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Multicenter evaluation of a new progastrin-releasing peptide (ProGRP) immunoassay across Europe and China

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ABSTRACT

Background: We performed a multicenter evaluation of the Elecsys® progastrin-releasing peptide (ProGRP) immunoassay in Europe and China.
Methods: The assay was evaluated at three European and two Chinese sites by imprecision, stability, method comparison and differentiation potential in lung cancer.
Results: Intermediate imprecision across five analyte concentrations ranged from 2.2% to 6.0% coefficient of variation. Good stability for plasma and serum samples was shown for various storage conditions. There was excellent correlation between the Elecsys® and ARCHITECT assays in plasma (slope 1.02, intercept −2.72 pg/mL). The Elecsys® assay also showed good correlation between serum and plasma samples (slope 0.93, intercept 2.35 pg/mL; correlation coefficient 0.97). ProGRP differentiated small-cell and non-small-cell lung cancer (NSCLC; area under the curve 0.90, 95% CI 0.87–0.93; 78.3% sensitivity, 95% specificity; at 84 pg/mL), with no relevant effects of ethnicity, age, gender or smoking. Median ProGRP concentrations were low in benign diseases (38 pg/mL), other malignancies (40 pg/mL) or NSCLC (39 pg/mL), except chronic kidney disease above stage 3 (>100 pg/mL).
Conclusions: Increased stability of the Elecsys® ProGRP assay in serum and plasma offers clear benefits over existing assays. This first evaluation of a ProGRP assay in China demonstrated comparable differentiation potential among different ethnicities.

1. Introduction

Tumor markers have been extensively studied in patients with lung cancer as a means to differentiate between the two major subtypes of lung cancer—non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC)—and, thereby, improve diagnosis and treatment selection [1–5]. NSCLC accounts for around 80% of all new lung cancer cases, with SCLC making up the remaining 20%. SCLC differs from NSCLC in having neuroendocrine differentiation, a higher tumor growth rate and earlier development of metastasis [6,7], and as such requires a different treatment approach. Of the two subtypes, NSCLC is more likely to present at an early stage, when surgery offers the best chance of cure [8]. The early-stage diagnosis of SCLC is very rare, meaning surgery is uncommon, but SCLC is highly sensitive to radiotherapy and chemotherapy [7]. Patients with SCLC often relapse; however, and 5-year survival

Abbreviations: NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer; NSE, neuron-specific enolase; ProGRP, progastrin-releasing peptide; NET, neuroendocrine tumors; MCT, medullary carcinoma of the thyroid; PUMCH, Peking Union Medical College Hospital; SST, serum separation tube; RST, rapid serum tube; CLSI, Clinical Laboratory and Standards Institute; UICC, Union for International Cancer Control; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; AUC, area under the curve; HSP, human sample pool.

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rates have remained constant in recent years [9]. The differential diagnosis of lung cancer subtype at initial presentation is critical to ensure appropriate therapeutic intervention.

Tumor biopsies are an essential component in the histologic differentiation of lung cancer. However, because of the submucosal location of many SCLCs, accurate tissue sampling can be difficult, and biopsies do not allow for early detection of the disease [10]. When diagnosed with limited-stage disease, approximately 20% of patients with SCLC can achieve long-term survival with aggressive chemotherapy and radiotherapy, versus just 5% when diagnosed at an advanced stage [10]. The analysis of tumor markers in plasma or serum samples offers clear benefits over histologic differentiation, including the potential for early detection of SCLC, and the chance to improve survival rates.

Neuron-specific enolase (NSE) and progastrin-releasing peptide (ProGRP) have proved most beneficial as tumor markers in SCLC [11,12]. Although NSE was historically the recommended tumor marker for SCLC [13], NSE also stains up to 80% of NSCLCs in tissue examinations and is elevated in the sera of 20–30% of patients with NSCLC [14]. Furthermore, NSE has low sensitivity, particularly in patients with disease confined to the hemithorax or ipsilateral mediastinum [15]. As NSE is present in platelets and erythrocytes, samples with hemolysis must be excluded and rapid storage of samples is essential [14]. ProGRP accurately discriminates between NSCLC and SCLC [16,17] and is rarely elevated in other malignant diseases or in benign conditions, except in patients with renal insufficiency, neuroendocrine tumors (NET) of the lung and medullary carcinoma of the thyroid (MCT) [16–22].

Evaluation of the first fully automated ProGRP ARCHITECT assay (Abbott Laboratories, Wiesbaden, Germany) was reported in 2009 [23]. Owing to the poor stability of ProGRP in serum on the ARCHITECT assay, which is believed to be due to thrombin-induced proteolysis, plasma samples are the recommended source material [24,25]. The Elecsys® ProGRP assay (Roche Diagnostics GmbH, Penzberg, Germany) is a new immunoassay designed to quantitatively determine levels of ProGRP in both human serum and plasma. As the two monoclonal antibodies in the Elecsys® ProGRP assay bind to epitopes in the ProGRP peptide that are relatively resistant to endoproteolytic cleavage [16,17,26] (Supplementary Fig. S1), serum samples as well as plasma samples can be used. Here we report on the technical and clinical performance of the Elecsys® ProGRP assay across a number of European and Chinese sites.

2. Materials and methods

Between August 2012 and September 2013, the Elecsys® ProGRP assay was evaluated at three European investigational sites in Amsterdam, Barcelona and Bonn, and two Chinese sites in Beijing (Peking Union Medical College Hospital [PUMCH] and Peking Xuanwu Hospital). Ethical approval/waiver was obtained from each institution before clinical study work began. All investigational sites conducted the study in accordance with the Declaration of Helsinki (rev. Tokyo, Venice, Hong Kong and Fortaleza 2013) and International Conference on Harmonisation Good Clinical Practice guidelines. The objective of the study was to evaluate the performance of the Elecsys® ProGRP assay in terms of imprecision, stability, method comparison and differentiation potential for SCLC.

2.1. Assay description

The Elecsys® ProGRP assay is an electrochemiluminescence immunoassay that uses a biotinylated ProGRP-specific mouse monoclonal antibody and a ruthenium-labeled ProGRP-specific mouse monoclonal antibody to capture and detect ProGRP in human serum and plasma. The assay is calibrated using the ProGRP CalSet (Roche Diagnostics) and has been standardized against the ARCHITECT ProGRP assay. Quality control is performed using two levels of PreciControl ProGRP (Roche Diagnostics). Both the calibrator and the control contain recombinant ProGRP.

2.2. Sample sources, preparation and handling

Samples were sourced from patients with previously untreated active disease, who had sufficient sample material available for analysis. No further demographic or histologic selection criteria were utilized. Lung cancer histologic types were classified according to the 1999 World Health Organization recommendations [27]. Differential diagnosis between SCLC and NSCLC was based on morphologic characteristics plus a positive CD56 and/or synaptophysin immunohistochemistry of the tumor. Lung cancer staging (TNM) was established according to international guidelines [28].

All sites collected samples and performed assay measurements. An additional German site (Institut für Klinische Pharmakologie GmbH, Kiel) contributed samples from apparently healthy individuals as a reference cohort. Owing to the low prevalence of SCLC in the patient population, differential diagnosis was based primarily on patient serum samples sourced from sample banks in Europe, whereas samples were collected prospectively in China (January to September 2013).

All three European sites and PUMCH used serum for clinical evaluation. Xuanwu used K2-EDTA primary tubes for plasma collection. The stability experiment was performed in Bonn and Amsterdam using K2-EDTA and serum separation tubes (SSTs). In addition, Amsterdam used rapid serum tubes (RSTs) for serum sampling in this experiment. The interior wall of the RSTs was coated with thrombin to promote rapid clotting. All studies were performed on **cobas® e**411 and **e**601 analyzers.

2.3. Statistical analyses

All analytical data were captured using the WinCAEv (Windows-based computer aided evaluation) software program. Demographic and clinical data were collected using the MACRO software program. All statistical analyses using clinical information were performed in the biostatistics department at Roche Diagnostics Penzberg using SAS (Statistical Analysis Software, version 9.2) and R (version 2.13.2). Outliers identified by visual inspection of the primary data set were re-measured.

2.4. Technical assessment

2.4.1. Interlaboratory survey

Three EDTA plasma sample pools were prepared by Roche R&D (~30, ~200 and ~1500 pg/mL) and distributed to all five investigational sites to evaluate differences in recovery and day-to-day variability between the laboratories in these three concentration ranges. The two levels of PreciControl ProGRP were also used as sample material. Each sample was measured in single determination over 10 days in each laboratory. The percentage recovery per sample was calculated as the measured concentration/all laboratory median $\times 100$.

2.4.2. Imprecision according to clinical laboratory and standards institute EP5-A2

Imprecision was assessed by means of the Clinical Laboratory and Standards Institute (CLSI) EP5-A2 guideline [29]. Three sample pools were prepared by each of the European sites with specified target concentration ranges of 7–60 pg/mL, 61–1000 pg/mL and 1001–5000 pg/mL. Approximately 27 mL of serum or plasma was required to prepare 84 × 300 µL aliquots. Samples were stored at −20 °C and used on the respective day of measurement. Repeatability and intermediate precision estimates were obtained by modeling the data according to the CLSI EP5-A2 variance component model. Between-day, between-run and repeatability variance components