The use of biomarkers in the diagnosis of lung cancer

L'utilisation de biomarqueurs diagnostiques dans le cancer du poumon

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Abstract

Using a simple 10 to 20 milliliters of blood sample in order to make the diagnosis of lung cancer is the dream of every patient and practitioner. In fact, even if tissue samples or bronchial liquid represent the gold standard for microscopic diagnosis, using less invasive procedures represented the aim of many researches published in the literature. The utility of biomarkers has been widely reported in screening context, mainly in association to low dose CT-scan, or in therapeutic context in order to highlight therapeutic targets or to change treatment in a context of resistance to target therapies. The use of biomarkers in a diagnostic context has been recently highlighted in the literature. The authors aimed to present a general review of different biomarkers that could be used in the diagnosis of lung cancer.

Key-words: lung cancer, biomarker, diagnosis

Résumé

Faire le diagnostic de cancer du poumon en utilisant un simple prélèvement de 10 à 20 ml de sang est le rêve de la plupart des patients et des praticiens. En effet, même si le prélèvement tissulaire et le liquide bronchique représentent le gold standard pour le diagnostic microscopique du cancer du poumon, l'utilisation de procédures moins invasives a représenté l'objectif de nombreux travaux de recherche publiés dans la littérature. L'utilisation de biomarqueurs a été largement discutée dans un contexte de dépistage, essentiellement en association avec la TDM thoracique à faibles doses, ou dans un contexte thérapeutique à la recherche de cibles thérapeutiques ou afin de modifier le traitement dans un contexte de résistance aux thérapies ciblées. L'utilisation de biomarqueurs dans un contexte de diagnostic a connu un regain d'intérêt récent dans la littérature. Les auteurs avaient pour objectif de faire une revue générale de la littérature concernant les différents biomarqueurs qui peuvent être utilisés dans le cadre du diagnostic de cancer du poumon.

Mots-clés: Cancer du poumon, biomarqueur, diagnostic

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INTRODUCTION

The diagnosis of lung cancer is based on microscopic findings. The latest World Health Organization classification has been published in 2021 and added mild modifications to the 2015 World Health Organization classification of lung tumours. The diagnosis is commonly made on biopsies, surgical specimen and bronchial liquid. Since a decade, biopsies have been considered as evils and risky procedures and many researches were performed in order to use noninvasive procedures in order to make a diagnosis of lung cancer. In this context, biomarkers represented a possible surrogate to tissue in order to perform the diagnosis [1–3]. Our aim was to present a general review of the current literature concerning the utility of biomarkers in the diagnosis of lung cancer.

WHAT IS THE DEFINITION OF BIOMARKERS?

A biomarker is a physical, biological or molecular state defining a new entity, a prognostic group or predicting a response to a treatment. A good biomarker implies an improvement in the take care process [2,4]

WHAT ARE THE BIOMARKERS SOURCES TO USE IN THE DIAGNOSIS OF LUNG CANCER?

Many biomarkers sources can be used in the diagnosis of lung cancer including bronchial liquid, sputum, urine, pleural or peritoneal fluid or blood. Liquid biopsy has changed the management of lung cancer and represented an alternative to tissue in order to highlight therapeutic targets [1]. It also gained a place in the diagnostic field of lung cancer.

WHAT ARE THE MAJOR BIOMARKERS ASSESSED FOR THE DIAGNOSIS OF LUNG CANCER?

The major diagnostic biomarkers of lung cancer are represented by:

- *Auto-antibodies* : they are defined as antibodies produced against abnormal tumor cell antigens. They are present in little amounts and are assessed using common laboratory techniques (ELISA). Many false negative cases are reported [4,5]

- *Circulating tumor cells*: circulating tumor cells are rare. They are always surrounded by 106 to 107 leucocytes. Their presence and amount are independent from the tumor stage or the microscopic subtype [6–9].

Devriese, et al. reported the presence of circulating tumor cells in 21 cases among 46 stage 4 non-small cell lung carcinomas and in 3 cases from 46 healthy patients [10]. Besides, Hofman P, et al. reported the presence of circulating tumor cells in 49% of the cases in stage I, 48% in stage II and 48% in stage III and 52% in stage IV tumors. The presence of circulating tumor cells is also independent from the histologic subtype. Hofman P, et al. reported circulating tumor cells in 47% of adenocarcinomas and in 40% of squamous cells carcinomas [9]. The major methods used in the detection of circulating tumor cells are represented by indirect methods and direct methods. Indirect methods are based on the biological properties of the tumor cells: the antigens expressed, the protein produced or the invasion potential. The direct methods are based on the physical properties of the tumor cells, their deformability or the density of their electrical changes. Many techniques have been reported in every method but the most reported ones consisted in CellSearch among the indirect methods and the ISET technique among the direct methods [1,7,11].

- *The exosomes* : They are vesicles measuring 40 to 100 nm. They are present in many biological liquids consisting of plasma, serum, sputum, bile, bronchoalveolar lavage (BAL) or amniotic liquid. They play a key role in the angiogenesis, vascular permeability, epithelial-mesenchymal transition and resistance to chemotherapy. The assessment of exosomes is based on extraction techniques which consist of precipitation, ultra-centrifugation, chip-microfluidics and detection techniques in order to assess their composition. The detection techniques consist of real-time PCR, ELISA, flow cytometry or western blot techniques. The major challenges of diagnosis are represented by the fact that the extracted exosomes aren't necessarily from tumor cells [12].

- *Micro-RNA* : They are tissue-specific and cellular type-specific. They have a role in the post-transcriptional regulation and are implicated in the carcinogenesis, the cell apoptosis or some viral infections. In addition to the messenger RNA, many non coding RNA molecules have been assessed including ribosomal RNA, transfer RNA, small nuclear RNA, small nucleolar RNA and other RNA including large RNA. Micro-RNA have been reported to play

a key role in the diagnosis of lung cancer [10, 11].

- **Proteomics** : The analysis of protein has represented an alternative to the DNA or RNA analysis because a same genome can produce different proteomes.

- **Metabolomics** : The analysis of different metabolites has been reported in the diagnosis of lung cancer. It consists in the assessment of different metabolites present in urine or sputum [11].

WHAT ARE THE ABNORMALITIES DETECTED IN ORDER TO DIAGNOSE LUNG CANCER?

The abnormalities assessed to diagnose lung cancer are those taking place during the carcinogenesis. Early, in the carcinogenesis process, loss of heterozygotie and instability of microsatellites followed by variation in the DNA methylation take place. The major abnormalities detected consist of the instability of microsatellites, DNA hyper methylation, microRNA expression and mutations in some genes including P53, KRAS and EGFR [2,4,5,12,13].

WHEN DO WE NEED TO USE DIAGNOSTIC BIOMARKERS IN LUNG CANCER?

After a review of the literature, using diagnostic biomarkers is needed in three major circumstances consisting of the early diagnosis and the assessment of indeterminate nodules diagnosed by the low dose CT-scan, early diagnosis of recurrences and the diagnosis of multiple pulmonary nodules.

- Early diagnosis and the assessment of indeterminate nodules diagnosed by the low dose CT-scan

Many biomarkers could be used for the early diagnosis and assessment of indeterminate nodules consisting of circulating tumor cells, circulating tumoral DNA, microRNA, auto-antibodies, proteomics and metabolomics.

- Circulating tumor cells are helpful in the early diagnosis of lung cancer. Hofman P, et al. reported the case of a 39-year-old patient with a past medical history particular for chronic broncho-pneumonitis who presented a normal CT-scan. Circulating tumor cells were observed in liquid biopsy. The patient was followed regularly and presented a pulmonary mass after 2 years of follow-up. The stage I tumor was resected surgically [14].

- Circulating tumoral DNA has no interest in the early diagnosis of lung cancer because they appear in late stages and necessitate techniques with a changed cutoff from 0.1 to 0.01% in order to introduce them in the early diagnosis [15].

- Epigenetics abnormalities have been reported by many authors. Ooki, et al. reported that the hypermethylation of 6 genes (SOX17, HOXA9, AJAP1, PTDGR, UNCX, MARCH11) reached a sensitivity of 96.7% and a specificity of 60% in the diagnosis of lung cancer [16]. Wielscher M, et al. reported a study about the methylation of 64 genes in the serum. This study included 204 patients counting 33 cancers, 68 interstitial pneumonias, 42 obstructive chronic pneumonitis, 61 healthy patients [17]. The study of the methylation reached a sensitivity of 88%, a specificity of 90% when comparing tumoral to healthy cases and a specificity of 88% when comparing chronic broncho-pneumonitis to interstitial pneumonias .

- Other authors reported that the gene methylation was variable according to the microscopic subtype. CDKN2A and MGMT genes were reported to be hypomethylated in adenocarcinomas and CDH13, RUNX3 and APC genes were reported to be hypermethylated in adenocarcinomas [18]

In another retrospective study reported by Zhou et al, the authors included 431 patients who presented stage I tumors with nodules <2 cm, patients with in situ carcinoma and healthy patients. The authors studied 4 regulators genes including GNAS, GRB10, SNRN and HM13. They reported a specificity and a sensitivity reaching respectively 92% and 95% in in-situ carcinomas, 91% and 100% in nodules measuring less than 2 cm [19]. These results seemed promising but the authors used different gold standards (biopsies in some patients, cytologies in others and surgical specimen in some patients). Besides, the study was retrospective and the authors followed the patients for 2 years.

- Different micro-RNA have also been reported in the literature. miR205 assessment in sputum has been reported to reach a sensitivity of 65% and a specificity of 90% [20]. Zhou, et al reported that the use of a microRNA panel (miR-17, miR-190, mi-R19a, mi-R19b, mi-R26b et miR-375) allowed to reach a sensitivity of 81% and a specificity of 80% [19]. Zheng et al reported that the use of micro RNA (miR-155, miR-197, mi-R182) reached a sensitivity of 81.33% and a specificity of 86,76%. Hennessey, et al. performed their research on mi-R15b, miR-27b and reached a sensitivity of 100% and a specificity of 84% [21]. Multicentric trials

(AEGIS-1 et AEGIS-2) were performed in order to compare the diagnostic potential of the bronchoscopy alone or in association to a classifier, in the early diagnosis of lung cancer. The authors used a brushing technique of normal epithelial cells during bronchoscopy and studied the expression of RNA using microarray. The prevalence of cancer in both cohorts reached 74 and 78%. Bronchoscopy wasn't diagnostic in 272/639 patients. The sensitivity and the specificity reached respectively 88 and 47% when using the classifier alone, 74% and 76% when using the bronchoscopy alone and 96% and 98% when using the bronchoscopy in association to the classifier [22].

Exosomal micro-RNA has also been assessed. Wu J, et al. reported that miR-181-5p, 30a-3p, 30e-3p, 361-50 were significantly present in adenocarcinomas and miR10b-5p, 15b-5p, 320b were significantly present in squamous cell carcinomas . They also reported an area under curve (AUC) reaching 0.89, 0.93 and 0.91 in respectively non small cell carcinomas, adenocarcinomas and squamous cell carcinomas [23]. Some authors reported that the quantity of exosomal miR was correlated to the tumor stage and was increased in patients with lung cancer in comparison to those that were healthy. Kim et al, reported elevated miR-126 and let-7a (in plasma and BAL) in adenocarcinomas [24]. Even if all these results seem promising, the gold standard wasn't consensual. It consisted in granulomatous lesions in some studies or normal tissues in others..

Yang Q, et al. performed a meta-analysis about the diagnostic and prognostic values of circular RNAs in lung cancer diagnosis. They included published studies from 2017 to 2020. All the studies included were Chinese. The gold standard used was the diagnosis made in tissue samples. The authors included case-control studies that were assessed using the QUADAS-2. Forty-five articles including 17 studies dealing with the diagnostic potential of circRNA were included. The authors reported a pooled sensitivity of 77%, a pooled specificity of 75% and an AUC of 0.83 [18]. Multiple other micro-RNAs have been reported in small-cell cancers with variable sensitivities and specificities.

- The utility of autoantibodies has been assessed in early diagnosis. Boyle P, et al. assessed a panel of antibodies and reported a specificity and a sensitivity reaching respectively 93% and 40% [25]. Wheelock E, et al. reported in a retrospective study about 1699 lung cancers with 61 stage I tumors, a sensitivity and a specificity reaching respectively 37% and 93% [26] .

- Proteomics have also been reported in early diagnosis of lung cancer. Many tests have been reported assessing protein panel in the blood. Nodify XL2 (Biodesix) is a test based on a 13-protein-proteomic classifier. The authors reported, in patients with a lung cancer incidence reaching 20%, a positive predictive value reaching 90% in benign lesions and in nodules ranging between 4 and 20 mm [5] .

Xpresys Lung was reported in the PANOPTIC study including 685 patients with a subgroup of patients presenting 6-30 mm nodules with a cancer probability inferior to 50%. The authors reported respectively a sensitivity, specificity, negative predictive value and likelihood ratio accounting for respectively 97%, 44%, 98% and 0.07. The authors reported the utility of the classifier in low-risk nodules [27]. Other biomarkers have also been reported in early diagnosis of lung cancer including the hypermethylation of p16 gene in sputum, the P53 mutations in squamous cell carcinomas, KRAS mutations in BAL in adenocarcinomas.

Metabolomics have also been used including the assessment of volatile organic compounds (VOC) in urine reaching respectively a sensitivity and a specificity ranging respectively between 36 to 95% and 60 to 97.6% [28] . The assessment of 6 metabolites in liquid biopsy has also been reported to reach an AUC of 0.97. The assessment of aspartic acid, pyruvic acid and sphingosine in urine have been reported to reach a sensitivity and a specificity ranging from respectively 77% to 93% and 90% to 97% [29].

- Early diagnosis of recurrences

In early diagnosis of recurrences, the monitoring of circulating tumor cells and the assessment of therapeutic targets have been reported to be efficient by sone authors [30].

- The diagnosis of multiple pulmonary nodules

The differentiation between multiple primaries and intrapulmonary metastases is easy when the nodules present different histologic subtypes. It can be challenging when all the nodules have the same subtype. Some authors reported the utility of assessing the clonality of P53 gene, the LOH of 3p, 5q, 9p, 11p, 13q, 17p ou 18 q chromosomes, the mutations of RAS gene, the assessment of a microsatellite instability or the assessment of EGFR mutations. In a meta-analysis, comparing the differentiation between multiple primaries and intrapulmonary metastases based on morphologic features, the authors reported a pooled specificity of 49% and a pooled sensitivity of 65% with an AUC reaching 0.62 [31]

CONCLUSION

Numerous research studies concerning diagnostic biomarkers in lung cancer have been published in the literature consisting mainly of retrospective observational studies with many methodological concerns. An integrative approach associating molecular biology, artificial intelligence is necessary in order to reach consensus. A better understanding of carcinogenesis is necessary in order to reach a consensus concerning the different biomarkers to assess, the techniques to use with respect to ethic principles and taking into account the cost of these techniques especially in low-income countries.

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