The influence of different blood samples treatment methods on pro-gastrin-releasing peptide

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Abstract
Pro-gastrin-releasing peptide (ProGRP) is the promising molecular tumor marker of small cell lung cancer (SCLC). Here we study the influence of different blood samples treatment methods on ProGRP.

Serum with and without separation gel and heparin plasma from 10 SCLC patients and 5 healthy individuals were assayed for ProGRP immediately and 2, 4, 6, 8, 24, and 48 hours after collection.

ProGRP of serum with and without separation gel and heparin plasma detected immediately was basically consistent, whereas there was a significant difference in the level of them assayed after 2 hours. No significant variation with time was observed in heparin plasma, but in serum with and without separation gel, ProGRP concentrations gradually declined with time, with statistical significance. When assayed within 2 hours, each time point of ProGRP in heparin plasma had no significant difference and the difference of ProGRP in serum separating gel existed at 1.5 hours.

Heparin plasma is the best option for clinical test of ProGRP. If serum with separation gel is used, optimization methods of turn-around-time which guarantee samples detected within 1 hour after collection can make results more instructive for clinical treatment.

Abbreviations: ANOVA = analysis of variance, GRP = Gastrin-releasing peptide, ProGRP = Pro-gastrin-releasing peptide, SCLC = small cell lung cancer, SD = standard deviation, TAT = turn-around-time.

Keywords: plasma, ProGRP, serum, TAT

1. Introduction
Small cell lung cancer (SCLC) is a kind of specific tumor with undifferentiated, high malignant, and complicated etiopathogenesis, characterized rapid proliferation, and high metastasis. Fortunately, it is sensitive to chemotherapy and has a good success rate with treatment. Gastrin-releasing peptide (GRP) is the gastrointestinal hormone which has the effect on promoting secretion of gastrin, widely in normal human brains, nerve fibers of stomach, and neuroendocrine tissues of fetal lungs. Previous studies have shown that low level of GRP can stimulate DNA synthesis in SCLC cells; therefore, GRP is deemed the independent growth factor of SCLC.[1] However, for the reason of the instability and short half-life, it is hard to detect GRP directly.

In recent years, it has been reported that the fragment (31–98) of pro-gastrin-releasing peptide (ProGRP) is the promising molecular tumor marker of SCLC, 3 kinds of common conserved sequence of carboxyl terminal of spliced variants of human ProGRP which represent the level of GRP.[2] There are a number of studies that have demonstrated the importance of ProGRP to the diagnosis, prognosis evaluation, and monitoring treatment of SCLC.[3–5]

Correct collecting and preservation conditions of the specimen should be given attention when the analysis of ProGRP in blood needs to be carried out, and good management of quality control before the test can make results more comparable, which contributes to meeting the needs of clinical diagnosis and treatment and alleviating the burden of patients. However, the influence of sample pretreatment on ProGRP test is less reported. In this study, we evaluate the influence of blood sample types and storing time on the test result of ProGRP and propose the preferred project to make results more instructive for clinical treatment.

2. Materials and methods
2.1. Clinical data and sample treatment
This study was approved by the Research Ethics Committee of ChangHai Hospital. All participants gave informed written consent. Ten SCLC patients who received treatment in ChangHai Hospital of the Second Military Medical University (Shanghai, China) in 2016 with increased ProGRP were included in this study and were divided into Group High and Medium. In addition, 5 healthy individuals with normal range ProGRP were
set as Group Low. Fasting venous blood samples were collected in heparin anticoagulant tubes (G), serum with (Y) and without (R) separation gel tubes (all purchased from Becton Dickinson and Company) at early morning, and then, serum and plasma were separated after 30 minutes’ standing (centrifugation with 3000 rcf/min, 10 minutes).

2.2. Detection methods

According to standard operating instructions of clinical laboratory, ProGRP was tested by Abbot automated chemiluminescence analyzer I2000sR and ProGRP reagents. Separated serum and plasma were assayed immediately, and preserved at room temperature. Then, they were detected respectively at 2, 4, 6, and 8 hours, and stored in sample bank at 2°C to 8°C to be detected at 24 h and 48 h.

2.3. Statistical methods

The unit of results is nanograms per milliliter and the range is 5 to 5000 ng/mL. Owing to the significant difference of ProGRP among patients, initial measured value of heparin anticoagulant tube was set as 100%, and residual rate could be calculated by the ratio of other measured value and initial measured value of heparin anticoagulant tube. Continuous variables were expressed as mean ± standard deviation (SD). All the analyses were performed using Prism6 (Graphpad software, San Diego, CA). Two-way analysis of variance (ANOVA) was used to determine the effect of tubes and storage time, and within each group or time point, 1-way ANOVA and Dunnett multiple comparisons were used. The level of statistical significance was set at \( P < .05 \).

3. Results

Blood samples were detected immediately and at 2, 4, 6, and 8 hours at room temperature. Regardless of high values (H), medium values (M), or low values (L), ProGRP of Y and R were significantly decreased with time (\( P < .05 \)), whereas ProGRP of G did not change obviously with time as there was no significant difference between measured values at each time point (\( P > .05 \), Fig. 1A–C).

To indicate the influence of sample types and storage time on ProGRP more directly, we set initial measured value of G as 100%, and residual rate was calculated by other measured values/initial measured value of G ratio (Fig. 2). The level of ProGRP instantly detected after collection was shown in Table 1 that no significant difference between G and Y was observed (\( P > .05 \)), but there was obviously difference between G and R (\( P < .01 \)). Moreover, there were significant differences between G and Y, R assayed after 2 hours (\( P < .05 \), Table 2).

Therefore, in this study we tried to shorten the storage time of Y to make results more instructive for clinical treatment. ProGRP of G and Y was tested immediately, as well as at 0.5, 1, and 1.5 hours. Our results suggested that each time point of G had no significant difference (\( P > .05 \)). In addition, detection of Y showed no obvious difference within 1 hour (\( P > .05 \)), whereas the difference existed at 1.5 hours (\( P < .05 \), Table 2).

| Table 1 |
|---|
| The remaining ProGRP in different sample types tested in ARCHITECT i2000 system. |
| | 0 h | 2 h | 4 h | 6 h | 8 h | 24 h | 48 h |
| G | 100 | 100.48±4.08 | 97.94±4.06 | 98.41±4.51 | 98.28±4.02 | 100.75±5.91 | 98.25±6.81 |
| Y | 99.28±5.51 | 84.93±7.54 | 78.02±8.33 | 75.33±9.43 | 72.10±9.99 | 64.09±12.66 | 55.69±13.60 |
| R | 77.00±8.76 | 65.41±5.63 | 58.25±7.40 | 55.08±8.53 | 50.25±8.60 | 45.37±9.54 | 38.16±8.35 |
The ProGRP results within 2 hours in ARCHITECT i2000 system.

|   | 0 h  | 0.5h | 1 h  | 1.5 h |
|---|------|------|------|-------|
| G | 100  | 100.19±4.50 | 99.26±2.01 | 99.23±3.24 |
| Y | 99.57±2.57 | 97.98±4.58 | 94.16±5.96 | 88.42±4.77 |

Each time point was compared with 0h, and P<.05 value is identified as significant level.

It has also been reported that the difference of concentration of ProGRP in serum and plasma is acceptable on Roche, with good consistency. From this, we similarly verified the result on Roche, and found that regardless of high values (H), medium values (M), or low values (L), measured values of G, Y, and R at each time point had no significant difference (P>0.05)(Fig. 3).

### 4. Discussion

Tumor markers gain much more focus in clinical treatment because of economy and convenience. The use of tumor markers can not only differentiate diagnosis for histologic types of lung cancer, but also make an appropriate treatment plan, and monitor therapy to ensure the effectiveness of therapy, whether change or not. Early diagnosis of tumor and metastasis can decrease mortality and medical costs.

ProGRP is a kind of new tumor marker, which is less exposed to diet and activity, so posture fixation and time are not required when taking blood. It has been reported that endogenous protease produced in coagulation process can degrade ProGRP in serum and plasma. ProGRP is detected by Abbot automatic chemiluminescence analyzer I2000sR is used to detect ProGRP in serum with separation gel tubes (Y) in ChangHai Hospital of the Second Military Medical University. Serum separating gel can separate the serum from nonanticoagulative blood and keep component in serum stable. It has been reported that blood glucose in vitro collected in serum separating gel tubes can remain stable for at least 48 hours. According to the data analysis in our study, it can be found that regardless of high values, medium values, or low values, ProGRP in serum separating gel tubes is significantly decreased with time and the measured value at each time point has significant difference.

Turn-around time (TAT) refers to the time from sample collection to report sent. Reducing sample storage time can optimize TAT. Our study indicates that there is a significant difference between ProGRP detected after 2 hours and immediate measurement. Through optimizing TAT, we add the ProGRP measurement from 0 to 2 hours, and find that ProGRP has no difference in 1 hour, which can be clinically accepted.

With the development of science and technology, and the improvement of medical care, the automatization and integration of clinical laboratory has been the tendency. As it is hard to detect blood samples immediately, TAT can be optimized if ProGRP cannot be assayed in plasma.

In conclusion, plasma samples are optimal choice when ProGRP is detected by Abbot automatic chemiluminescence analyzer. If serum samples are used, we can optimize TAT to ensure the detection within 1 hour, making results more acceptable. When ProGRP is detected by Roche automatic electrochemiluminescence immunoassay system, there is no difference between plasma and serum samples.

The factors affecting ProGRP in blood samples in this study are explored preliminarily and the date provided in this study is not absolute, just representing the trend of change of ProGRP in blood samples because of determinative error and individual difference.

### Author contributions

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