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Measuring adrenal autoantibody response: Interlaboratory concordance in the first international serum exchange for the determination of 21-hydroxylase autoantibodies☆

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Abstract 21-hydroxylase autoantibodies (21OHAb) are the gold standard immune marker to identify patients with clinical or subclinical autoimmune Addison's disease (AAD). No assessment of interlaboratory concordance has been made for 21OHAb measurement. Serum samples from 267 patients with primary adrenal insufficiency and from 83 healthy control subjects were distributed to four independent laboratories that determined presence and titer of 21OHAb, by using radiobinding assays with either *in vitro* translated ^{35}S-radiolabelled or ^{125}I-radiolabelled autoantigen. Cohen's κ of inter-rater agreement ranged from 0.857 to 0.983, showing a very good concordance of the positive/negative score among the four laboratories. Passing–Bablok regression showed a good agreement of 21OHAb titers arranged by ranks, but important discrepancies emerged at the Bland–

Abbreviations 21OHAb, 21-hydroxylase autoantibodies; AAD, Autoimmune Addison's Disease; ACA, Adrenal Cortex Autoantibodies; AHC, adrenal hypoplasia congenita; ALD, adrenoleukodystrophy; APS, autoimmune polyendocrine syndrome; AUC, area under ROC curve; CI, confidence interval; IAN, Italian Addison Network; ICC, intra-class correlation coefficient; PAI, primary adrenal insufficiency; RC, repeatability coefficient; ROC, receiver-operating characteristic; TBC, tuberculosis; T1DM, type 1 diabetes mellitus.

☆ *Disclosure statement:* RSR Ltd is a manufacturer of medical diagnostics including kits for 21OH autoantibodies.

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Altman plot, as the repeatability coefficient was much higher than the laboratory cut-offs, which indicates that results from different laboratories cannot be used interchangeably. A standardization international program for 21OHAb measurement is strongly needed.

1. Introduction

Primary adrenal insufficiency (PAI) affects approximately 1 in 7000 individuals [1,2]. The primitive adrenocortical deficit has an heterogeneous etiopathogenesis and may develop through several distinct mechanisms, including autoimmunity, infiltrative adrenalitis, adrenoleukodystrophy (ALD), genetic disorders, metastasis, adrenal hemorrhage, surgery, sepsis, infections or toxic agents [3,4]. Autoimmune Addison's Disease (AAD) is caused by an autoimmune process responsible for the selective destruction of adrenal cortex cells [5,6]. Although AAD is the result of a T-cell mediated process, adrenal autoimmunity is made evident by the appearance of circulating adrenal cortex autoantibodies (ACA), that represent the best immune marker to identify patients with AAD [5,6]. ACA have little or no pathogenetic role [5–8], but their detection in human serum is clinically useful for both the etiological classification of PAI [9] and the identification of subjects at high-risk for future development of clinical AAD [10–16].

From 1963 until the middle of the 1990s, ACA were exclusively detected by means of the indirect immunofluorescence technique on cryostatic sections of human or animal adrenal glands [17]. The identification of the steroidogenic enzyme 21-hydroxylase as the main autoantigen identified by ACA [18,19] led to the development of sensitive and specific immunoassays for the detection of 21-hydroxylase autoantibodies (21OHAb) in human serum [20–22]. Several subsequent studies have demonstrated that 21OHAb is the gold standard immune marker for diagnosis of clinical and pre-clinical AAD [5,6,10–16,20–29]. In Europe, from 1974 to 2010, over 2000 patients with PAI have been reported in the literature as having been evaluated for adrenal autoantibodies [5,6]. The prevalence of adrenal autoantibodies ranged from 44.5 to 94% in different studies [5,6].

The Italian Addison Network (IAN) has developed a comprehensive flow-chart for the etiological classification of PAI which takes into consideration immunological, biochemical and imaging data [9]. In this flow-chart, the analysis of adrenal autoantibodies, and more specifically of 21OHAb, plays a major role to discriminate autoimmune from non-autoimmune forms of the disease [9]. Studies on subjects with organ-specific autoimmune diseases have shown that not only the presence, but also levels, of 21OHAb may have clinical relevance in the estimates of future risk for development of the clinical signs of AAD [13].

In the IAN study already mentioned [9], a comparison of 21OHAb assays in two independent laboratories and of ACA in two other independent laboratories was performed. The concordance rate of the two laboratories that performed 21OHAb assays was higher than that of the two laboratories that performed ACA assays [9]. As an extension of the previous IAN study, a novel study was planned in which four independent laboratories (two from Italy and two from other countries) performed 21OHAb assays in a large series of samples from patients with PAI and healthy control subjects. The aim of the present study was specifically that of comparing 21OHAb results generated by different laboratories using their own internal standard sera and in-house calculated cut-offs, to define the need for future standardization programs aimed at harmonizing the methods and identifying a common standard serum. Accordingly, serum samples were consecutively collected from PAI patients irrespective of disease duration or etiology.

Table 1 21OHAb positivity in different clinical forms of PAI, in 4 different laboratories.

	PG	RM	CAR	DEN
Idiopathic	168/205 (82%)	168/205 (82%)	173/205 (84.4%)	170/205 (82.9%)
APS1	14/15 (93.3%)	14/15 (93.3%)	13/15 (86.7%)	14/15 (93.3%)
ALD	0/8	0/8	2/8 (25%)	0/8
AHC	0/1	0/1	0/1	0/1
Post-sepsis	0/1	0/1	0/1	0/1
Post-surgical	0/1	1/1	0/1	0/1
Post-TBC	0/36	0/36	1/36 (2.8%)	1/36 (2.8%)
Healthy control subjects	0/83	0/83	2/83 (2.4%)	0/83

AHC: adrenal hypoplasia congenita; ALD: adrenoleukodystrophy; APS1: autoimmune polyendocrine syndrome type 1; Post-TBC: post-tuberculosis primary adrenal insufficiency.

2. Materials and methods

2.1. Serum samples

Serum samples from 267 patients (107 males and 160 females) with PAI and from 83 healthy control subjects (35 males and 48 females, median age 39 years, range 21–55 years) were collected by the IAN, as previously described [9], and stored at −70 °C until subsequently used for the present study. At the time of sample collection, the median patient age was 49 years (range, 6–87 years) and the median disease duration was 5 years (range, 0–53 years). In all patients, clinical symptoms and signs of PAI were associated with low basal cortisol (<3 μg/dl) and high basal ACTH (>100 pg/ml) levels. For statistical purposes, PAI patients were subdivided into two groups: a) clinically idiopathic/APS1 (n=220; this group included 15 cases with autoimmune polyendocrine syndrome type 1), and b) with demonstrated non-autoimmune etiology (such as post-infiltrative adrenalitis, n=36, ALD, n=8, adrenal hypoplasia