Significance of secretory leukocyte peptidase inhibitors in pleural fluid for the diagnosis of benign asbestos pleural effusion

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Research Article

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Abstract

Background

Secretory leukocyte peptidase inhibitor (SLPI) is a biomarker present in the respiratory tract that protects against tissue destruction and aids wound healing. However, it is difficult to distinguish early-stage malignant pleural mesothelioma (MPM) from benign asbestos pleural effusion (BAPE) presenting as pleural effusion in diagnostic imaging. More biomarkers of pleural effusion are needed to identify early-stage MPM. We examined whether SLPI in pleural effusion can be used to distinguish BAPE from MPM and other conditions that involve pleural effusion.

Methods

We measured levels of SLPI, hyaluronic acid (HA), soluble mesothelin-related peptides (SMRP), galectin-3, CCL2, and CYFRA21-1 in 51 BAPE patients, 37 MPM patients, 77 patients with pleural effusions due to non-small cell lung cancer (LCa), and 74 patients with other pleural effusions diagnosed at Okayama Rosai Hospital.

Results

SLPI levels in pleural fluid of BAPE patients were significantly lower ($p < 0.0001$) than those in patients with MPM, LCa, and other pleural effusions. The area under curve (AUC) for SLPI's ability to distinguish BAPE from MPM was 0.902, with a sensitivity of 82.4% and a specificity of 86.5%. These values were not only favorable, but were better than the AUC for the ability to distinguish BAPE from HA (0.802), and SMRP (0.746). Galectin-3 levels were significantly lower in patients with BAPE compared with those in patients with MPM and the other two diseases, whereas CCL2 levels were significantly higher in patients with BAPE compared with patients with MPM and the other two diseases. Moreover, CYFRA21-1 levels were significantly lower in BAPE patients compared with levels in patients with MPM and LCa. Using these six markers enabled BAPE to be distinguished from MPM and other diseases. As a single marker, SLPI proved to be superior to HA and SMRP for the diagnosis of BAPE.

Conclusions

The measurement of pleural fluid SLPI as well as HA and SMRP is useful as a biomarker to diagnose BAPE, which needs to be distinguished from early-stage MPM.

Background

Benign asbestos pleural effusion (BAPE), an inflammatory lesion of the pleura caused by asbestos fibers, was first reported in 1964 by Eisenstadt [1]. As the underlying mechanism of BAPE has not been

elucidated, no treatment methods have been established. A differential diagnosis of BAPE and early-stage lesions of malignant pleural mesothelioma (MPM) can be difficult. In patients who present pleural effusion such as macroscopic and neoplastic pleural thickening, with no abnormal findings on computed tomography (CT) of the chest, accurately identifying the lesion site can be challenging, even with thoracoscopic biopsy. When sufficient tumor tissue cannot be obtained from a biopsy site, it can be difficult to make a histopathological diagnosis. In such instances, the detection of p16 gene deletion using fluorescent in situ hybridization (FISH) may be useful [2, 3]. In addition, demonstrating BRCA1-associated protein 1 deletion in cell nuclei as well as deletion of the p16 gene is reportedly important [4].

In the diagnosis of MPM, elevated levels of hyaluronic acid (HA) [5, 6] and soluble mesothelin-related peptides (SMRP) [7, 8] in pleural fluid are reportedly useful for differentiation from other diseases. Secretary leukocyte peptidase inhibitor (SLPI) is a serine protease inhibitor found in the respiratory tract and in the mucous of the cervical canal, nasal discharge, and saliva. Its physiological function is associated with wound healing and the prevention of tissue destruction, and it is considered a promising biomarker of acute renal impairment following heart surgery [9].

In BAPE, which is an inflammatory lesion of the visceral pleural caused by asbestos fibers, the significance of SLPI in pleural fluid has yet to be confirmed. Here, we measured SLPI levels as a biomarker for BAPE, which is difficult to differentiate from MPM, and obtained results superior to those involving other markers.

Methods

We used pleural fluid of 51 patients with a definite diagnosis of BAPE, 37 MPM patients (31 with epithelial MPM and six with sarcoma-type conditions), 77 patients with malignant pleural effusions due to non-small cell lung cancer (LCa), 27 patients with heart failure (HF), and 47 patients with bacterial pleurisy (IF) diagnosed at the Okayama Rosai Hospital from 2015 to 2019. The 74 patients with HF and IF were considered “other” patients.

BAPE can be diagnosed, according to the criteria of Epler et al., [10] in individuals with a history of occupational exposure to asbestos, and in whom the presence of pleural effusion can be confirmed. However, a diagnosis of BAPE is based on the presence of an exudate with no cause other than asbestos exposure, deaminase and carcinoembryonic antigen in pleural fluid, as well as cytology, pleural plaque findings and, depending on the patient, pleural biopsy results [11].

We measured SLPI as a reagent using a human SLPI Quantikine enzyme-linked immunosorbent assay (ELISA), HA using latex coagulating nephelometry, SMRP using Lumipulse CLEIA, galectin-3 using a human galectin-3 ELISA kit (PromoKine®), CCL2 using a human MCP-1 ELISA Kit (PromoKine®), and CYFRA21-1 using a Lumipulse® chemiluminescent enzyme immunoassay.

Significant differences between each disease were determined using a nonparametric Kruskal–Wallis test in accordance with Dunn’s post hoc test, and a p value < 0.05 was deemed a significant difference. The

reliability of each marker was evaluated using a receiver operating characteristics (ROC) curve. The cut-off value was determined based on the curve, and the specificity and sensitivity were calculated. Analyses were performed using R and GraphPad Prism statistical software.

All participants provided written informed consent before inclusion in the study.
This study was approved by the 11th research ethics committee of the Japan Organization of Occupational Health and Safety on June 18,2018 (No.9).
All study procedures were carried out in accordance with the principles of Declaration of Helsinki.

Results

As shown in Fig. 1, The SLPI level was 21.19 ng/mL in BAPE patients, which was significantly lower than 118.62 ng/mL in MPM patients, 85.04 ng/mL in LCa patients, and 77.45 ng/mL in those with other diseases.

The HA level was 30,000 ng/mL in BAPE patients, which was significantly lower than the 89,200 ng/mL in patients with MPM. On the other hand, the HA level was 23,400 ng/mL in LCa patients, and 17,750 ng/mL in others, indicating a significantly higher level in BAPE patients (Fig. 2).

The SMRP level was 6.9 nmol/L in BAPE patients, which was significantly lower than the 16.7 nmol/L in MPM patients and significantly higher than the 4.9 nmol/L in others. On the other hand, in LCa patients, the SMRP level was roughly comparable at 6.2 nmol/L ($p < 0.512$), with no significant difference observed (Fig. 3).

When an ROC curve was drawn to confirm the reliability of SLPI to differentiate BAPE and MPM, and the cut-off value was 82.9 ng/mL, we found that sensitivity was 82.4%, specificity was 86.5%, and the area under the curve (AUC) was 0.902, indicating that SLPI was an effective differential marker (Fig. 4). However, with a cut-off value of 47,100 ng/mL in the ROC curve for HA, sensitivity was 77%, specificity was 75%, and the AUC was 0.802 (Fig. 5), which while useful, were inferior results to those of SLPI. Furthermore, with a cut-off value of 9.03 ng/mL in the ROC curve for SMRP, sensitivity was 72.3%, specificity was 71.4%, and the AUC was below 8 (0.746; Fig. 6). Compared with results in BAPE patients, this suggests that SLPI is a more effective differential marker than HA and SMRP, which are known as differential markers for mesothelioma.

Regarding other differential markers, the measured level of galectin-3 was 8.4 ng/mL (0.9–61.7) for BAPE, which was significantly lower than 32.4 ng/mL for MPM patients, 15.5 ng/mL for LCa patients, and 13.7 ng/mL for others (Fig. 7).

The CCL2 level was 4.44 pg/mL (0.49–20.76) in BAPE patients, which was significantly higher than 2.15 pg/mL for MPM patients, 1.37 pg/mL for LCa patients, and 1.07 pg/mL for others (Fig. 8). However, the data differed according to histological type, with a level of 1.5 pg/mL for epithelioid MPM patients, and

9.5 pg/mL for sarcomatoid MPM patients. This can be attributed to the fact that three out of six patients with sarcomatoid MPM had relatively high levels of between 12.9 and 18.2 pg/mL.

The CYFRA21-1 level was 15.6 ng/mL for BAPE patients, 75.9 ng/mL for MPM patients, 37.8 ng/mL for LCa patients, and 9.2 ng/mL for others. That is, while levels were significantly lower ($p < 0.03$) compared with MPM and LCa, they were significantly higher ($p < 0.0001$) for others.

In a comparison of the lymphocyte occupancy rates of the cellular components of pleural fluid, the rate was 88% for BAPE patients, 64.5% for MPM patients, 58% for LCa patients, and 68% for others, indicating that a significantly higher ($p < 0.0001$) rate of lymphocyte occupancy was associated with BAPE compared with all other diseases.

Discussion

The underlying mechanism for BAPE, which is an inflammatory condition of the visceral pleura caused by asbestos fibers, has yet to be elucidated. However, it is a condition that must be differentiated for the early diagnosis of MPM. BAPE is an exudative pleural effusion, and diagnosis is based on a history of occupational exposure to asbestos, findings of pleural plaque on imaging, and elimination of other possible causes using pleural fluid markers, cytology, and pleural biopsy.

Early-stage lesions of MPM often present with pleural effusion only; however, as the disease progresses, imaging findings such as pleural rind patterns may suggest malignant tumors. In early-stage lesions, neoplastic pleural thickening is typically not presented, making it difficult to differentiate from other diseases, particularly mediastinitis. Treatments considered for MPM include the use of CDDP + pemetrexed combination therapy and nivolumab as second-line therapy; however, no treatment method greatly improves prognosis. Because diagnosis of early-stage lesions and surgical treatment can improve prognoses, when patients with a history of asbestos exposure present with pleural effusion, BAPE should be considered for a differential diagnosis of MPM.

By focusing on BAPE, and differentiating with other diseases, we found that SLPI in pleural fluid was a significant indicator.

Tests of the ability of each pleural fluid marker to differentiating BAPE from MPM revealed that in BAPE, the following are observed:

1. In differentiation from MPM, SLPI, galectin-3, CYFRA21-1, SMRP, and HA exhibited significantly lower level, whereas CCL2 exhibited significantly higher levels. Moreover, if the percentage of lymphocytes was at least at 80%, this is suggestive of BAPE.
2. In differentiation from LCa, SLPI, galectin-3, CYFRA21-1, and HA levels were significantly lower, whereas CCL2 levels were significantly higher, and the presence of abundant lymphocytes was suggestive of BAPE.

3. In differentiation from other diseases (IF, HF), SLPI and galectin-3 levels were significantly lower, whereas CCL2, CYFRA21-1, SMRP, and HA ware significantly higher, and if the percentage of lymphocytes was high, then BAPE should be considered.

SLPI exhibited significantly lower values for these three conditions.

Although HA and SMRP reportedly serve as differential markers for MPM and other diseases, upon drawing an ROC curve, SLPI had an AUC of 0.902, indicating higher reliability than HA, which had an AUC of 0.802, and SMRP, which had an AUC of 0.746. Furthermore, galectin-3 showed significantly lower values for the three conditions, whereas CCL2 showed significantly higher values for the three. CYFRA21-1 showed significantly lower values for MPM and LCa, and significantly lower values for other diseases.

Combining pleural effusion markers with pleural fluid cytology, chest CT, and positron emission tomography–CT images facilitates the differentiation of BAPE from MPM and LCa, which are malignant tumors. However, in early-stage MPM, many patients do not exhibit significant uptake on chest CT or PET-CT, and it has been reported that MPM is diagnosed by searching for the presence of p16 gene mutations using FISH in histopathology specimens, and cytology tools when more than a certain number of homozygous deletions are confirmed [2, 12]. However, in MPM patients, the rate of diagnosis by pleural effusion cytology is much lower than in malignant pleural effusion caused by malignant tumors such as lung cancer. Therefore, even if tumor cells are detected, markers that suggest MPM are needed. In the past, such markers included osteopontin [13] and fibulin-3 [14, 15], but at present, they are rarely evaluated.

As a marker for differentiating pleural effusion in MPM, SLPI only appears in a report by Blanquart et al. (2013) [16]; however, in their report, three types of markers (CCL2, galectin-3, and SMRP) were reportedly effective, and if they are used properly, MPM could be differentiated from other diseases that cause pleural effusion, with an AUC of 0.968. However, Blanquart et al. used three types of markers, rather than a single marker. For SLPI alone, the AUC was 0.706, which was the lowest among the markers examined, and its significance was not evaluated.

With regard to MPM, CCL2 levels in pleural fluid were high and reportedly increased as disease progressed [17]. We have reported that high levels were found in serum in advanced-stage MPM [18]. In the present study, we examined CCL2 in pleural fluid, and found significantly higher levels in BAPE patients compared with patients with LCa and other diseases. However, with respect to MPM, which should be associated with high levels, we found significantly lower levels compared with BAPE ($p < 0.016$), in contradiction to Blanquart et al., [16] who reported that the levels differed according to histological type, with 2.82 ng/mL in epithelial mesothelioma, and 16.73 ng/mL in sarcomatoid mesothelioma. Our patients included 31 with epithelial mesothelioma and six with sarcomatoid mesothelioma (indicating overwhelmingly more patients with epithelial mesothelioma), and the mean level was therefore low at 2.15 pg/mL. Of the 37 MPM patients included in the study, three had sarcomatoid mesothelioma, and while some patients had a high level, the level in epithelial cases was 0.4

to 3.0 pg/mL, indicating significant individual variation. We therefore intend to conduct another study with a larger subject sample.

Galectin-3 not only showed high levels in MPM patients but in those with LCa as well, and we therefore suspect that it can be used to rule out malignancy because low levels are found in BAPE patients [19]. Similarly for CYFRA21-1, high levels are common in pleural effusions, even in early-stage MPM, and therefore, even if there was no malignant pleural thickening on imaging, early-stage MPM should be considered because CYFRA21-1 appears to be a marker that can serve to warrant a thoracoscopic biopsy [20].

When comparing HA, which is a biomarker of mesothelioma, and SMRP by focusing not on MPM but BAPE, we found a significantly high AUC at 0.902 for SLPI, and significantly low values compared to 0.802 for HA and 0.746 for SMRP. Even on its own, SLPI was deemed a superior marker for differentiating BAPE and MPM compared with HA and SMRP. We also found that it was useful for differentiating LCa, and HF, or IF. Differential diagnosis of LCa can be based on cytology or tumor markers such as carcinoembryonic antigens and CYFRA21-1; for tuberculosis pleurisy among IF, it can be based on adenosine deaminase; and for inflammatory pleurisy, differential diagnosis can be achieved based on neutrophilia in pleural fluid.

Because SLPI levels were significantly lower in BAPE patients compared to patients with pleurisy caused by other diseases such as MPM, it may be an effective a screening marker for the diagnosis of BAPE, which is important in a differential diagnosis of early-stage MPM.

Conclusions

Levels of SLPI in the pleural fluid of BAPE patients were significantly lower compared to patients with MPM, LCa, and other diseases. In differential diagnosis of early-stage lesions in MPM patients, we propose the inclusion of SLPI as a pleural effusion test item along with HA and SMRP.

List Of Abbreviations

AUC Area under curve

BAPE Benign asbestos pleural effusion

CT Computed tomography

FISH Fluorescent in situ hybridization

HA Hyaluronic acid

HF Heart failure

IF Infection

MPM Malignant pleural mesothelioma

ROC Receiver operating characteristics

SLPI Secretory leukocyte peptidase inhibitor

SMRP Soluble mesothelin-related peptides

Declarations

Ethics approval and consent to participate

All participants provided written informed consent before inclusion in the study. This study was approved by the 11th research ethics committee of the Japan Organization of Occupational Health and Safety on June 18,2018 (No.9). All study procedures were carried out in accordance with the principles of Declaration of Helsinki.

Consent for publication

All participants approve publication.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

TK & NF: Contributed for evaluation of these data. YK: Contributed for the measurement of biomarker.

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Figures

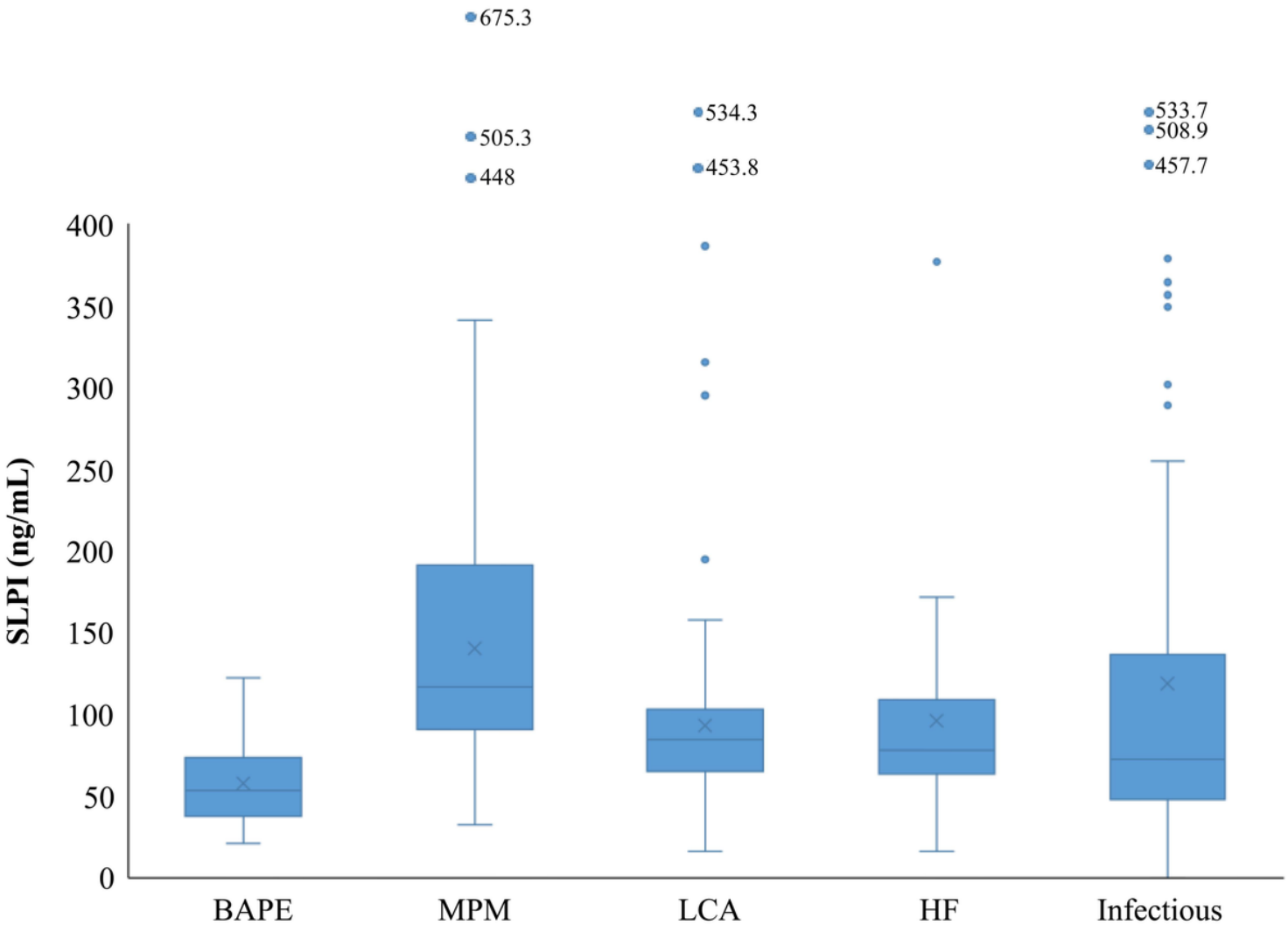

Figure 1

SLPI levels of pleural effusion in BAPE, MPM, LCA, HF and IF patients. SLPI levels in BAPE patients were significantly lower ($p < 0.0001$) than in patients with MPM, LCA, HF and IF

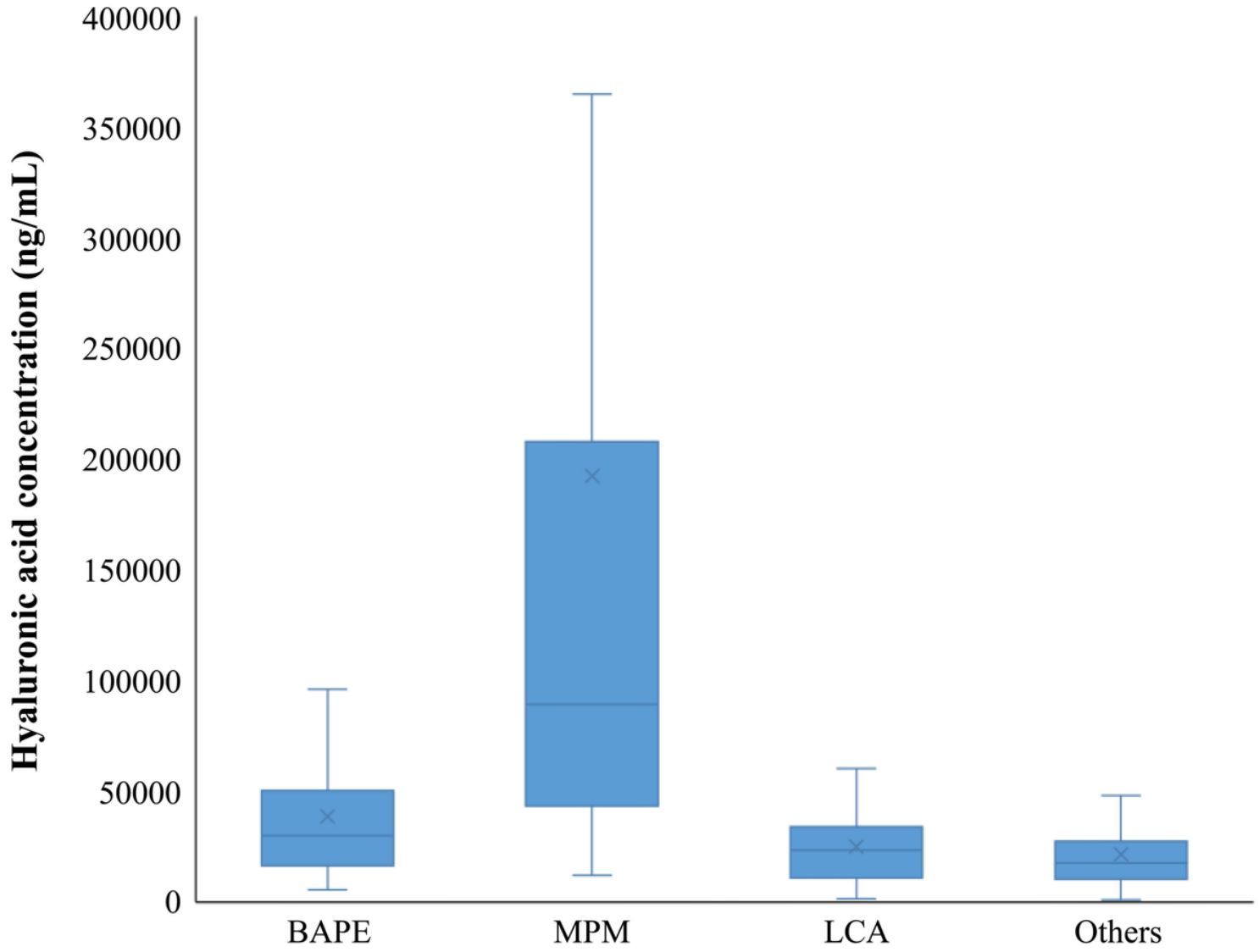

Figure 2

HA levels of pleural effusion in BAPE, MPM, LCA and other diseases patients. HA levels in BAPE patients were significantly lower ($p < 0.004$) than in patients with MPM, LCA, and other diseases ($p < 0.0001$)

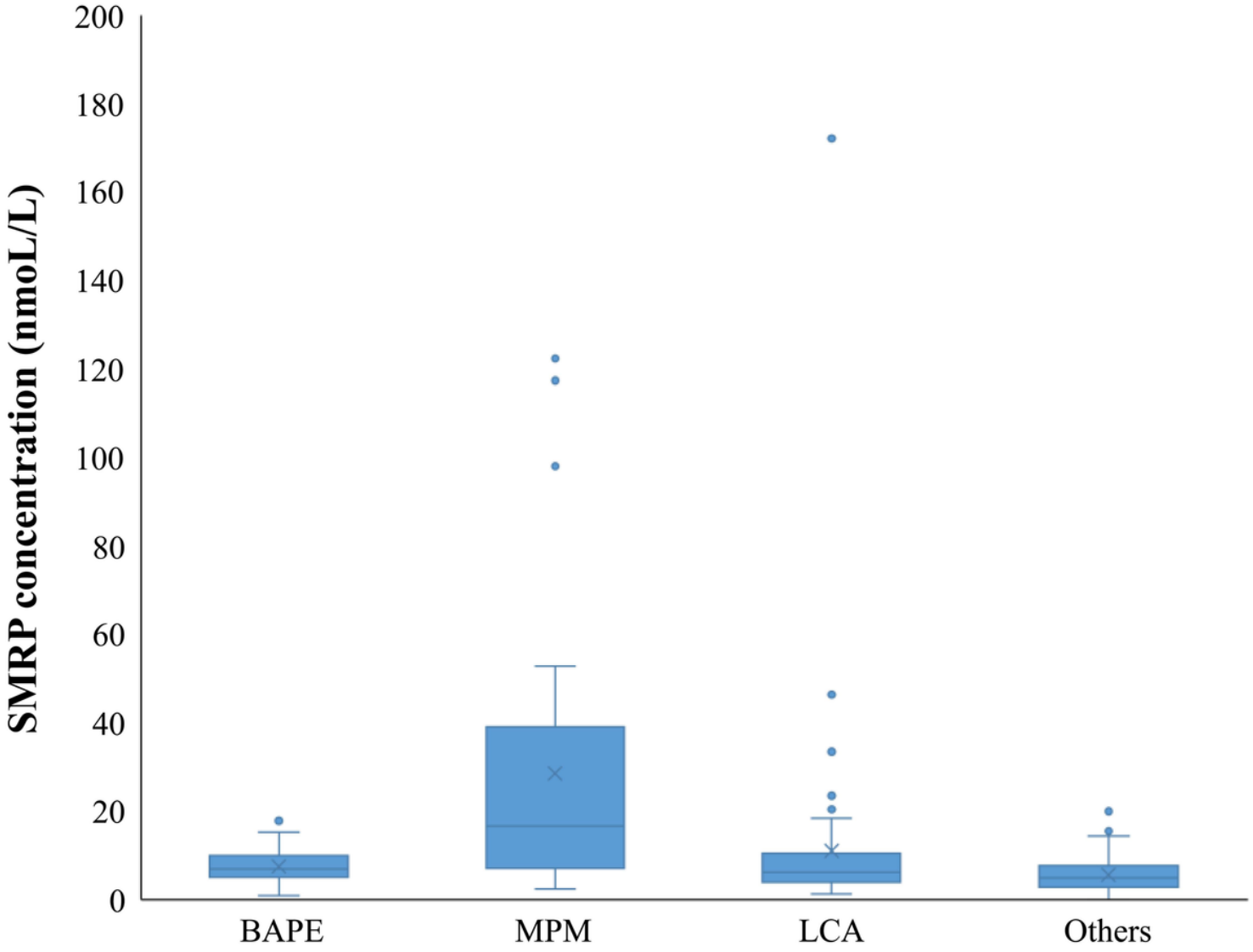

Figure 3

SMRP levels of pleural effusion in BAPE, MPM, LCA, IF patients. SMRP levels in BAPE patients were significantly lower ($p < 0.0001$) than in patients with MPM, HF, and IF ($p < 0.001$). No significance difference was found when compared with levels in LCA patients ($p < 0.512$).

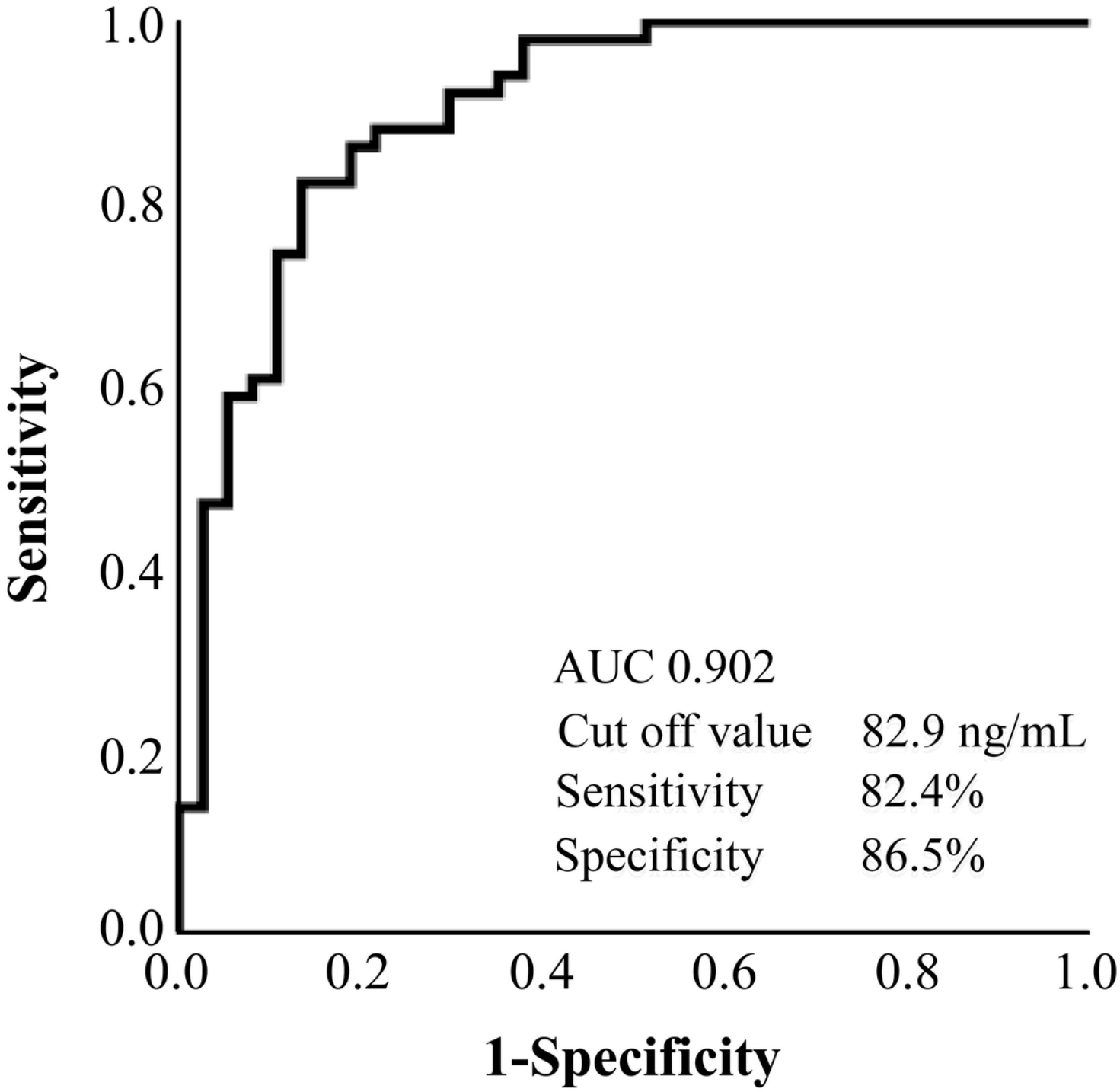

Figure 4

The ROC curve between BAPE and MPM for SLPI. The ROC curve between BAPE and MPM for SLPI demonstrates significance, with an AUC of 0.902 and a cut-off of 82.9 ng/mL.

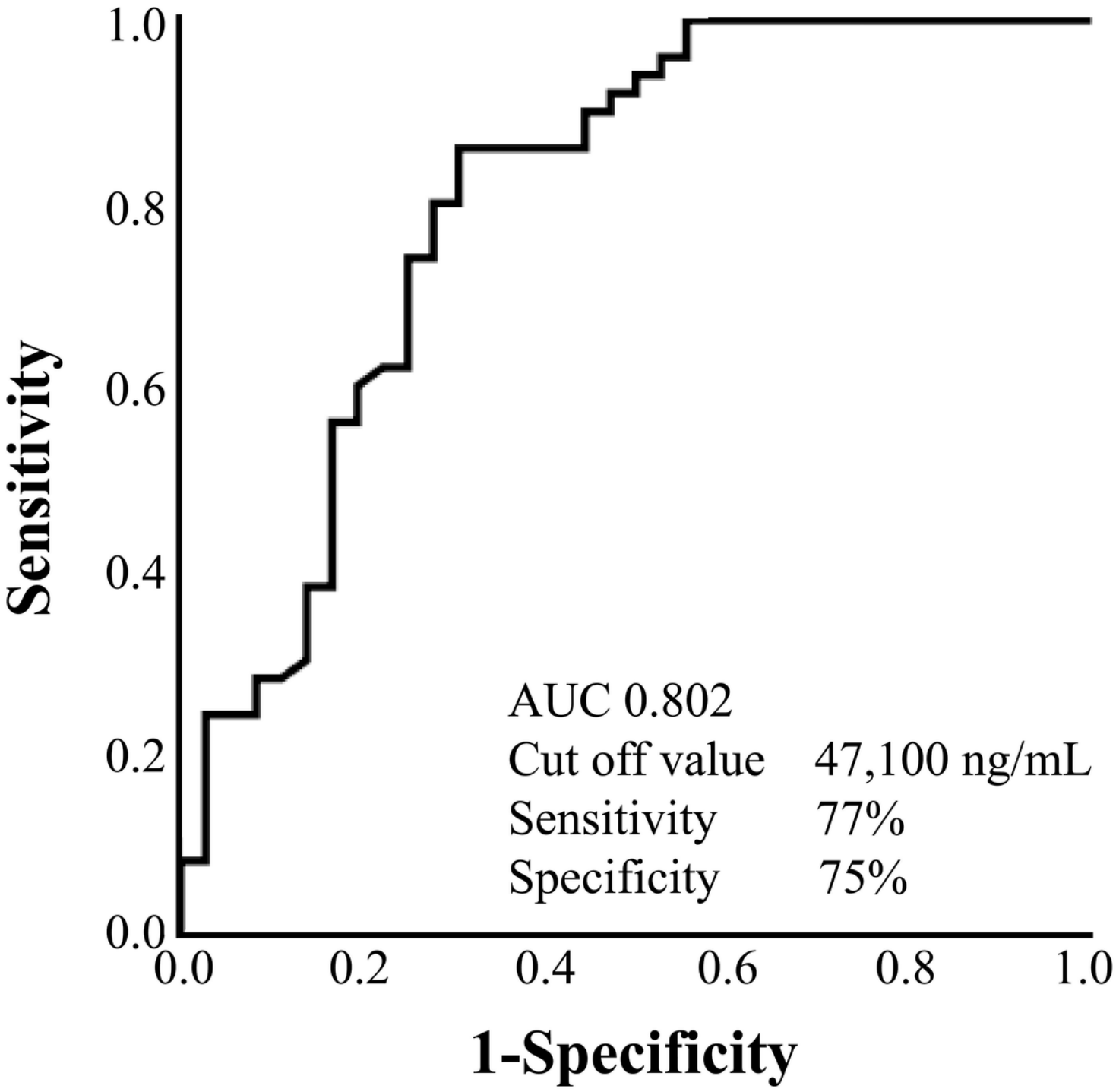

Figure 5

The ROC curve between BAPE and MPM for HA. The ROC curve between BAPE and MPM for HA demonstrates significance, with an AUC of 0.802 and a cut-off of 47,100 ng/mL.

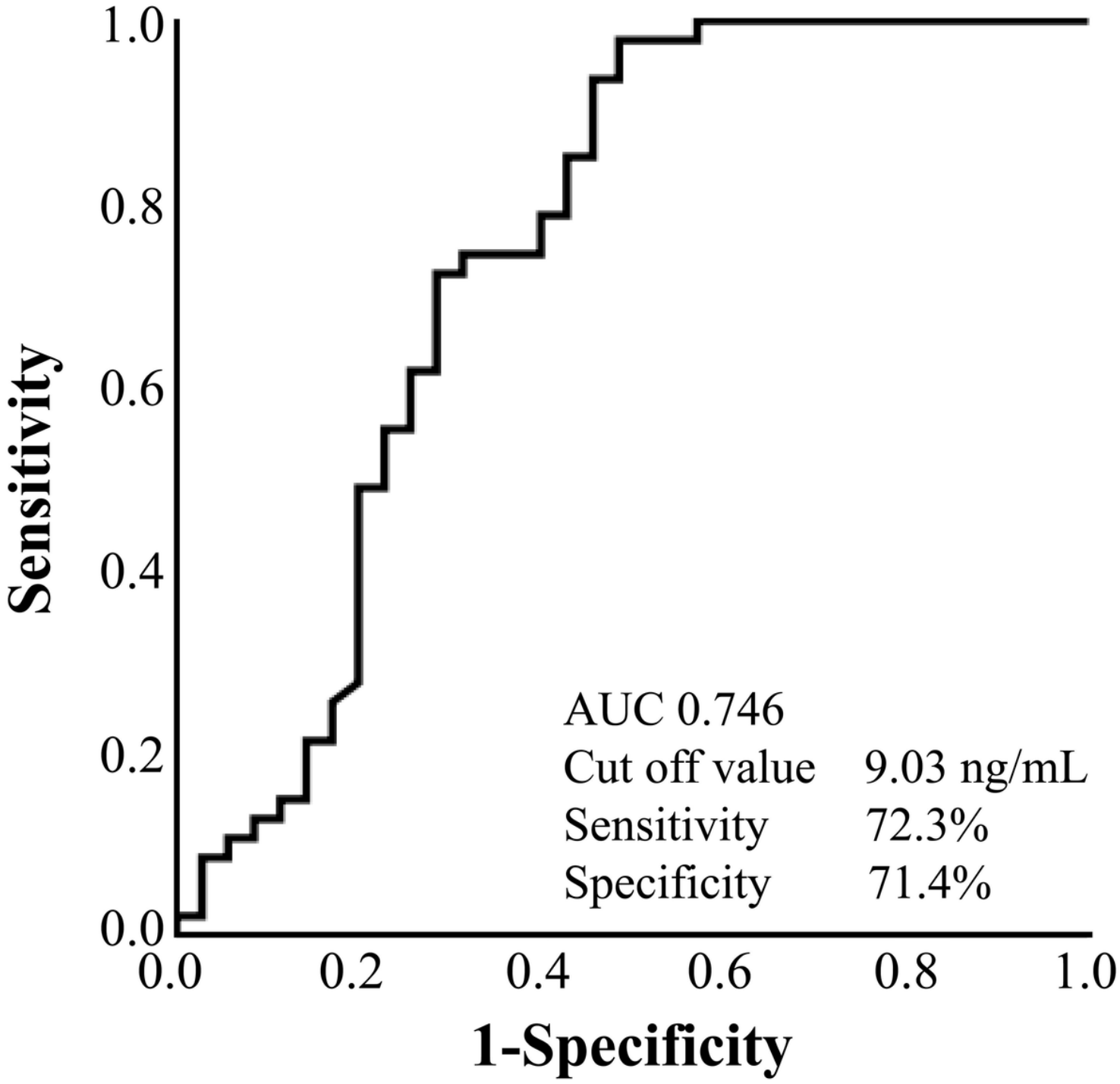

Figure 6

The ROC curve between BAPE and MPM for SMRP. The ROC curve between BAPE and MPM for SMRP demonstrates significance, with AUC of 0.746 and a cut-off of 9.03 ng/mL.

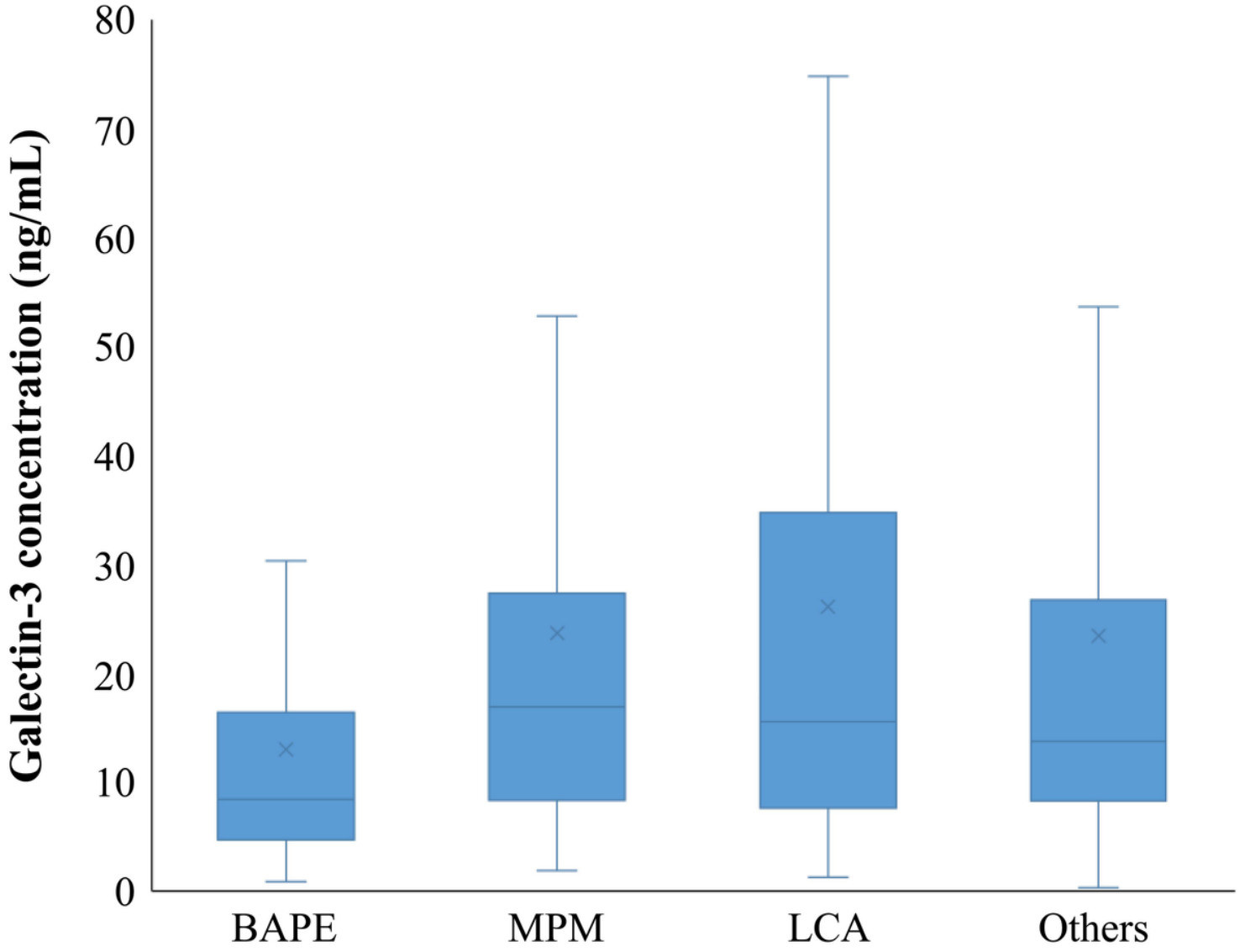

Figure 7

Galectin-3 levels of pleural effusion in BAPE, MPM, LCA and other diseases patients. Galectin-3 levels in BAPE patients were significantly lower than in patients with MPM ($p < 0.004$), LCA ($p < 0.001$), and other diseases ($p < 0.002$). However, the difference was not as significant as that associated with SLPI.

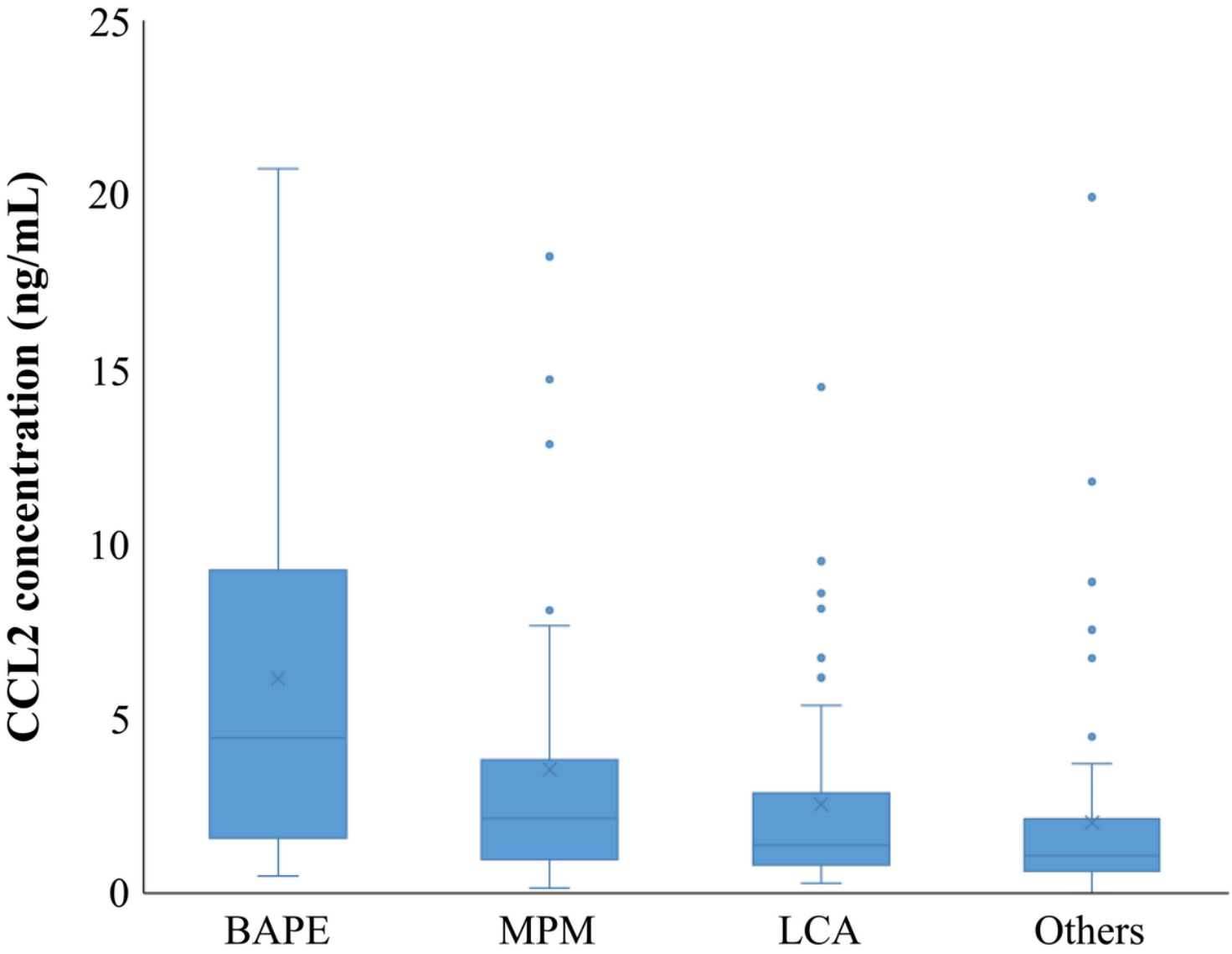

Figure 8

CCL2 levels of pleural effusion in BAPE, MPM, LCA and other diseases patients. CCL2 levels in BAPE patients were significantly higher than in patients with MPM ($p < 0.02$), LCA ($p < 0.0001$), and other diseases ($p < 0.0001$). However, the difference was not as significant as that found for SLPI in MPM patients.