Development of a novel and rapid measurement system for growth differentiation factor-15, progranulin, and osteopontin in uterine sarcoma

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Abstract. Uterine sarcomas are rare and aggressive gynecologic tumors with poor prognosis; therefore, early diagnosis is crucial for therapy. However, it is very difficult to distinguish uterine sarcomas from leiomyomas which are common benign uterine tumors. Therefore, the development of a diagnostic method that utilizes reliable biomarkers to distinguish uterine sarcomas from leiomyomas is important so as to identify the rare tumors. The candidate factors as novel biomarkers were searched for in public databases and a pilot study was performed for confirmation. Growth differentiation factor-15 (GDF15), progranulin, and osteopontin were identified as candidate biomarkers for diagnosing uterine sarcoma. Thus, developing a rapid and easy method to measure these factors could help establish a screening system for uterine sarcomas. In this study, we developed a novel measurement system for these factors using a compact chemical luminescence immunological automatic analyzer POCube™. This assay system, which is based on the flow-through membrane immunoassay, completes the whole process and generates results within 15 min. Serum concentrations of these factors measured via POCube™ correlated well with those measured using enzyme-linked immunosorbent assay (r = 0.994 for GDF15, r = 0.992 for progranulin, and r = 0.976 for osteopontin). The POCube™ system provides rapid and easy measurement of these factors, thereby facilitating uterine sarcoma diagnosis.

Key words: POCube, Growth differentiation factor-15, Progranulin, Osteopontin, Uterine sarcoma

UTERINE SARCOMAS are rare and aggressive gynecologic tumors that have poor prognosis. In such cases, early diagnosis is crucial as complete resection of the tumors is one of the most important therapeutic measures [1]. On the other hand, uterine leiomyomas are very common benign uterine tumors that occur in about one-third of women over the age of 30 [2]. It is very difficult to distinguish uterine sarcomas from leiomyomas solely via imaging analyses [3]. Therefore, the development of a diagnostic method that utilizes reliable biomarkers to distinguish uterine sarcomas from leiomyomas is important so as to identify the rare tumors. Circulating growth differentiation factor-15 (GDF15) has been reported to be an effective biomarker that distinguishes highly malignant uterine sarcomas from benign uterine leiomyomas [4]. The finding also suggests the possibility of some other circulating proteins, in addition to GDF15, as valuable biomarker candidates useful for the preoperative identification of uterine sarcoma. We identified progranulin and osteopontin, in addition to GDF15, as candidate biomarkers for diagnosing uterine sarcoma. In this study, we developed a novel measurement system for these factors using a POCube™, a fully automated immunological analyzer. The POCube™ system provides rapid and easy measurement of these factors, thereby facilitating uterine sarcoma diagnosis.
Material and Methods

Bioinformatics analysis
To identify candidate biomarkers for diagnosing uterine sarcoma, public databases of gene expression were explored. Subio Platform programs (Subio Inc., Amami, Japan) were used to analyze the GEO (GSE13399 for 23 leiomyoma patients and 23 healthy controls) and TCGA (57 uterine sarcoma patients) databases. We acquired several candidate factors such as GDF15, progranulin, and osteopontin, which are considered to be secretory proteins that exhibit significantly different expression levels of the corresponding protein-coding gene in uterine sarcoma and leiomyoma.

ELISA
ELISA kits for GDF15, progranulin, and osteopontin were purchased from R&D Systems (Minneapolis, MN, USA). The blood samples were collected within 2 weeks before surgery except for the post-operative collections. The serum concentrations of these bioactive factors were measured according to the manufacturer’s instructions. The protocol was approved by the ethical committee of the University of Fukui Hospital, and written informed consent was obtained from all study patients.

POCube™
The POCube™ is a fully automated and compact immunological analyzer developed by Toyobo Co. Ltd. (Osaka, Japan) [5]. Briefly, the principle of this system is based on the flow-through membrane immunoassay using two antibodies, where one is biotinylated and the other is conjugated to alkaline phosphatase (ALP). Antibodies and physiologically-active antigens for GDF15, progranulin, and osteopontin were purchased from R&D Systems. The antibodies were labeled using biotin-labelling and alkaline phosphatase-labeling kits according to the manufacturer’s instructions (Dojindo, Kumamoto, Japan). The immune complexes are trapped by an anti-biotin Ab-coated filter membrane in a reaction vessel and the ALP activity is measured via the luminescence output.

Results and Discussion

We conducted a pilot study using ELISA to measure GDF15, progranulin, and osteopontin from the blood samples of patients with gynecologic tumors. The serum concentrations of these bioactive factors (GDF15, progranulin, and osteopontin) were significantly higher in patients with uterine sarcomas than those with leiomyomas (Fig. 1). The receiver operating characteristic (ROC) analysis also show the specificity and sensitivity of these measurement for the diagnosis of uterine sarcoma (Table 1). Clinical characteristics of patients with uterine sarcoma are shown in a Supplemental Table 1.

Next, we further developed a novel, rapid, and easily operable measuring system for these factors using a POCube™. This assay system completes the whole process and generates results as luminescence counts within 15 min. Furthermore, the assay requires only 10 μL of
Table 1  ROC analysis of GDF15, progranulin, and osteopontin in differentiating between uterine sarcoma and leiomyoma

| Bioactive factors | Cut-off value (ng/mL) | AUC   | Sensitivity (%) | Specificity (%) |
|-------------------|-----------------------|-------|-----------------|-----------------|
| GDF15             | 1.17                  | 0.979 | 100             | 88.9            |
| Progranulin       | 54.7                  | 0.877 | 91.7            | 83.3            |
| Osteopontin       | 76.8                  | 0.722 | 50.0            | 94.4            |

AUC: area under the curve

Fig. 2  Standard curve for measurement of GDF15, progranulin, and osteopontin using the POCubeTM.
Standard curve with different concentrations of the recombinant bioactive factors for calculation of GDF15, progranulin, and osteopontin.

Fig. 3  Correlation between the POCubeTM and ELISA assays for GDF15, progranulin, and osteopontin.
Serum concentrations of GDF15 ($n = 8$), progranulin ($n = 26$), and osteopontin ($n = 10$) were measured using ELISA and POCubeTM. Each dot in the figure represents a pair of results for one serum sample.

undiluted serum for the experiment. Standard curves were obtained based on the measurement of seven or eight serial reference standards (Fig. 2). Data from GDF15, progranulin, and osteopontin yielded working ranges of 0.1–6 ng/mL, 6–400 ng/mL, and 5–160 ng/mL, respectively. The accuracy, precision and reliability of the immunoassay for these bioactive factors using the POCube system are shown in Supplemental Tables 2–19.

To evaluate the correlation between the POCubeTM and ELISA assays, the samples were analyzed for each factor using both methods. The correlation between the...
POCube™ and ELISA assays for serum concentrations of GDF15, progranulin, and osteopontin were $y = 0.972x + 0.123$, $r = 0.994$ ($n = 8$); $y = 1.041x + 6.412$, $r = 0.992$ ($n = 26$); and $y = 1.032x + 3.548$, $r = 0.976$ ($n = 10$), respectively (Fig. 3). These results strongly indicated the feasibility of POCube™ in measuring these proteins in place of ELISA.

The POCube™ is suitable for clinical specimens because it is a fully automated and rapid (within 15 min) measurement system that only requires small sample amounts (~10 μL). Using this system, even physicians and nurses who do not have special techniques could measure the concentrations of these bioactive factors at the hospital. Notably, a three-factor combined data analysis achieved a more accurate diagnosis of uterine sarcoma than that obtained using single-factor analysis. Further study is needed to determine the individual and/or combined cut-off values in order to distinctly diagnose uterine sarcoma and leiomyoma. This assay could also be used for post-operative management of uterine sarcoma. In fact, the serum concentrations of GDF15, progranulin, and osteopontin seem to be lower after surgery than before (Supplemental Fig. 1). Therefore, this novel and rapid measurement system can contribute to the early detection and prognosis management of uterine sarcoma.

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Disclosure Statement

The authors declare no conflict of interests.

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