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Techniques au quotidien

Analytical and clinical performance of an automated chemiluminescent immunoassay for direct renin measurement: comparison with PRA and aldosterone assays

Performances analytiques et cliniques d'une méthode automatique en chemiluminescence pour la mesure de la rénine directe : comparaison avec les mesures de l'activité plasmatique de la rénine et de l'aldostérone

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Abstract

The analytical performance of a fully automated chemiluminescentimmunoassay (CLIA) by Nichols Institute Diagnostic for direct renin, was evaluated. Within-assay imprecision (CV) resulted 5.6% at 4.7 μU/ml; 1.9% at 33.7 μU/ml and 2.3% at 194.9 μU/ml; between-assay imprecision 12.4% at 5.3 μU/ml; 8.7% at 32.4 μU/ml, 8.2%at 90.2 μU/ml, 5.5% at 196.5 μU/ml. Direct renin CLIA showed good linear relationship with direct renin IRMA and with plasma renin activity (PRA). The clinical performance of direct renin CLIA, PRA and aldosterone assays was tested by ROC analysis in patients with heart failure (HF). For mild HF, areas under the curve were: PRA = 0.733, direct renin = 0.671, aldosterone = 0.789; for severe HF: PRA = 0.855, direct renin = 0.792, aldosterone = 0.801. In conclusion, direct renin CLIA measurement showed good analytical performance (shorter turn around time, better precision and practicability than IRMA and PRA) and good clinical performance in HF patients with different severity of disease similar to that of aldosterone and PRA measurement.

Résumé

Nous avons évalué la performance analytique de la méthode chemiluminescent (CLIA) et complètement automatique de l'Institut Diagnostic Nichols pour la mesure de la rénine directe. L'imprécision dans les essais (CV) était 5,6 % à 4,7 μU/ml ; 1,9 % à 33,7 μU/ml et 2,3 % à 194,9 μU/ml ; l'imprécision parmi les essais 12,4 % à 5,3 μU/ml ; 8,7 % à 32,4 μU/ml ; 8,2 % à 90,2 μU/ml ; 5,5 % à 196,5 μU/ml. La rénine directe CLIA était bien corrélée avec la rénine directe IRMA et avec l'activité plasmatique de la rénine (PRA) aussi. Nous avons aussi évalué la performance clinique de la rénine directe CLIA, PRA et aldostérone avec l'analyse ROC entre patients avec décompensation cardiaque légère ou grave comparés avec des sujets sains. Dans le premier cas les aires sous les courbes étaient PRA = 0,733, rénine directe = 0,671, aldostérone= 0,789; dans le second cas: PRA = 0,855, rénine directe = 0,792, aldostérone = 0,801. Notre conclusion est que la mesure de la rénine directe avec CLIA montrait une acceptable performance analytique (plus bref temps de réponses, meilleure précision et praticabilité en

Abbreviations: ACE, angiotensin converting enzyme; AUC, area under the curve; CI, confidence interval; CLIA, chemiluminescentimmunoassay; EF, ejection fraction; HF, heart failure; IRMA, immunoradiometricassay; NYHA, New York Heart Association; PRA, plasma renin activity; RAAS, renin–angiotensin–aldosterone system; RIA, radioimmunoassay; RLU, relative light unit; ROC, receiver operating characteristic; S.D., standard deviation; TAT, turn around time; WHO, World Health Organization.

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comparaison avec IRMA et PRA) et une acceptable performance clinique dans les patients avec décompensation cardiaque comparable avec celles de la PRA et de l'aldostérone.

Keywords: Chemiluminescent immunoassay; Direct renin; Plasma renin activity; Aldosterone; Heart failure

Mots clés : Chemiluminescent immuno essai ; Rénine directe ; Activité plasmatique de la rénine ; Aldostérone ; Décompensation cardiaque

1. Introduction

Renin is an aspartyl protease mainly produced in the juxtaglomerular apparatus of the kidney; it is released in response to various physiological factors such as sodium depletion, decrease in blood volume and pressure and β adrenergic stimulation. Renin catalyses the conversion of angiotensinogen to the biologically inactive angiotensin I which is converted by the angiotensin converting enzyme (ACE) to the biologically active angiotensin II. Angiotensin II is considered the most powerful vasoconstrictor agent and the main regulator of aldosterone synthesis and secretion, promoting the reabsorption of sodium and secretion of potassium. Renin circulates in two different forms, prorenin, the biologically inactive precursor of renin, and active renin [20,21]. Renin, angiotensin I, angiotensin II, ACE and aldosterone are collectively known as the renin–angiotensin–aldosterone system (RAAS).

Under normal conditions and in many pathological situations, the amount of renin determines the activity of the whole RAAS. Due to difficulties associated with the measurement of plasma concentration of angiotensin II, the effector peptide of RAAS, the determination of plasma renin activity (PRA), which measures enzyme ability to convert angiotensinogen in angiotensin I, has been widely adopted in clinical practice. However, PRA assay is time, temperature, pH and substrate dependent and needs ice-cooling during assaying and transport of plasma samples. Active renin assay, instead, measures the actual concentration of renin in plasma, therefore, is not dependent on angiotensinogen concentration. Moreover, to avoid cryoactivation of prorenin in renin, samples must be handled at room temperature [23,6]. Therefore, the introduction of active renin measurement could result in simplification and shortening of the diagnostic procedure. Nichols Institute Diagnostic has developed a chemiluminescent immunoassay (CLIA) for plasma active renin (direct renin) which is enough sensitive to detect low renin samples, minimizes interference by prorenin and can be performed in less than 1 h, using the commercially available automated Advantage® analyzer.

The use of renin measurement is recommended in clinical practice for the diagnosis of secondary forms of atrial hypertension, and for primary aldosteronism, in association with plasma aldosterone measurement [1,11,13,26]. The aldosterone/PRA ratio is a crucial step in differentiating among the causes of hypertension and hypokalemia [25,27]. However, renin assay is widely used in pathophysiological studies, concerning clinical conditions in which RAAS activity is altered [8,15,24]. Heart failure (HF) is characterized by the activation of several vasoconstrictive/sodium retentive systems, including the sympathetic nervous system, endothelins, vasopressin, and RAAS [3,9,12]. The neuro-endocrine activation, initially compensatory, has detrimental effects in patients with chronic HF, promoting ventricular and vascular remodeling, and causing peripheral and visceral oedemas, fatigue, dyspnoea, life-threatening arrhythmias. This provides the rationale for use of "neuro-endocrine" drugs in HF, namely those counteracting the activation of RAAS and adrenergic overactivity. Moreover, the observation that hemodynamic and clinical improvement during optimal pharmacological treatment is paralleled by reduction in plasma levels of neuro-endocrine agents (such as RAAS), may represent the rationale for the measurement of these levels in monitoring and tailoring the therapy in patients with HF.

In this study the analytical performance of the direct renin CLIA was evaluated and then compared to a traditional immunoradiometric assay (IRMA) and to PRA radioimmunoassay (RIA). Moreover, the clinical performance of the direct renin was compared to PRA and aldosterone in patients with HF, classified according to New York Heart Association (NYHA), using age-matched healthy subjects as control group.

2. Materials and methods

2.1. Sample collection

For direct renin determinations, EDTA blood collection tubes (after 30' of supine position at 08.00 h) were not pre-chilled or stored on ice; blood was processed at room temperature in non-refrigerated centrifuge and plasma was rapidly stored at –20 °C if not immediately assayed. For RIA measurements of PRA and aldosterone, blood was collected into pre-chilled venipuncture tubes or plastic syringes, put into polypropylene tubes containing EDTA and placed into ice-bath. Samples were rapidly separated by centrifugation at 4 °C for 10 min and plasma was aliquoted and stored at –20 °C without delay until use.

2.2. Study population

Normal ranges were calculated by enrolling 25 healthy subjects, 58 ± 8 years old.

In order to assess the clinical performance of aldosterone, PRA and direct renin assays by ROC analysis, we compared