value of less than 0.05 was considered as statistically significant.

To assess the precision of the new Elecsys® cortisol II assay, three internal controls were run each day, at three levels: PCU1 (109 nmol/L) and PCU2 (333 nmol/L) from Roche Diagnostics, and Immunoassay-plus control 1 (741 nmol/L) from Biorad. The inter-assay CV for the new assay was 1.9% at 109 nmol/L, 2.8% at 333 nmol/L and 2.8% at 741 nmol/L. The intra-assay CVs was 1.1% and 1.3% at 108 and 426 nmol/L respectively.

To directly compare results obtained using the two assays, we performed a Deming regression analysis, using all 863 samples. The best-fit regression line was given by the equation $y = 0.6965x + 3.972$ (Fig. 1A), $r^2 = 0.9592$, with 95% confidence intervals (CI) of –0.7696 to 8.714 for the y-intercept and 0.6869 to 0.7061 for the slope. Consistent with earlier data, there was therefore a large proportional bias between the two assays (Bland Altman representation) (Fig. 1B), values with the newer assay being approximately 30% lower over most of the working range.

To determine the optimal cut-off for a stimulation test (Synacthen® test or ITT) using the new assay, we first classified our 109 samples as “normal” or “abnormal” on the basis of the results with the older (Elecsys Cortisol) assay, a normal response being defined as being 500 nmol/L or above (independent of the clinical status of the patient). To assess the ability of

http://dx.doi.org/10.1016/j.anndo.2016.05.002
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Table 1
Variation in sensitivity and specificity of the new Elecsys Cortisol II assay for classifying normal and abnormal stimulation test responses at different cut-off levels (taking the old Elecsys Cortisol assay result as the reference).

<table>
<thead>
<tr>
<th>Assay and cut-off</th>
<th>Abnormal response</th>
<th>Normal response</th>
<th>False positivesa</th>
<th>False negativesb</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old assay 500 nmol/L</td>
<td>n = 20</td>
<td>n = 89</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol II 500 nmol/L</td>
<td>61</td>
<td>48</td>
<td>41/89 (46%)</td>
<td>0</td>
<td>100</td>
<td>54</td>
</tr>
<tr>
<td>Cortisol II 400 nmol/L</td>
<td>30</td>
<td>79</td>
<td>10/89 (11.2%)</td>
<td>0</td>
<td>100</td>
<td>88.8</td>
</tr>
<tr>
<td>Cortisol II 374 nmol/L</td>
<td>26</td>
<td>83</td>
<td>6/89 (6.7%)</td>
<td>0</td>
<td>100</td>
<td>93.3</td>
</tr>
<tr>
<td>Cortisol II 350 nmol/L</td>
<td>16</td>
<td>93</td>
<td>0</td>
<td>4/20 (20%)</td>
<td>85</td>
<td>100</td>
</tr>
</tbody>
</table>

a Samples classified as normal by the old assay but falsely considered as abnormal by the new assay.
b Samples classified as abnormal by the old assay but falsely considered as normal by the new assay.

the new Elecsys Cortisol II assay to correctly classify these same samples, we then constructed a ROC curve using the results of the new assay, by plotting “sensitivity” against “(1-specificity)” with the cut-off set at different levels.

The ROC curve had an AUC of 0.9966 (95% CI: 0.9899–1.003). As shown in Table 1, if the current cut-off of 500 nmol/L is used with the new assay, sensitivity (for detecting abnormal cases) is 100% but specificity is only 54%, implying that 46% of normal cases would be wrongly classified as abnormal (insufficient) responders – an unacceptable false-positive rate.

If the cut-off is lowered, specificity improves, initially with no loss of sensitivity. A point is reached however (below 374 nmol/L) beyond which any further gain in specificity incurs a reduction in sensitivity to below 100%. Since we considered the overriding aim was to not miss any abnormal cases, this point determines the minimum cut-off level. Based on this study of more than 100 stimulation tests, we therefore propose that the cut-off for use with the new Cortisol II assay should be 374 nmol/L.

Similar analysis of the 28 suppression test results gave a ROC curve with an AUC of 1.000 (95% CI: 1.000–1.000). Unlike the stimulation test results, however, the distribution of normal and abnormal values was such that even if the current cut-off of 50 nmol/L was used for the new assay, sensitivity and specificity were still both 100%. There was therefore no advantage in altering the existing cut-off to the ROC-derived equivalent concentration of 43 nmol/L, which was obtained from a small cohort.

In summary, the new Elecsys® Cortisol II assays gave great results in term of intra-assay imprecision and reproducibility (intermediate conditions). Furthermore, we have confirmed a systematic bias with respect to the current Cortisol assay, such that values obtained are around 30% lower, necessitating a revision of the current cut-off for assessment of stimulation test results. Based on our ROC analysis, and the precision of the new assay, we recommend the following approach:

- for all suppression tests, the threshold of 50 nmol/L may be retained;
- for stimulation tests the new cut-off should be 374 nmol/L.

Funding sources

Roche Diagnostics kindly provided reagents for cortisol assays.

Disclosure of interest

The authors declare that they have no competing interest.
References


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