Correlation between matrix metalloproteinase 9 and ¹⁸F-2-fluoro-2-deoxyglucose-positron emission tomography as diagnostic markers of lung cancer†

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Abstract

OBJECTIVES: This study was conducted to evaluate the diagnostic role of matrix metalloproteinase 9 (MMP9) measured in bronchoalveolar lavage (BAL), serum and tissue samples of patients with indeterminate lung lesions and its correlation with F-18-2-fluoro-2-deoxyglucose-positron emission tomography (¹⁸FDG-PET) findings in diagnostic work.

METHODS: MMP9 levels (ng/ml) in serum and BAL were analysed using enzyme-linked immunosorbent assay in 60 consecutive patients with lung mass. ¹⁸FDG-PET was performed on all patients and a standard uptake value (SUV) threshold of 2.5 was used to differentiate benign from malignant lesions. In tissue samples of resectable patients, MMP9 expression was also revealed by immunohistochemical staining.

RESULTS: Twenty patients had benign disease and 40 patients had malignant lesions, of which 7 (17.5%) were classified as Stage I, 18 (45%) as Stage II, 7 (17.5%) as Stage III and 8 (20%) as Stage IV. MMP9 levels in serum were significantly higher in malignant than in benign lesions (673 ± 182 versus 309 ± 96, respectively, \( P < 0.0001 \)), and were significantly higher in patients with metastatic disease than in patients of other stage groups; no significant difference was found between different histological types. MMP9 levels in BAL were higher in malignant than in benign lesions (502 ± 137 versus 325 ± 118, respectively, \( P = 0.001 \ )); no significant differences were found between different stages or histological groups. In patients with malignant lesions, MMP9 levels in BAL were inversely correlated with FEV1 (volume that has been exhaled at the end of the first second of forced expiration) and FVC (forced vital capacity of maximally forced expiratory effort) values. In patients with SUV > 2.5, MMP9 levels in serum and BAL had a sensitivity, specificity, positive predictive value and negative predictive value of 73, 100, 100 and 81% (cut-off point of 601; area under the curve (AUC): 0.7) and 94, 100, 100 and 83% (cut-off point of 745; AUC: 0.9), respectively. In patients with SUV < 2.5, MMP9 levels in serum and BAL had a sensitivity, specificity, positive predictive value and negative predictive value of 94, 100, 100 and 75% (cut-off point of 601; area under the curve (AUC): 0.7) and 70, 100, 100 and 73% (cut-off point of 321; AUC: 0.7), respectively. Of the 26 tumour samples, 9 (34%) showed positive immunohistochemical staining for MMP9.

CONCLUSIONS: The measurement of MMP9 levels helps to differentiate benign from malignant lung mass. Its use in combination with PET study adds further information to the diagnosis work-up of lesions to select patients who may or may not benefit from additional invasive procedures.

Keywords: Matrix metalloproteinase 9 • ¹⁸FDG-PET • Lung cancer

INTRODUCTION

Making a definitive preoperative diagnosis in patients with indeterminate pulmonary lesions is still a challenge in clinical practice. Current tests for the diagnosis, with regard to their performance characteristics and complication rates, are far from ideal. Bronchoscopic or computed tomography-guided tissue sampling often yields a specific malignant diagnosis but suffers from sampling bias, which dictates additional work if biopsy results are non-diagnostic in patients with a high pretest probability of malignancy [1, 2]. The associated pneumothorax rate, though high, infrequently leads to significant morbidity. In recent years, ¹⁸F-2-fluoro-2-deoxyglucose-positron emission tomography (¹⁸FDG-PET) has emerged as a powerful diagnostic tool for diagnosis, staging and restaging of lung cancer. However, it has some

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limitations. $^{18}$FDG in malignant ground glass and partially solid lesion is variable and cannot be used reliably to distinguish benign from malignant lesions. Otherwise, false-negative PET scanning results are uncommon but may occur with carcinoid tumour, bronchoalveolar carcinoma (BAC) and early stage disease. Conversely, false-positive lesions have been reported to include hamartoma, pneumonia, caseating granulomas, sarcoidosis, amyloidosis, talc pleurodesis, rounded atelectasis, pleural fibrosis and atherosclerosis [3, 4]. Thus, other biomarkers that provide further information for diagnosing lung lesions are desirable.

Matrix metalloproteinases (MMPs) are a group of enzymes that take part in the metabolism of extracellular matrix components, tissue repair and cell migration. They play important roles in tumour invasion and metastasis and are also involved in initial stages of tumour development by regulating cell proliferation, apoptosis, angiogenesis and immune surveillance. Matrix metalloproteinase 9 (MMP9) belongs to the family of gelatinases that occupy special position because of their ability to degrade both elastin and type IV collagen—a major component of the basal membrane that may be the first barrier to tumour metastasis [5]. MMP9 is expressed in many different tumours, and elevated levels of MMP9 in circulation have been reported in patients with gastric, colon, breast and prostate cancer [6]. In the case of lung cancer, however, many questions still remain regarding the clinical significance of this enzyme.

Therefore, in this study we measured MMP9 levels in bronchoalveolar lavage (BAL) and serum of patients with indeterminate lung lesions diagnosed by computed tomography (CT) and $^{18}$FDG-PET scans with the aim of differentiating benign from malignant lesions. The expression of MMP9 was also determined in cancerous tumour samples of patients undergoing surgical resection by immunohistochemical staining. Finally, we examined the clinical use of such enzymes by investigating the relationship between MMP9 levels in serum and BAL by PET. The aim was to determine whether MMP9 adds further information to PET findings in diagnosing lung malignancy.

**MATERIALS AND METHODS**

**Study design**

This study included a series of consecutive patients with indeterminate lung lesions who were scheduled to undergo diagnostic bronchoscopy at the Thoracic Surgery Unit of the Second University of Naples between January 2008 and October 2010. Patients underwent diagnostic examinations to obtain a diagnosis of lung lesions. MMP9 levels were measured in serum samples and in BAL before surgery; In tissue samples of patients undergoing surgical resection by immunohistochemical staining. Finally, MMP9 expression was correlated with the final diagnosis of the lesion, and among patients with lung malignancy, with spirometric values (Fig. 1). The protocol of this study was approved by the Hospital Ethics Committee of the Second University of Naples and written informed consent was obtained in all cases before beginning the study.

**Patients characteristics**

This study included 60 patients (38 men, 22 women; age range: 32–83 years; mean age ± standard deviation (SD): 66 ± 9 years) with single lung mass diagnosed by a CT scan. All lesions of our study group were ‘indeterminate’ because they were non-calciﬁed and no specific diagnosis could be achieved on the basis of morphological imaging. The clinical assessment included a physical examination, hematological and biochemical screening, a whole body CT scan, radionuclide bone scan, fibre-optic bronchoscopy with bronchoaspirate and/or brushing and/or bronchial biopsy. Yet, all patients underwent an $^{18}$FDG-PET scan as a component of their clinical evaluation. Patients who showed signs of metastases in mediastinal lymphnodes in a CT scan and/or a PET underwent a transbronchial needle aspiration biopsy during bronchoscopy and/or mediastinoscopy before surgery.

Clinical staging was carried out according to the revised (1997) TNM system and later revised following the guidelines of the International Association for the Study of Lung Cancer (IASLC) proposed in 2009 [7]. All cancer was diagnosed pathologically. Benign diagnosis was determined pathologically or radiologically with serial CT. Finally, patients with resectable lung malignancy underwent surgical resection and dissection of lymph nodes.

**Serum samples**

Blood samples were collected from an antecubital vein by routine venipuncture. The samples were allowed to stand for 30 min for clotting, followed by centrifugation at 3000xg for 5 min, and then stored in deep refrigeration at −80°C until MMP9 levels were measured (ng/ml).

**BAL samples**

Bronchoscopy was performed with the patients in supine position, under local anaesthesia in standard fashion using a videobronchoscopy (model BF XT160, Olympus; Tokjo, Japan). The bronchoscope was wedged at the point of division of the bronchus that showed abnormal shadows on CT scan and/or bronchoscopic findings within large airways. Physiological saline at body temperature was fed through the internal channel of the fibrobronchoscope in variable quantities, although 150 ml of saline in three 50-ml aliquots was recommended. The BAL obtained was quickly transferred to the laboratory, where it was processed in <60 min. It was subsequently centrifuged (500xg, 10 at 4°C) and the supernatant was collected in appropriate sterile tubes and stored at −80°C until analysis. The resulting
pellet was sent to the Pathological Anatomy Department for a cytological study and histological classification.

**Measurement of MMP9 levels in serum and BAL**

MMP9 levels in serum and BAL were measured by the same technologist using a commercially available human MMP9 enzyme-linked immunosorbent assay kit according to the manufacturer’s instructions. The technologist was blinded of final diagnosis.

**MMP9 immunohistochemical staining**

Expression of MMP9 was confirmed immunohistochemically in tumour samples of patients undergoing surgical resection. Each sample was fixed with peroxide-lysine-paraformaldehyde fixative for 18–24 h at 4°C after treatment with monensin in RPMI-1640 at 37°C for 3 h and then embedded in paraffin wax. Immunohistochemical staining was performed using the standard linked streptavidin biotin method (LSAB), using an automatic Ventana Benchmark™, with mouse antihuman monoclonal antibodies, to determine MMP9 expression, according to the manufacturer’s instructions.

The slides were examined under a Leitz Laborlux light microscope using objectives with ×10 and ×40 magnifications. Immunohistochemical staining was analysed semi-quantitatively by verifying the distribution of stained cancer cells in the centre of the tumour. In addition, the immunohistochemical staining of the tumour-infiltrating leucocytes and macrophages was considered. The tumours were divided into four groups as follows: +C/+IP, homogeneous staining of cancer cells and inflammatory cells; +C−/IP, heterogeneous staining of cancer cells; −C/IP+, no staining of cancer cells but positive inflammatory cells accompanying the cancer cells; and −C−/IP, no staining of cancer cells and inflammatory cells.

**18FDG-PET**

After a 6-h fast, each patient received 18.5 MBq (0.5 mCi)/10 kg of body weight of 18FDG intravenously. Serum glucose levels just before the injection of 18FDG were ≤120 mg/dl in all patients. Imaging started 50–70 min after tracer injection using a three-dimensional dedicated PET (Siemens EXACT HR+ or ART) scan from the base of the brain to the level of the proximal thighs. Transmission scans using a 68Ge pin or 137Cs source were also performed and allowed the calculation of attenuation correction factors that were used to correct the 18FDG emission data. The attenuation corrected and non-corrected emission data were reconstructed with an iterative reconstruction algorithm using an ordered subset expectation maximization method. Two physicians of nuclear medicine, blinded from each other and from the diagnosis of the lesion, interpreted the examination. Disagreements were resolved by consensus, with a third observer as referee. The images, reconstructed in transaxial, sagittal and coronal planes, were visually compared with the chest CT scan. Any obvious foci of increased 18FDG uptake compared with background were considered positive for malignancy. For semi-quantitative analysis, regions of interest were defined manually on the transaxial tomograms that showed the lesion’s highest uptake to be at the middle of the tumour. The regions of interest placed on the lesion encompassed all pixels that had uptake values ≥80% of the maximum uptake in that slice and the average standard uptake value (SUV) was calculated as tumour concentration of tracer per injected tracer dose per body weight. An SUV threshold of 2.5 was used to differentiate benign from malignant lesions as reported elsewhere [3, 4].

**Statistical analysis**

Data are presented as mean ± SD. Comparisons of MMP9 levels between patients with benign and malignant lesions, and between different histological types, were performed using Mann–Whitney U-tests. The multiple comparisons between the stage groups were performed by a one-way factorial analysis of variance (ANOVA) with the Bonferroni post hoc test. The relationships between MMP9 expression and spirometric values (FEV1 (volume that has been exhaled at the end of the first second of forced expiration) and FVC (forced vital capacity of maximally forced expiratory effort) presented as the percentage of predicted value) were valued using the Spearman rank correlation test. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated in the standard manner for PET results. In patients with SUV > 2.5 (classification variable) and in those with SUV < 2.5 (classification variable), values of sensitivity, specificity, PPV and NPV with a 95% confidence interval (95% CI) of MMP9 levels (variables) in serum and BAL were calculated using receiver operating characteristic analysis. A value of P < 0.05 was considered statistically significant. MedCalc® statistical software was used for analysis.

**RESULTS**

Sixty patients were enrolled in this study. The characteristics of the study population are summarized in Table 1. Of the patients enrolled, 40 (66%) patients had malignant lesions: 15 squamous cell carcinomas, 20 adenocarcinomas (4 BACs), 4 large-cell carcinomas and 1 mixed (large-cell carcinoma and microcita). The diagnosis was obtained by bronchoscopic biopsy (n = 17) and fine needle aspiration biopsy CT-guided for 20 patients, whereas the diagnosis was made postoperatively by a negative cytolgy for 3 patients. A total of 7 (17.5%) patients were classified as Stage I, 18 (45%) as Stage II, 7 (17.5%) as Stage III and 8 (20%) as Stage IV. Of 40 patients, 26 were judged as operable, and in all cases the tumour was completely removed. The remaining 14 patients were judged as inoperable because of the locally advanced (n = 5) or metastatic (n = 8) disease; in a patient with early stage disease, the resection was medically contraindicated. A total of 20 (34%) patients had a benign disease: 8 pneumonia sequelae, 2 active tuberculosis, 3 tuberculosis, 3 chronic abscesses, 2 hamartoma and 2 fibrotic nodule. In 3 patients (2 tuberculoma and 1 hamartoma), the diagnosis was established by means of surgical resection, whereas in the remaining 17 patients, the benign nature was confirmed by the absence of malignant cells in specimens obtained by invasive procedures and the reduction or disappearance of the lesion on radiological follow-up after medical therapy.
MMP9 levels in serum

MMP9 levels in serum were significantly higher in malignant than in benign lesions (673 ± 182 versus 309 ± 96, respectively, *P* < 0.0001; Fig. 2A). Among malignant lesions, adenocarcinomas + BACs had MMP9 levels of 643 ± 198, squamous carcinomas had MMP9 levels of 713 ± 183 and large-cell carcinomas + mixed carcinomas had MMP9 levels of 683 ± 39. However, a comparison between the different histological groups showed no significant difference (Fig. 2B).

Regarding the stage of disease, MMP9 levels in the serum of patients with Stage I, II, III and IV disease were 530 ± 62, 637 ± 45, 688 ± 48 and 897 ± 38, respectively. MMP9 levels were significantly higher in patients with Stage IV disease than in those with Stage III (*P* = 0.02), Stage II (*P* = 0.02) and Stage I (*P* = 0.01) disease. However, no significant difference was found between the other stage groups (Fig. 2C).

MMP9 levels in BAL

MMP9 levels in BAL were higher in malignant than in benign lesions (502 ± 137 versus 325 ± 118, respectively, *P* = 0.001; Fig. 3A). Among malignant lesions, adenocarcinomas + BACs had MMP9 levels of 500 ± 119, squamous carcinomas had MMP9 levels of 473 ± 166 and large-cell carcinomas + microcitoma had MMP9 levels of 494 ± 112. However, a comparison between the different histological groups showed no significant difference (Fig. 3B).

Regarding the stage of disease, MMP9 levels in BAL of patients with Stage I, II, III and IV disease were 476 ± 19, 501 ± 71, 539 ± 60 and 553 ± 67, respectively. However, no significant difference was found between different stage groups (Fig. 3C).

Correlation between MMP9 levels and respiratory function

Among patients with lung malignancy, values of FVC and of FEV1 were 73 ± 12 and 74 ± 13, respectively. A significant inverse correlation was found between MMP9 levels in BAL and FVC (*r* = −0.332; *P* = 0.03; 95% CI: 0.584–0.02; Fig. 4A) and FEV1 (*r* = −0.39; *P* = 0.01; 95% CI: 0.625–0.088; Fig. 4B) values. An inverse correlation was also seen between MMP9 levels in serum and BAL (*r* = −0.222; *P* = 0.1; 95% CI: 0.499–0.096; Fig. 4C) and FEV1 (*r* = −0.243; *P* = 0.1; 95% CI: 0.516–0.741; Fig. 4D) values, but it was not significant.

Immunohistochemical staining of tumour samples

Immunohistochemical staining for MMP9 was attempted in 26 tumour samples of patients undergoing surgical resection (24 with early stage disease and 2 with locally advanced disease). A total of 17 patients with early stage disease showed negative staining and were classified as −C/IP−. Of the tumour samples, nine (34%) showed positive immunohistochemical staining for MMP9 and were categorized as +C/IP+ (two advanced cancers), +C/IP− (two early cancers) and −C/IP+ (five early cancers) (Fig. 5).

Diagnostic accuracy of MMP9 expression according to PET findings

Of the total number of patients, 41 had SUV > 2.5 (38 malignant lesions and 3 benign lesions; prevalence of disease: 93%) and 19 had SUV < 2.5 (17 benign lesions and 2 malignant lesions; prevalence of benign lesion: 89%). The sensitivity, specificity, PPV and NPV of PET findings were 95 (38/40), 85 (17/20), 92 (38/41) and 90% (17/19), respectively.

In patients with SUV > 2.5 (classification of variable), MMP9 levels (variable) in serum had a sensitivity, specificity, PPV and NPV of 73 (95% CI: 56–86), 100 (95% CI: 15–100), 100 (95% CI: 87–100) and 81% (95% CI: 2–48), respectively (cut-off point of 601; area under the curve (AUC) 0.7; Fig. 6A), in diagnosing lung malignancy. In the same group of patients, MMP9 levels in BAL had a sensitivity, specificity, PPV and NPV of 94 (95% CI: 82–99), 100 (95% CI: 15–100), 100 (95% CI: 90–100) and 85% (95% CI: 6–93), respectively (cut-off point of 745; AUC 0.9; Fig. 6B).

In patients with SUV < 2.5 (classification of variable), MMP9 levels (variable) in serum had a sensitivity, specificity, PPV and NPV of 94 (95% CI: 71–99), 100 (95% CI: 29–100), 100 (95% CI:...
78–100) and 75% (95% CI: 19–99), respectively (cut-off point of 240; AUC: 0.9; Fig. 6C). In the same group of patients, MMP9 levels in BAL had a sensitivity, specificity, PPV and NPV of 70% (95% CI: 44–89), 100% (95% CI: 29–100), 100% (95% CI: 73–100) and 73% (95% CI: 7–77), respectively (cut-off point of 321; AUC: 0.7; Fig. 6D).

**DISCUSSION**

MMP9 is capable of degrading extracellular matrix components—the first barrier against tumour metastasis—thus facilitating tumour cell migration through basement membranes [5, 6]. A limited number of studies [8–10] exist concerning MMP9 expression in the serum of patients with lung carcinomas, and the results are inconclusive. Yet, only a previous report investigated MMP9 levels in BAL of patients with benign and malignant lesions [11], whereas, to our knowledge, there are no studies regarding an integrated approach using MMP9 expression and PET findings for the clinical evaluation of indeterminate lung lesions.

First, in our study we observed a significant elevation of MMP9 levels in the serum of patients with lung malignancy and those with benign lesions, in line with the studies by Laack et al. [8], Jumper et al. [9] and Iizasa et al. [10]. MMP9 levels in BAL were also higher in patients with lung cancer than in the control group, conversely to the data of Koç et al. [11] who found no significant difference. This contrasting result may be due to the difference between the groups of patients with benign disease. In the study by Koç et al. [11], the control group included disease as bronchiectasis and tuberculosis that were not present.
or present in fewer numbers, respectively, in our population. In such diseases, MMP9 has been shown to be involved as it leads to inflammation and tissue destruction.

Among patients with lung cancer, no relationship was observed between MMP9 levels in serum and BAL and the different histological types. Our findings are supported by other studies [8, 9] that reported no significant association between MMP9 levels in serum and BAL and the different histological types of non-small-cell lung cancer (NSCLC). Conversely, Ylisirnio et al. [12] found that MMP9 levels in serum was lower in patients with small-cell lung cancer (SCLC) than in patients of other histological groups, but these data are not comparable with our study that did not include patients with SCLC.

Regarding tumour stage, we found that it was significantly correlated with MMP9 levels in serum but not with MMP9 levels in BAL. The correlation between MMP9 levels in serum and the stage of disease is not a surprising result considering that MMP9 is a key regulator of the metastatic process as reported above. It remains unclear why MMP9 levels in BAL are not correlated with the stage of disease. Probably, MMP9 expression during the metastatic process may be only in part due to local production by tumour tissue whereas the major elevation of MMP9 levels is due to secretion by inflammatory [13, 14] and blood [15] cells stimulated by the same cancer cells through the production of regulatory factors including cytokines. Activated cells expressing MMP9 may be detectable by blood serum but not by BAL analysis because they are present in a vascular bed.

MMP9 has been implicated in parenchymal lung destruction and repair processes of diverse pulmonary disease, such as emphysema, and chronic obstructive pulmonary disease that are also associated with lung cancer [16]. Because evidence suggests that lung cancer prevalence increases as FEV1 decreases, we evaluated the correlation between MMP9 expression and respiratory function in patients with lung malignancy. We found that FEV1 and FVC were inversely correlated with MMP9 levels in BAL samples in agreement with the results of other studies in patients with asthma [17], emphysema and chronic obstructive pulmonary disease [16]. Conversely, we did not observe significant correlation of MMP9 levels in serum and spirometric values, suggesting the increase in MMP9 activity was restricted to the inflamed area of the lung. Elevated production of MMP9

Figure 4: The scatter diagram shows an inverse significant correlation between MMP9 levels in BAL and FVC ($r = -0.332; P = 0.03; 95\% CI: 0.584-0.02$; A) and FEV1 ($r = -0.39; P = 0.01; 95\% CI: 0.625-0.088$; B) values. No significant correlation was seen between MMP9 levels in serum and FVC ($r = -0.222; P = 0.1; 95\% CI: 0.499-0.096$; C) and FEV1 ($r = -0.243; P = 0.1; 95\% CI: 0.516-0.741$, D) values. Spotted line: 95% CI.

Figure 5: Immunohistochemical staining for MMP9 showed positive inflammatory cell (macrophages) accompanying cancer cells (×10 magnification; inset: ×40 magnification).
by alveolar macrophages and neutrophils from patients with chronic lung disease has been reported [16]. As BAL is a direct analysis of lung response to inflammation, it explains why we found larger levels of MMP9 in BAL than in serum samples.

Second, the presence of activated inflammatory cells expressing MMP9 is confirmed by our immunohistochemical studies. We found that 9 of 26 (34%) tissue samples of patients with resected cancer (2 advanced and 7 early cancers) showed positive immunohistochemical staining for MMP9 whereas 17 tissue samples of patients with early cancers showed negative immunohistochemical staining for MMP9.

The negative immunohistochemical staining (−C/IP−) for MMP9 in 17 patients with early stage disease and the positive immunohistochemical staining (+C/IP+) for MMP9 in 2 patients with locally advanced stage cancers are not surprising data considering that MMP9 expression is correlated with the tumour stage as above mentioned. That may explain why in some patients of our study population the frequency of tumour samples expressing MMP9 is much less than the frequency of samples with elevated MMP9 levels in plasma and BAL.

Conversely, the interesting data is the expression of MMP9 in seven tumour samples from patients with early stage disease, for whom we would expect a negative local expression of MMP9 considering the early stage of the disease. However, in five of these cases we found that MMP9 was expressed not by tumour cells but by infiltrating inflammatory cells alone (−C/IP+) as macrophages, in line with the study by Iizasa et al. [10] who report five cases in which macrophages stained positive for MMP9 in the tumour samples.

The presence of activated MMP9 inflammatory cells would indicate that these tumours may have potential metastatic activity despite the early stage, in agreement with data from the studies by Kodate et al. [18] and Cox et al. [19]. In association with MMP9 expression, the same tumours presented a high SUV (>5.5). Multiple studies have observed that patients with lung cancers that are highly metabolic, as measured by the degree of 18FDG uptake, tend to have a more aggressive clinical course than those with lung cancers that have a low metabolic rate [20–22]. Thus, MMP9 expression associated with SUV as a prognostic factor in operable NSCLC should be investigated in the future in larger studies.

Third, our data suggest that MMP9 expression combined with PET results would improve the accuracy of PET imaging in diagnosing lung lesions to select patients who may or may not benefit from additional invasive procedures. The approach of patients with lung lesion is determined to a large extent by the likelihood that a lesion in a particular patient is a lung cancer. PET examination is especially advocated if a patient is older than 55 years and has smoked more than 15 pack-years according to the criteria used in CT screening studies [23, 24]. An SUV threshold of 2.5 is usually used to differentiate benign from malignant lesions as in this study [3, 4]. In line with the data of the literature [3, 25], in our study population we find that the sensitivity, specificity, PPV and NPV of PET findings were 95 (37/39), 85 (18/21), 89 (38/42) and 91% (16/18), respectively.

Two tubercoloma and a hamartoma had false-positive results on PET examinations (SUV > 2.5), and in all cases the pathological diagnosis was obtained after surgical resection. Conversely, two
cancers (both BACs) had negative results on PET examinations (SUV < 2.5), and were diagnosed by bronchoscopy and aga-
biopsy CT-guided. Thus, in clinical practice it should keep in
mind that 18F-DG-PET may fail to differentiate benign
from malignant lesions, and thus any additional markers for
MMP9 would be of great clinical utility.

In patients highly suspected of having lung cancer according
to PET results (SUV > 2.5), MMP9 levels >601 and/or >745 in
serum and BAL, respectively, might increase the risk of the lesion
being malignant. This is because the high PPV (100%) translates
into minimal false-positive results. Thus, in such cases, if it is dif-
ficult to have a preoperative pathological diagnosis, a rational
approach might be to directly perform surgical resection to
reduce the morbidity from invasive diagnostic procedures. Conversey, in patients with MMP9 levels <601 and/or <745 in
serum and BAL, respectively, positive 18F-DG PET results should
be interpreted with caution, especially if patients have low
epidemiological risk for lung cancer (younger than 55 years and
a smoking history of less than 15 pack-years) and/or come from
areas with high prevalence of inflammatory diseases such as
tuberculosis that are well known to result in high SUV uptake on
PET images. In such cases, before proceeding to surgical resec-
tion, further invasive diagnostic strategies are required to avoid
unnecessary thoracotomy. This is because MMP9 expression has
a high value of NPV (81 and 83% for serum and BAL, respective-
ly) that translates into fewer false-negative results.

In patients less suspected of having lung cancer according
to PET results (SUV < 2.5), low levels of MMP9 in serum (<240)
and in BAL (<321) might confirm the strategy of conservative
management with radiological follow-up rather than invasive
risks procedures in the light of the value of NPV (75 and 73%
for serum and BAL, respectively). Conversely, MMP9 levels
>240 in serum (PPV of 100%) and >321 in BAL (PPV of 100%)
associated with PET negative results would require invasive
strategy, especially in patients with high epidemiological risk
for cancer to avoid the risk of missing cancer at an early stage.
This is because PET findings may be often negative in
case of BAC and typical carcinoid tumours; although these are
low-grade carcinomas, there is ample evidence that such tumours do progress, metastasize and are eventually lethal if
not resected at an early stage.

Study limitations

There are several limitations in this study. First, in our study
collection the prevalence of malignant lesions is higher than
that of benign lesions (66 versus 34%, respectively). The preva-
ience of malignant lesions might be related to selection bias
because our study population includes patients with lung mass
and not only with coin lesion. In addition, all patients are
referred from a thoracic surgery unit with prevalence of cancer
stage. This is because our population does not include only coin lesions;
therefore, in the future the clinical utility of association between
PET examination and MMP9 expression should be ongoing in a
study group with small nodules to diagnose cancer at an early stage.

Third, PET and MMP9 results are not stratified according to
lesion size, which is a limitation for PET evaluation.

CONCLUSION

Our preliminary data show that the measurement of MMP9
levels in serum and in BAL significantly helps in the differenti-
ation of cancer as compared to masses that are not cancerous.
The measurement of MMP9 levels in serum is correlated with
the tumour stage but not with the different histological types. In
addition, its use in combination with other biomarkers and PET
study would add further information in the diagnosis of lung
lesions to select patients who may or may not benefit from ad-
ditional invasive procedures. However, further studies with a
larger number of patients are required in the future to explore
the clinical diagnostic role of MMP9 as a marker of lung
malignancy.

Conflict of interest: none declared.

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