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DETECTION PHENOTYPIQUE ET MOLECULAIRE DES COLONISATIONS BRONCHIQUES PERIOPERATOIRES EN CHIRURGIE THORACIQUE ONCOLOGIQUE

Jury

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AVANT PROPOS :

Le format de présentation de cette thèse correspond à une recommandation de la spécialité Maladies Infectieuses et Microbiologie, à l'intérieur du Master des Sciences de la Vie et de la Santé qui dépend de l'Ecole Doctorale des Sciences de la Vie de Marseille.

Le candidat est amené à respecter des règles qui lui sont imposées et qui comportent un format de thèse utilisé dans le Nord de l'Europe et qui permet un meilleur rangement que les thèses traditionnelles. Par ailleurs, la partie introduction et bibliographie est remplacée par une revue envoyée dans un journal afin de permettre une évaluation extérieure de la qualité de la revue et de permettre à l'étudiant de commencer le plus tôt possible une bibliographie exhaustive sur le domaine de cette thèse.

Par ailleurs, la thèse est présentée sur article publié, accepté ou soumis associé d'un bref commentaire donnant le sens général du travail. Cette forme de présentation a paru plus en adéquation avec les exigences de la compétition internationale et permet de se concentrer sur des travaux qui bénéficieront d'une diffusion internationale.

Professeur Didier RAOULT

« Une main habile sans la tête qui la dirige est un instrument aveugle ».

Claude Bernard

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Aux membres du Jury

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A mes parents

A Sophie

A mes deux enfants, Camille et à Etienne

A ma famille

A mes amis

Table des Matières

RESUME

Les complications respiratoires restent la première cause des complications postopératoires en chirurgie thoracique oncologique. Le développement des ces complications sont le plus souvent de nature infectieuse. Leur fréquence reste élevée (30 %) et représente la première cause de mortalité hospitalière. Des données récentes suggèrent que ces complications respiratoires soient liées à une colonisation périopératoire des voies aériennes. Plusieurs travaux fondés sur l'analyse phénotypique de mise en culture traditionnelle démontrent l'existence d'une colonisation bronchique proximale chez près de 40 % des malades. Néanmoins, les liens entre colonisation et complications respiratoires restent controversés. Une des principales limites demeure les méthodes de cultures employées qui ne permettent l'identification que d'une faible partie (< 1%) des espèces microbiologiques potentiellement existantes dans la biosphère. Nous avons formulé l'hypothèse que des techniques de biologie moléculaire d'amplification universelle des ADN présents dans les échantillons suivies du clonage des produits de PCR et du séquençage de ces clones, appliquées à des échantillons obtenus des bronches distales et de biopsies pulmonaires, permettraient l'identification de pathogènes bactériens, viraux ou émergents. Nos résultats suggèrent que l'identification précise et exhaustive de ces colonisations ne peut être réalisée que par une approche moléculaire moderne, innovante et systématique. Cette approche permet d'envisager, d'une part, un lien plus précis entre colonisation et complications respiratoires et d'autre part, l'identification de pathogènes difficilement cultivables ou émergents.

Mots-clés : Cancer du poumon, Cancer de l'œsophage, Pneumopathie nosocomiale, Syndrome de détresse respiratoire aigue, Colonisations bronchiques, Complications respiratoires, Biologie moléculaire

ABSTRACT

Phenotypic and molecular detection of perioperative airways colonizations in patients submitted for thoracic oncologic surgery

Postoperative respiratory complications remain the most frequent and serious complications, as well as being the primary cause of hospital death after thoracic oncologic surgery. Their incidence is relatively high and concern near 30 % of patients submitted for surgery. These complications are notoriously infectious and airways colonizations (AWC) have been suggested to be an essential first step in the pathogenesis of this respiratory morbidity. Previous studies have documented that AWC are presents in near 40 % of cases. However, correlation between AWC and respiratory complications remains controversial. One of the limits is the traditional phenotypic methods of cultures that precludes for definitive conclusions when considering that majority of microbiological species required modern and innovating techniques of culture to be identified. Recent data have demonstrated that 99% of organisms seen microscopically are not cultivated by routine techniques and required molecular techniques to be identified. We have postulated that instead of culture test, molecular detection (DNA genes amplification and sequencing of the bacterial 16S ribosomal RNA) applied to distal bronchial samples or to lung biopsies, should allow identifying bacteria, virus or emerging pathogens. Our results suggest that molecular culture-independent techniques applied in the context of AWC will provide in the future a great opportunity to precise correlation between colonization and respiratory complications and to the other hand, to discover new and/or emerging pathogens that are currently unknown.

Key-words : Lung cancer, Esophageal cancer, Nosocomial pneumonia, Acute respiratory distress syndrome, Airways colonization, Respiratory complications, Molecular biology.

Objectifs de l'étude

La chirurgie thoracique oncologique s'adresse pour l'essentiel aux patients présentant un cancer du poumon et de l'œsophage. Le traitement chirurgical reste le traitement de référence des stades précoces de ces tumeurs lorsqu'elles sont jugées résécables. Bien que différentes du point de vue technique, ces deux pathologies, ont en commun une même approche chirurgicale par voie thoracique. Il en découle une similitude dans la gestion du risque opératoire.

Avec les progrès de l'anesthésie-réanimation et la standardisation des techniques chirurgicales, la mortalité opératoire de ce type chirurgie a été profondément modifiée depuis ces dernières décennies. Les progrès dans l'évaluation de la maladie, la précocité du diagnostic dans les populations à risque et dans une prise en charge thérapeutique plus agressive ont permis de prolonger l'espérance de vie de ces patients. La mortalité opératoire actuelle dans des centres de haut volume se situe entre 4 et 10 % pour ces deux types de chirurgie, dépendant étroitement des co-morbidités et du type d'exérèse *(Bernard et al, 2001) (Birkmeyer et al, 2002)*.

Parmi les complications postopératoires, les complications respiratoires arrivent au premier rang des complications et représentent la première cause de mortalité hospitalière de la chirurgie thoracique oncologique *(Brunelli et al, 2008)(Doty et al, 2002)*. De nombreuses études se sont attachées à comprendre leurs mécanismes physiopathologiques *(Ra et al, 2008) (Ferguson et al, 2002) (Kozower et al, 2010)*. Cependant il existe un important problème de définition et l'analyse de leur incidence par comparaison aux études existantes est difficile. Ces complications respiratoires sont multifactorielles. Il existe des facteurs préopératoires liés aux patients, à leurs co-morbidités, à leurs fonctions respiratoires et aux antécédents de tabagisme. Il existe des facteurs peropératoires liés à l'acte chirurgical et à la gestion de l'anesthésie. Enfin, il existe des facteurs postopératoires dépendant de la prise en charge en réanimation, la réhabilitation par kinésithérapie, la prise en charge de la douleur et l'expérience de chaque centre *(D'Journo et al, 2008)*. La fréquence de ces complications reste élevée et stable depuis de nombreuses années. Elle est estimée à près de 30 % des patients opérés. La mortalité liée à ce type de complications varie et peut atteindre près de 50 % dans sa forme la plus grave : le syndrome de détresse respiratoire aigue (SDRA) *(Doty et al, 2002)(Alam et al, 2007).*

Ces complications respiratoires sont le plus souvent de nature infectieuse et doivent être considérées comme nosocomiales. Des données récentes suggèrent que ces

complications respiratoires soient liées à une colonisation des voies aériennes pendant la période périopératoire *(Belda et al, 2005) (Cabello et al, 1997) (Ioanas et al, 2002) (Schlusser et al, 2006 et 2008)*. La colonisation bronchique est définie par l'identification de germes au niveau des voies aériennes supérieures, que l'on peut cultiver par des méthodes classiques de mise en culture chez des patients asymptomatiques. Cet état résulte d'un équilibre entre les défenses de la muqueuse de l'arbre respiratoire en limitant et non en éliminant le micro-organisme responsable. L'infection pulmonaire serait le résultat d'une baisse des défenses cellulaires et humorales de l'hôte, dans des situations spécifiques, où un inoculum bactrien de quantité et de virulence suffisante pourrait entrainer le point de départ du processus infectieux *(Torres et al, 2006)*. En chirurgie pulmonaire, des études préliminaires évaluent la fréquence de ces colonisations entre 20 et 41 % des malades proposés à une chirurgie d'exérèse. Mais le lien entre colonisation et complication reste controversé. Aucune donnée n'est disponible pour les patients proposés pour une chirurgie œsophagienne.

Les conclusions de ces travaux restent limitées. En effet, les études publiées à ce jour n'ont été fondées que sur des méthodes d'identification microbiologique phénotypique, c'est-à-dire reposant sur l'analyse traditionnelle classique de mise en culture. Or on estime que ces analyses phénotypiques ne permettent d'identifier que seulement 1 % des espèces microbiologiques potentiellement existantes dans la biosphère *(Pace, 1997)*. Par ailleurs, 99 % des espèces bactériennes connues sont difficilement identifiables par des techniques de mise en culture traditionnelle. Cette limitation dans l'identification des souches incriminées explique en partie que le lien clinique entre colonisation bronchique et survenue d'une complication respiratoire demeure incertain à ce jour. Les limitations inhérentes aux différentes techniques d'identification microbiologique suggèrent que l'analyse précise et exhaustive des colonisations des voies respiratoires ne peut être réalisée que par une approche systématique, en particulier l'amplification universelle des ADN présents dans les échantillons suivie du clonage des produits de PCR et du séquençage de ces clones. Cette approche a été rarement utilisée dans la littérature et seulement sur un nombre limité d'échantillons. Ces méthodes d'analyse moléculaire n'ont jamais été appliquées à l'identification de colonisations bronchiques dans le cadre de la cadre de la chirurgie thoracique oncologique.

Le but de cette thèse est de caractériser par des méthodes phénotypiques et moléculaires, les colonisations bronchiques périopératoires en chirurgie thoracique oncologique.

- Dans une première partie, nous dresserons un bilan de l'information existante sur les colonisations bronchiques par une revue générale de la littérature consacrée à la chirurgie oncologique pulmonaire. Nous synthétiserons les données existantes dans une méta-analyse combinant les résultats d'une série d'études indépendantes et prospectives sur un même problème donné. En révisant l'ensemble des données publiées à ce jour et en augmentant le nombre de cas étudiés, notre objectif sera de tirer une conclusion globale sur un lien clinique et statistique potentiel entre colonisations bronchiques et complications respiratoires.

- Dans une deuxième partie, nous étudierons, par des méthodes phénotypiques et sérologiques, la fréquence des colonisations bronchiques bactériennes et virales chez des patients opérés d'un cancer de l'œsophage. Il n'existe à ce jour aucune donnée disponible dans la littérature.

- Dans une troisième partie, compte tenu des limites représentées par les méthodes phénotypiques, nous avons formulé l'hypothèse que des techniques de biologie moléculaire d'amplification universelle des ADN présents dans les échantillons suivies du clonage des produits de PCR et du séquençage de ces clones, appliquées à des échantillons obtenus des bronches distales et de biopsies pulmonaires de patients opérés d'un cancer du poumon, permettraient l'identification de pathogènes bactériens, viraux ou émergents. Cette approche permettrait d'envisager un lien plus précis entre colonisations bronchiques et complications respiratoires, et permettrait l'identification de pathogènes difficilement cultivables ou émergents.

Résultats

Article 1

II.1. Article 1

Airways colonizations in patients undergoing lung cancer surgery

II.2.1 Introduction

Dans cette première partie, nous avons effectué une revue exhaustive de la littérature concernant l'information existante sur les colonisations bronchiques en chirurgie d'exérèse pulmonaire pour cancer du poumon.

Le cancer du poumon représente la première cause de décès par cancer dans le monde quelque soit le sexe *(Jemal et al, 2004)*. Le traitement curatif repose pour l'essentiel sur la chirurgie dans les formes localisées de la maladie. Avec les progrès réalisés en chirurgie et en anesthésie, le nombre de patients proposés pour une chirurgie va vraisemblablement augmenter en élargissant les indications aux patients présentant un risque opératoire potentiel considéré comme « risque intermédiaire ». Malgré l'amélioration des techniques opératoires et de la prise en charge postopératoire, la chirurgie continue d'exposer le patient à un risque potentiel de complications et de décès. La mortalité de la chirurgie pulmonaire dépend du type d'exérèse : elle est estimée entre 0 et 4 % après segmentectomie, entre 0 et 10 % après lobectomie et entre 3 et 21 % après pneumonectomie *(Bernard et al, 2001) (Doddoli et al, 2005) (Birkmeyer et al, 2002)*. Parmi les complications postopératoires, les complications respiratoires représentent la première cause de mortalité hospitalière dans ce type de chirurgie. La mortalité varie de 22 % et 75 % dans les formes les plus graves *(Alam et al, 2007)*. Les raisons de ces complications sont multiples et complexes *(Stephan et al, 2000)*. Il existe un certain nombre de données cliniques indiquant que ces complications respiratoires ne forment qu'un même continuum d'une même pathologie mais s'exprimant selon des degrés variables de sévérité. La part commune demeure à l'évidence infectieuse *(Watanabe et al, 2004) (Wada et al, 1998)*. Néanmoins, la documentation microbiologique postopératoire reste difficile avec un nombre critique de pneumopathies ou de SDRA qui demeurent non documentés. Dans la mesure où la documentation postopératoire est un élément déterminant dans l'élaboration d'un modèle physiopathologique compréhensible de ces détresses respiratoires, un effort certain doit être envisagé. Cette documentation requiert un haut niveau d'expertise en maintenant un haut niveau de suspicion infectieuse incitant à la pratique de méthodes invasives telle que la fibroscopie bronchique pour la documentation microbiologique (Schlusser et al, 2006).

Plusieurs études ont posé l'hypothèse que ces complications respiratoires avaient pour origine probable une colonisation bronchique préalable *(Belda et al, 2005) (Cabello et al, 1997)(Ioanas et al, 2002) (Schlusser et al, 2006 et 2008)*. Les patients proposés pour ce type de chirurgie cumulent en effet les facteurs de risque reconnus de cette colonisation. Ils sont souvent fumeurs actifs ou sevrés, présentent une bronchite chronique ou un emphysème et sont porteurs d'un cancer du poumon avec ces conséquences locales et générales sur l'état immunologique et l'obstruction bronchique.

Plusieurs études ont évalués cette colonisation en chirurgie d'exérèse pulmonaire *(Yamada et al, 2010), (Wansbrough-Jones et al, 1991), (Belda et al, 2005), (Cabello et al, 1997), (Ioanas et al, 2002), (Schlusser et al, 2006 et 2008)*. Néanmoins, la grande variation dans les méthodes de documentation, d'analyses microbiologiques et dans l'interprétation des résultats limitent toutes possibilités d'obtenir une information homogène et de qualité. Cette colonisation bronchique est supposée être un élément prépondérant dans les mécanismes des pneumopathies postopératoires, où toutes les conditions sont réunies pour favoriser l'infection : abolition de la toux, douleur, réactions inflammatoires, micro-inhalations. Néanmoins, même si une colonisation préopératoire peut être mise en évidence, son lien avec le développement de complications postopératoires est incertain. Le niveau de preuve dans la littérature qui en résulte reste faible.

Le but de cette première étude était de faire une revue exhaustive des données existantes sur les colonisations bronchiques en chirurgie d'exérèse pulmonaire pour cancer. L'objectif était d'analyser les méthodes de prélèvements, les méthodes d'identification microbiologiques et les facteurs de risque reconnus de la colonisation bronchique. Pour approfondir notre étude, nous avons synthétisé les données existantes dans une méta-analyse combinant les résultats d'une série d'études indépendantes et prospectives sur un même problème donné. En révisant l'ensemble des données publiées à ce jour et en augmentant le nombre de cas étudiés, notre objectif était de tirer une conclusion globale sur un lien clinique et statistique potentiel entre colonisation et complications respiratoires.

II.1.2 Article

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Airways colonizations in patients undergoing lung cancer surgery

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Abstract

Lung cancer remains the main leading cancer-related cause of death in the world. For early stage tumor, surgery stands out as the best curative option offering the greatest change for cure. Despite improvement of per and postoperative management, surgery continues to carry out a high morbidity with a significant mortality. Among postoperative complications, respiratory failures (nosocomial pneumonia, acute respiratory distress syndrome) are currently the most frequent and serious, as well as being the primary cause of hospital death, after a lung resection for cancer. Because infectious etiologies have been highly incriminated in the development of these pulmonary complications, microbial airways colonizations (AWC) are supposed to be an essential first step in the pathogenesis of these failures occurring in hospitalized and chronically ill individuals. These patients fulfill all the predisposing factors to bronchial colonizations and are particularly exposed to development of respiratory failures in the postoperative setting, when secretion clearance and cough reflex are impaired. Under immunosuppressive condition, AWC should act in a manner that increases its ability to stimulate micro-organisms and increase the risks of superimposed infections. Few studies have addressed the problem of the AWC in patients submitted for lung cancer surgery. Because of several limitations, especially the lack of exhaustive microbiological studies, the conclusions that can be reached remain inconclusive. This review aims to report the existing literature on this critical and controversial point, focusing on their specific incidence, their predisposing factors, their correlation with development of respiratory failures and in turn, the reliability of the current antibiotic prophylaxis for their prevention.

INTRODUCTION

Lung cancer is the main leading cancer-related cause of death worldwide in both genders [1-3]. To date, current curative strategies are based mainly on surgery which offers the greatest chances of cure for patients with an early stage disease. With the advances made in anesthesia and surgery, the number of patients submitted to surgery is expected to increase continuously, including in marginally operable candidates.

Despite improvements in the per and postoperative care, surgery continues to carry out a high morbidity and a substantial mortality. Surgery-related mortality ranges between 0 and 4 % after segmentectomy, 0 and 10 % after lobectomy and 3 to 21 % after pneumonectomy [4-8]. Among postoperative complications, respiratory failures remain the most frequent and serious, being the primary cause of in-hospital death in 22 to 75% of the cases [4]. These facts have stimulated continuous efforts in the prevention, diagnosis and treatment of postoperative pulmonary complications.

Postoperative respiratory complications are a heterogeneous group of diseases with various causes and pathogenic mechanisms. Reasons for this pulmonary morbidity are multifaceted, and those due specifically to surgery are probably very difficult to segregate from those due to the perioperative anesthetic management, to postoperative events and to the patient himself. However, there is growing evidence that these different forms of respiratory failures should have a common substratum, notoriously infectious, with similar clinical presentations [4, 5, 9]. The clinical frame includes tracheobronchitis, pneumonia and acute respiratory distress syndrome (ARDS). Pneumonia is considered as the main concern of a dramatic continuum leading to ARDS and represents near one third of all care-related infections. The incidence of pneumonia after a lung resection is estimated between 2-20% with a related-mortality between 15–75% [4]. Incidence of these respiratory failures has poorly varied since the last decade and their severity remains invariably stable over time. A vast majority of these respiratory failures are mostly bacterial but a part of them is frequently undetermined with inconclusive postoperative microbiological results [6, 10].

Because infectious etiologies have been highly incriminated in the occurrence of these respiratory failures, airways colonizations (AWC) are supposed to be an essential first step in the pathogenesis of nosocomial pneumonia and ARDS occurring in hospitalized and chronically ill individuals fulfilling all predisposing factors to bronchial colonization [10-14]. Bronchial colonization would be the result of an equilibrium in which the host's defenses succeed in limiting, but not eradicating, the microorganisms adhering to the bronchial epithelium. Lung infection would be the result of a lowering of the host's cellular and humoral defenses at a specific time or of the presence of an inoculum in a sufficient quantity or of a sufficient virulence to produce it [14-19]. AWC is supposed to facilitate the development of pneumonia in the postoperative setting, when secretion clearance and cough reflex are impaired. Under immunosuppressive condition, such as surgery, colonizations of the respiratory mucosal surface act in a manner that increases its ability to bind micro-organisms and increase the risks of superimposed infections.

Few studies have addressed the problem of the AWC in patients submitted to lung cancer surgery [10-14]. Because of several limitations, conclusions that can be reached remain inconclusive. This review aims to report the existing literature on this critical and controversial point, focusing on their specific incidence, their predisposing factors, their correlation with development of respiratory failures and in turn, the reliability of the current antibiotic prophylaxis for their prevention.

MATERIALS AND METHODS

English-language reports of published studies on airways colonization in lung cancer surgery by cross-referencing the following medical subject headings (MeSH) keywords and text words: airways colonizations, lung cancer, respiratory complications, nosocomial pneumonia, thoracic surgery, ARDS, bronchoscopy, antibioprophylaxis. Databases searched included PubMed, EMBASE and Cochrane Database of Systematic Reviews. Bibliographies of original articles were manually reviewed for additional articles. Non-English language

reports were also identified in PubMed using the same keywords in order to supplement our search.

We conducted a meta-analysis which evaluates the published literature in a qualitative and quantitative way by comparing and integrating the results of different studies. The main goal of the meta-analysis was to assess the correlation between preoperative AWC and occurrence of postoperative respiratory complications. Studies included in the meta-analysis had to assess AWC in patients submitted for lung surgery and had to provide related-numbers of respiratory complications in both colonized and non-colonized group. Meta-analysis was carried out using odds ratio (OR) and confidence interval (CI) 95 %. The OR represents the odds of a positive association between AWC and postoperative respiratory complications in the colonized group in comparison to the non-colonized group. An OR of up to 1 if the 95% CI does not include the value 1 or 0 was considered as statistically significant at the $p < 0.05$ level. In our study both fixed and random effect models were employed. The fixed effect model is based on the assumption that the AWC in each study is constant, whereas the random effect model is based on the assumption that there is variation between studies. Thus, the ratios calculated in the random effect model are more conservative than those calculated in the fixed effect model. In a meta-analysis of surgical research, the random effect model is preferable due to a number of sources of heterogeneity including various risk profiles, selection criteria for each surgical technique, study design, study start date, and duration of follow-up. Heterogeneity between studies was investigated by the standard chi-squared Q-test. Analysis was performed by SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL, USA), Microsoft Excel 2002 (Microsoft Corporation, USA), and Medcalc 11.3 (MedCalc Software, Mariakerke, Belgium).

INCIDENCE OF AIRWAYS COLONIZATIONS (AWC)

Colonization is classically defined by isolation of micro-organisms into airways with positive cultures in asymptomatic patients. Patients are considered to have colonization whenever a microorganism is isolated above specific thresholds: $\geq 10^2$ cfu/mL when

investigated by protected specimen brush (PSB) or ≥ 10^4 cfu/mL for bronchial aspirates [11, 12].

In order to provide an evidence-based statement on their clinical relevance in the setting of lung cancer surgery, it is obviously necessary to describe the incidence of these colonizations in different clinical situations.

In healthy non-smokers patients

Healthy non-smokers are generally considered as free from bacterial colonization of the lower airways [20, 21]. Nevertheless, there is very little information dealing with this issue. Cabello et al. have found that only 1 of 10 healthy and asymptomatic subjects had a true respiratory pathogen isolated from his lower airways [11]. Using PSB and bronchoalveolar lavage (BAL) to study the distal airways of eight healthy individuals, Kirkpatrick have found that only 1 BAL specimen yielded 1 potential pathologic micro-organism (PPMs) [22]. These studies have underlined the efficacy of lung defenses in healthy non smoker's individuals in maintaining the near-sterility of the lower airways.

In smokers and in COPD patients

In healthy smokers, bacterial colonization is frequent between 29 to 33 % [15,23]. Qvarfordt has demonstrated that 29 % of asymptomatic smokers were colonized and this was comparable to the fraction of colonized chronic obstructive pulmonary diseases (COPD) patients [23-25]. In COPD patients, Monso has demonstrated that 25% of 40 stable COPD patients had colonization of the distal airways, mainly of *H influenzae* and *S pneumoniae* [25]. It has been speculated, particularly in COPD patients, that the persistence of microorganisms in distal airways could worsen the evolution of the chronic underlying disease [15, 16, 19]. Similar results were reported by Riise [24] studying 18 COPD patients where 12 have presented a documented colonization (66 %). Cabello has reported a higher rate of colonization in COPD patients (83%) [11]. However, 76% of the colonizing agents were non-PPMs belonging to the oropharyngeal flora.

In lung cancer patients

Colonization patterns in patients with bronchogenic carcinoma do not differ from those found in COPD patients, indicating that both populations are very similar. Reporting on 33 patients, Cabello have reported that 42 % of his patients were colonized (36 % of PPMs and 64% of non-PPMs) [11]. Liaw has shown that Gram negative bacilli and anaerobes may be found in distal airways below the bronchial obstruction [26]. Hirakata has examined the bacterial colonization of the upper respiratory tract of 110 patients with primary lung cancer, 75 patients with non malignant lung diseases and 45 healthy volunteers [27]. The frequency of bacterial colonization of the upper respiratory tract was significantly higher in lung cancer patients (59.1%) than in non malignant lung diseases patients (37.3%) and healthy volunteers (37.8%). The frequency of gram-negative colonization was significantly higher in lung cancer patients than in the other subjects.

In lung cancer patients undergoing surgery

A systematic review of the literature is exposed in table 1. This summarizes 6 prospective studies and 1 retrospective study.

In 1991, Wansbrough-Jones has investigated 75 patients subjected to pulmonary or pleural resection by performing cultures from samples obtained by preoperative BAL [28]. He found pathogenic micro-organisms in 23 %, with *H influenzae* found most frequently. Of the 75 patients, 11% developed infectious respiratory complications in the postoperative outcome. The likelihood of developing postoperative chest infection was 42% in those patients whose lavage culture was positive for bacterial pathogens compared to 4.8% for those whose culture was negative. In 2002, Sok designed a prospective study in order to verify the origin of the pathogens that cause pleuropulmonary infections after lung cancer resection [29]. He studied samples of sputum 3 days before, during and 3 days after the operation. The infections appeared at 4.3 days. In 75% of cases, the microorganisms that caused the lung infections were Gram-negative bacilli and *Candida albicans*. These were isolated in 18, 13 and 63% of sputum samples, pre-, intra- and postoperatively, respectively. He also found a strong association between the pathogens found in the sputum obtained

3 days after the operation and those found in the sputum of patients who developed a lung infection. The author suggested that the colonization of the airway generally occurs during the postoperative period, from the patient's oral cavity, pharynx and hypopharynx.

In 2002, Ioanas investigated 41 patients with resecable cancer [13]. In his prospective observation based on PSB, he found that 41 % presented a bacterial colonization mainly by PPMs (36%). Body mass index (BMI) > 25 and proximal location of the tumor were the sole two independent factors of the AWC in the multivariate analysis. However, he did not find any correlation between AWC and postoperative infection in his univariate analysis. In the prospective evaluation of Belda in 2005, based on bilateral PSB before thoracotomy, AWC was present in 65 of 78 patients (83%) [12]. Microbiological agents included PPMs in 36 % and non-PPMs in 72%. He found a positive correlation between airways colonization and postoperative pulmonary complications on multivariate analysis. These results have been confirmed in 2006 by Schussler. In an observational and prospective study, he investigated 168 patients with resecable lung cancer [10]. Based on bilateral bronchoscopic aspirates, he demonstrated AWC in 22.8%. The bacterial agents were mainly *H influenzae* (61%), *S pneumoniae* (29 %) and polymicrobial (29%). He also confirmed a positive correlation between AWC and postoperative pulmonary complications on multivariate analysis.

Recently, Dancewicz has reported AWC in 59 % of his 44 patients [30]. However none of their patients developed postoperative pulmonary infections. At last, Yamada reported in 2009 the results of a retrospective evaluation of 626 patients where all the patients had pre and postoperative sputum samples or aspirates [31]. AWC was present in 10.5% in non-COPD patients and in 20 % in COPD patients. Again, the authors found on the multivariate analysis a positive association between AWC and postoperative pulmonary complications.

ASSOCIATION BETWEEN AIRWAYS COLONIZATIONS AND POSTOPERATIVE PULMONARY COMPLICATIONS

Despite a huge variability in its incidence (10.5 to 83 %), there is an indisputable presence of colonizing agents of lower respiratory tract in patients undergoing lung cancer surgery [10-14, 28-30]. However, correlations between these findings and their clinical impacts remain controversial.

Table 2 and Figure 1 present the results of our meta-analysis including 7 selected studies and including 1083 patients. Of them, 291 were considered as colonized patients (26 %) and the remaining 792 patients constituted the non-colonized group (74%). Postoperative respiratory complications occurred in a total of 149 patients (13%): 66 (23%) patients in colonized group and 83 (10.4%) in non-colonized group. Five of the 7 studies summarized in Table 1 and 2 find a positive correlation between AWC and postoperative pulmonary complications [12, 10, 28, 29, 31]. Three of these 5 studies demonstrate a strong correlation where AWC appeared as an independent factor on the multivariate analysis [10, 12, 31]. Only two studies find a lack of correlation between airways colonizations and postoperative respiratory failures [13, 30]. Results of our meta-analysis indicated an odd ratio of 2.3 (1.58-3.48 CI 95%) in the fixed model and 2.44 (1.45-4.11 CI 95%) in the random model. To conclude, based on the existing information summarized in our meta-analysis, there is reasonable evidence documenting a correlation between preoperative AWC and postoperative respiratory failures.

If a statistical correlation between AWC and postoperative complications is likely evident on different multivariate analysis [10, 12, 31], it is not the case from a microbiological view point. Correlation between microbiological agents found pre and postoperatively is not clear. Ioanas have pointed out the problem finding only two positive cultures of the 7 patients developing postoperative pulmonary complications. These two cultures did not correspond to those isolated preoperatively in bilateral PSB [13]. This problem was also reported by Sok that demonstrated that preoperative positive cultures were mainly Gram-positive (77%) or

Gram-negative bacteria (20%) whereas postoperative positive culture were mainly Gram-negative (55%) and Gram-positive bacteria (37%) [29]. In contrast, some authors have demonstrated an association between pre and postoperative cultures [10, 28]. Wansbrough-Jones found that 7 patients developed postoperative pneumonia with the same agents identified preoperatively [28]. In Schussler's series, among the nine colonized patients who developed documented postoperative pneumonia, a concordance between the bacteria responsible for colonization and postoperative pneumonia could be proven in six cases (85%) [10]. Belda also found a correlation between AWC and postoperative pneumonia [12]. Comparing the PPMs isolated during the perioperative examination and those isolated at the onset of infection, total concordance of PPMs was obtained in 21%, partial concordance was obtained in 21%, and no concordance occurred in 58%.

On the basis of these results, it is reasonable to consider that the relationship between perioperative colonization and postoperative infections is controversial and relatively weak, showing partial or total coincidence in only 42% cases of infection [12]. Moreover, patients without perioperative colonization by PPM are still at risk for postoperative respiratory infection. This moderate microbiological correlation, contrasting with the clinical evidence supported by prospective evaluation including multivariate analysis, emphasizes the imperfections of the current microbial sampling methods and the limitations of the traditional microbial cultures used [32]. Furthermore, it supports the current concept of multifaceted origins of the respiratory failures where additional "hits" are participating to occurrence of respiratory failures. Indeed, others mechanisms may be involved in the onset of postoperative respiratory failures and especially the postoperative inflammatory response. This has been suggested for postoperative pulmonary complications in esophageal cancer patients submitted to surgery [33].

MICROBIOLOGICAL AGENTS

Colonizing agents

What remains unclear is why postoperative documented pathogens are frequently different from those isolated in the preoperative period. From our literature review (Table 1), most series have reported *H influenzae*, *S pneumoniae* and *S aureus* as the most commonly identified colonizing agents into the airways of lung cancer patients [10, 12, 13, 28-31]. Recently, Yamada et al. reported the distribution of preoperatively isolated PPMs into the airways of 626 patients submitted to lung cancer surgery. They divided colonizing agents according to PPMs related to community-acquired pneumonia (CAP) (*H influenzae* (3.5 %), *S pneumoniae* (3%), *M catarrhalis* (2%)) and PPMs related to nosocomial pneumonia (NP) (*S aureus* (2%), *P aeruginosa* (1%) and non fermenting Gram negative bacteria (1.8%) [31]. They confirmed that PPMs-CAP represented the main preoperative colonizing agents but they also emphasized that PPMs-NP were the main causative pathogens in the occurrence of respiratory failures.

Agents identified in postoperative outcome

Postoperative documentation of pathogenic agents is problematic. Between 29 to 50 % of postoperative pulmonary infections remain not documented [10, 12]. If documentation has been achieved, pathogenic bacteria are in most instances those classically reported for early hospital-acquired pneumonia [10, 12, 13, 28-31]: *H influenzae* (3-66%), *S pneumoniae* (3-29%), and *S aureus* (8-19%). A polymicrobial aetiology is recognized between 15 and 33% of patients. *Enterobacter* and *Pseudomonas* species are responsible for less 10 % of cases. In previous studies performed so far to assess microbiological characteristics of postoperative pulmonary infections, results were somewhat similar. Team from Mayo Clinic [6] found that *Streptococcus* species and *H influenzae* were responsible for 50% of all postoperative pulmonary complications, whereas gram-negative pathogens (other than *Haemophilus* species) accounted for 31% of pneumonias. In the experience of Sok [29], gram-negative pathogens (other than *Haemophilus* species) were responsible for 71% of postoperative pulmonary complications and Streptococcus species were found in only 10%

of cases. In the Schussler's experience, culture of intraoperative bronchial aspiration showed classical cultures: *H influenzae* (41.7%), *S pneumoniae* (25%) and other streptococci (12.5%) [10]. He also found that 22.2% of *S pneumoniae* strains had a decreased sensitivity to penicillin G and 26% of *Haemophilus* strains were ß-lactamase positive, these last figures being in agreement with available data on resistance in the setting of both community-acquired respiratory infection and of medically treated lung cancer [34, 35].

Unexpected agents

To our knowledge, previous reports have only focused their investigation on bacteriological cultures [10, 12, 13, 28-31]. This constitutes a strong limitation because bacteria do not represent the sole microbial agents in a broad spectrum of potential pathogens. In fact, in the large microbial biodiversity, virus and fungi might represent also potential pathogenic micro-organisms. Our group has reported the results of a prospective observational study based on preoperative bronchoscopic BAL in patients submitted for esophageal cancer surgery [36]. Our data suggested that preoperative AWC was relatively common (30%). Thirteen of the 45 BAL patients (28%) had a preoperative bronchial colonization by either PPMs (16%) or non-PPMs (13%). Among PPMs, cytomegalovirus (CMV) was cultured from BAL in four patients. One of the striking results of our study was the 9% incidence of preoperative CMV detection in BAL group patients, and the 42% incidence of postoperative CMV infection, documented by open lung biopsy, in those patients who experienced ARDS postoperatively. CMV infection is a well-known problem in patients treated by high-dose chemotherapy for haematologic diseases, in HIV-infected patients, and in lung transplantation recipients [37]. Data on cancer patients are scarce, but CMV infection has been incriminated particularly if steroids were a component of the therapy [38]. We have previously reported CMV as a possible cause of ventilator associated pneumonia and ARDS [39]. Acquisition of the virus arises progressively from an early age, and in developed countries the overall seroprevalence is 30–70%. However, there is a huge variance in the incidence of CMV infection depending mainly of local ecology. Homosexual men, poor socioeconomic groups, and residents of developing countries, have seroprevalence rates that can exceed 90% [37].

Others unexpected agents participating to AWC should be considered (HSV2, Candida Albicans…) but data are unavailable.

METHODS OF MICROBIOLOGICAL ANALYSIS

Methods of assessment and microbiological analysis constitute two strong limitations in the investigation of AWC in lung cancer patients.

Methods of sampling

There is a general agreement that methods of microbiological assessment and identification of colonizing agents are not optimal. Studies summarized in table 1 emphasize the variance between the different sampling methods used. For most studies, AWC have been investigated by proximal samples such as sputum or tracheal aspiration [29, 31], by bronchoscopic samples [10, 12, 13, 30] or by samples in the resected specimen [10, 13, 28]. As for diagnosis of ventilatory aquired pneumonia (VAP), most authors consider bronchoscopic sampling methods with quantitative cultures as the best reliable method to determine optimal assessment of potential colonization [32]. This technique provides a good assessment of both proximal and distal airways. As a result, incidence of AWC when performed with endoscopy is increased compared to others traditional techniques. However, this method of assessment is exposed to potential contamination from oro-pharyngeal flora. In contrast, when cultures are obtained from distal airways, at best from lung parenchyma or from BAL performed in lung resected specimen, incidence of AWC is lower even negative [10]. This suggests that incidence of AWC is strongly dependant of sampling methods used and second, that bronchial colonization should be limited to proximal airways.

Methods of cultures

Another limitation of the previous studies assessing AWC is related to the methods of microbiological identification. Knowledge of microorganisms in AWC and more generally in the environment has depended in the past mainly on studies of pure cultures in the laboratory. However, there are several limitations with culture methods. First, depending of the type of sample, isolation and/or detection of potential microbial pathogens could be

difficult in complex microbial samples contaminated by other bacteria or fungi. Second, culture methods are usually limited to a few selective or non selective media for which many uncultured pathogens and anaerobes could be missed on these media and thus could not be detected. Recent data have demonstrated that 99% of organisms seen microscopically are not cultivated by routine techniques and required modern and innovating techniques to be identified [40, 41]. It seems critical in this context to develop new media that could be more permissive for isolation of new bacteria. Thus, development of new molecular techniques, such as 16S rRNA gene amplification and sequence directly from samples may be useful and will provide a new point of view of the problem. This has been successfully used for pleural infection [42] and in pneumonia [43]. Moreover, evaluation of the potential microbial diversity in these samples may be also assessed using molecular techniques including 16S rRNA gene clonal library sequencing or 16S rRNA gene pyrosequencing as recently exemplified in the context of cystic fibrosis [44-46]. To date, there is no specific data on genetic and molecular assessment of AWC in patients submitted to lung cancer surgery. Finally, recent metagenomic studies of the human microbiome using high-throughput sequencing analysis are currently ongoing in many clinical microbiology areas including gut and skin surface [47-49]. Such tool has been also recently used to characterize respiratory tract DNA viral communities in cystic fibrosis and non-cystic fibrosis patients [50].

FACTORS PREDISPOSING TO AIRWAYS COLONIZATIONS

There are numerous descriptions of risk factors for pneumonia that can coincide in the perioperative period and that would act by altering the balance between the host's defense system and the causal microorganism in some way (alteration of the nutritional state, old age, deteriorated of predicted FEV1, chest pain, type of lung resection etc…) (Figure 2) [51-56]. AWC could act as causal or an additive element but what constitute their predisposing risk factors remains unclear.

1- Smoking and COPD

The physical properties of cigarette smoke promote the deposition of particles in the lower airways, where they affect respiratory defense mechanisms at multiple levels [50]. In healthy smokers, bacterial colonization is frequent (33 %) [15]. The proinflammatory effects of smoking overlap those induced by bacterial infection, especially neutrophilic infiltration of the airways. In-ex smokers, a persistent inflammatory process is seen in the central and small airways of which is indistinguishable from inflammation seen in current smokers [58-60]. Ongoing inflammation is present in BAL fluid and in bronchial biopsies among ex-smokers with COPD [61]. Therefore, to reliably distinguish the proinflammatory effects of bacterial colonization from those related to active smoking requires limiting study to only ex-smokers.

For patient submitted to surgery there is growing evidence that smoking cessation should be undergone weeks prior to surgery. In a prospective study, Barrera has provided evidence that smoking cessation in the week's immediately prior undergoing thoracotomy for the resection of lung cancer conferred a beneficial advantage decreasing the risks of pulmonary infections. Non-smokers had lower rates of all pulmonary complications and pneumonia than did all smokers [62]. However the study was not design to provide definitive conclusions in term of microbiological AWC.

2- Intubation and oro-pharyngeal contamination

Patients submitted to lung cancer surgery are subjected to selective tracheobronchial intubation, mechanical ventilation and, in many cases, several bronchoscopies to check or reposition the tube used for the selective intubation or aspiration of secretions. In a prospective trial including 194 patients, Sok supported that the mechanism of airway contamination does not occur during anaesthesia but rather in the early postoperative period secondary to silent aspiration, reflux, overspill and manipulation in the ICU [29]. There were similar strains and a similar distribution of pathogens found in the postoperative sputum and in samples collected from oropharyngeal cavity to identify the causative agent of postoperative complications. The strong correlation between the postoperative sputum

microbiology and the subsequent postoperative infective complications indicates that the oral cavity, pharynx and hypopharynx rather than the lung itself are the sources of pathogens.

It has been stressed, however, that in esophageal surgery the most obvious route for infection to reach the upper airway is inoculation from upper alimentary tract (UAT). In the prospective evaluation by Sharp, there was a definite correlation (66% of cases) between pathogens of UAT content collected at operation and those responsible for postoperative infection [63]. Further investigations comparing AWC agents and colonizing agents belonging from upper alimentary tract are needed to draw definitive conclusions.

3 - Previous chemoradiotherapy

Some authors have postulated that preoperative chemoradiation increases the risk of postoperative complications [7, 64, 65] with subsequent leucopoenia, anorexia, weight loss and interstitial pneumonitis. It was shown recently that chemoradiotherapy leads to immunosuppression by severely impairing proliferative capacity of T lymphocytes [65]. On the other hand, radiation-induced tissue damage could make the lung parenchyma more vulnerable to postoperative complications and to AWC [64-66]. To the best of our knowledge, no specific data is available on AWC after neoadjuvant CRT in lung cancer. Our group has reported the results of a prospective study based on bronchoscopic BAL after neoadjuvant CRT in esophageal cancer [36]. Our results have confirmed that AWC was present after CRT (30%) and unexpected agents such as CMV could have been encountered.

4 - Preoperative hospital admission

Garibaldi was the first to demonstrate in 1981 that prolonged preoperative hospital stay was an independent factor of postoperative pneumonia among 520 patients submitted for elective thoracic or upper gastro-intestinal surgery [67]. Since then, prolonged preoperative hospital stay was frequently suggested as a patient characteristic associated with increased surgical site infection risk [68]. However, length of preoperative stay is likely a surrogate for severity of illness and co-morbid conditions requiring inpatient work-up and/or therapy before the operation. To date, there is no specific data available evaluating the impact of prolonged preoperative admission on AWC.

ANTIBIOTIC PROPHYLAXIS IN LUNG CANCER SURGERY

Considering that AWC should be a determining element in a broad spectrum of incriminated factors of respiratory failures, its existence raises the question of the accuracy of current antibiotic prophylaxis used in lung cancer surgery. From our literature review summarized in Table 3 and Figure 3, existing evidence on what constitutes the best appropriate antibiotic prophylaxis in this kind of surgery is poor. To our knowledge there are 9 prospective randomized controlled trials and two large prospective studies tackling the problem with different antibiotic regimens [70-80]. The conclusions that can be reached from these previous studies join the conclusion from Torres [14]: “The ideal regime for antibiotic prophylaxis for the prevention of respiratory infections was not discovered and this is still the case 20 years later”.

This is due to several reasons: First, there is no data concerning specifically lung cancer surgery and all the studies have included a mixed of different lung resections excluding only infectious diseases. Secondly, basic comparison between first-generation cephalosporin (cefazolin, cefalotin) or penicillin versus placebo has shown no significant difference in term of pulmonary complications. This argument raises the question of the rational of targeted antibiotic prophylaxis preventing mainly wound infections in the setting of pulmonary surgery where prevention of respiratory complications is of paramount importance. Thirdly, documented microbiology of postoperative pulmonary complications is restricted to only 5 of the 11 reported studies. Fourthly, despite a clear definition of postoperative pneumonia, there is a huge variance in the postoperative incidence of infectious events. This probably emphasizes the differences between surgical team, differences in anesthetic management and probably the specific local hospital ecology.

However, despite inhomogeneous data, there are significant messages:

1) AWC detection could act as a clinical based-evidence to target the antibiotic prophylaxis according to specific local ecology. On this basis, every thoracic surgery departments should define their specific ecology according to their inherent population. Boldt and colleagues, in 1999, suggested that the best schedule for antibiotic prophylaxis will

depend, among other factors, on the bacteria that are present at the time when these patients undergo surgery [79]. They conducted a randomized prospective study in which they compared ampicillin-sulbactam and cephazoline according to their specific local ecology. Every patient was subjected to a preoperative bronchial aspirate through a double-light tube in order to carry out a microbiological study and determine whether isolated germs were sensitive to the antibiotics used. The isolated microorganisms were mainly H. influenzae, S. pneumoniae, S. viridans, S. aureus and K. pneumoniae. Patients from ampicillin-sulbactam group had significantly fewer pulmonary infections than patients from cefazolin group. In the first group, all the isolated bacteria were sensitive to the antibiotic used, while in the second group, eight out of the 25 isolated bacteria were not sensitive.

2) The antibiotic prophylaxis recommended should include micro-organisms colonizing airways but include also agents coming from oropharyngeal cavity. As it has been shown by Sok, the colonization of the airway by pathogens in patients operated for lung cancer resection is basically produced during the postoperative period via the oral cavity, pharynx and hypopharynx. Therefore, scheduled prophylaxis administered in the perioperative period would not strictly be applicable to respiratory infections after a lung resection and might include oropharyngeal flora [29]. In France for example, the recommended antibiotic prophylaxis remains first- or second-generation cephalosporin [81]. This prophylaxis has been shown to decrease the incidence of wound infections and to be effective in the prevention of postoperative empyema. However, it is likely not sufficient to be effective on the broad spectrum required in prevention of respiratory complications.

3) Antibiotic prophylaxis should be based on more than one single dose. Bernard et al, in a randomized prospective study on patients subjected to lung surgery, have compared two prophylaxis regimens with cefuroxime [72]. They observed that administration every 6 h for 48 h, as opposed to administration in a single dose, significantly reduced the number of respiratory infections and empyema. In Contrast, Olak comparing the efficacy of two regimens of Cefazolin (signe shot vs 48h) in preventing pulmonary infections in lung-resection surgery, found no significant differences between the two groups [75].

4) Changing of antibiotic prophylaxis should be well documented by clear prospective and randomized controlled trial avoiding selection and increase of bacterial resistance. Recently, Schussler et al. have reported in a large sequential study over 18 months, a comparison between first-generation cephalosporin and a short-term (24 h), high-dose (6 g) course of amoxicillin–clavulanate (AC), which targets bacteria most often responsible for preoperative colonization (S.Pneumonia an H.Influenzae) [80]. They observed a significant decrease in the incidence of both microbiologically documented and non-documented POP, as well as significant decrease in the overall need of postoperative antibiotics. The authors suggested the use of amoxicillin-clavunate in clinical practice. However, without depicting actual susceptibility results of “resistant” gram-negative pathogens to both cefamandole and amoxicillin-clavulanate, it is not possible to ascertain whether one would have enhanced activity against these pathogens. Because gram-negative susceptibility varies profoundly among varying geographic areas, the results in one center would be difficult to extrapolate universally.

CONCLUSIONS

Current evidences on AWC in patients submitted for lung cancer surgery remain controversial and weak. Their incidence is considered as frequent, estimated between 10 to 83 %, but their association with postoperative respiratory infections is unclear. It seems that patients with AWC are at risk to develop respiratory failures but if they do, they may develop infection to pathogens related to nosocomial infection. It is likely evident that airways colonization is more largely dependant of several epidemiologic factors, of the population constituting the country, of local ecology and of national policy of curative antibiotic treatment. Moreover, airways colonizations are not limited to bacteria only. Part of pathogenesis of respiratory failures might be largely influenced by others potential pathogenic microorganisms notoriously viral species. Strong limitations continue to preclude to definitive conclusions and every effort should be made to investigate this critical issue.

Therefore, AWC detection should be encourage whenever possible to target the antibiotic prophylaxis according to specific local ecology. Progress in microbiology cultures and molecular analysis should deserve further information in a near future. We believe that molecular culture-independent techniques applied in the context of AWC will provide in the future a great opportunity to discover new and/or emerging pathogens that are currently unknown.

Clinician should keep into their mind this old and evident adage: we find only what we are looking for.

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Figure 1: Meta-analysis of selected studies investigating airways colonizations (AWC) and postoperative respiratory complications (PCR).

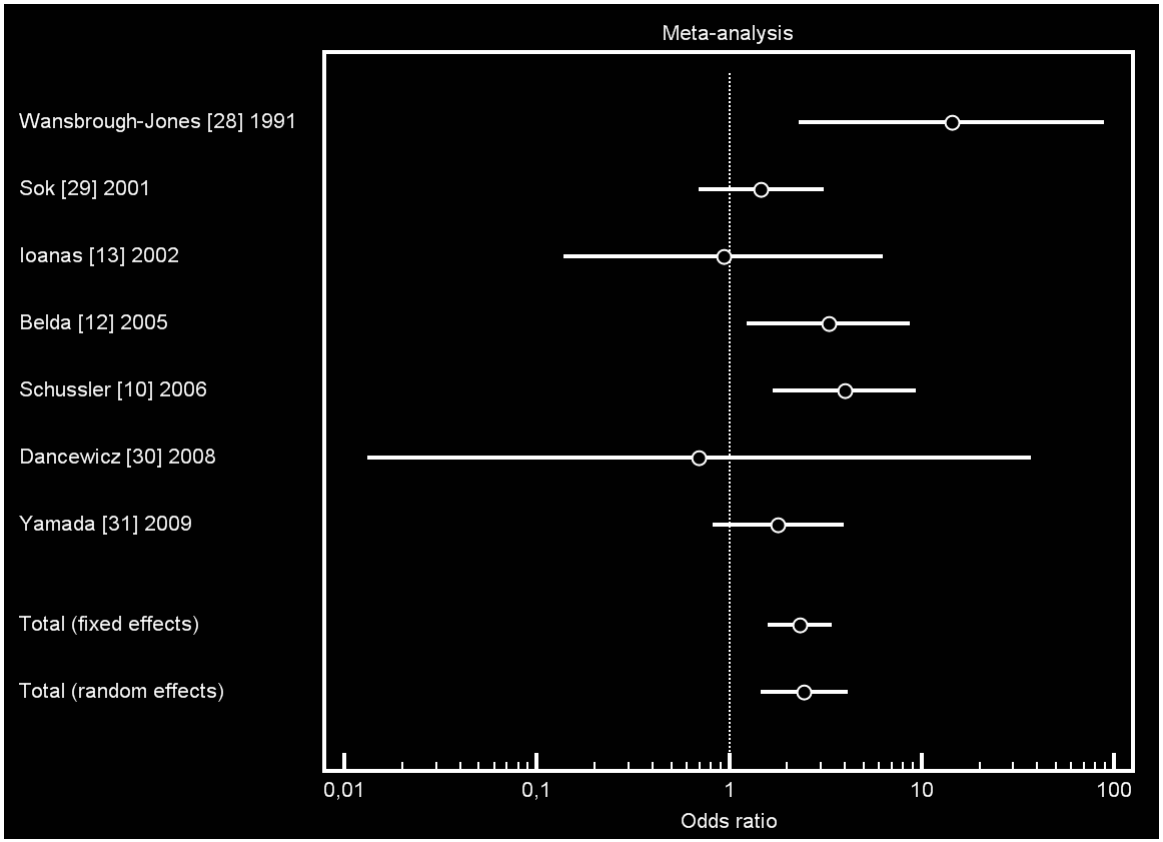

Figure 2 : Main clinical factors affecting occurrence of airways colonizations (AWC)

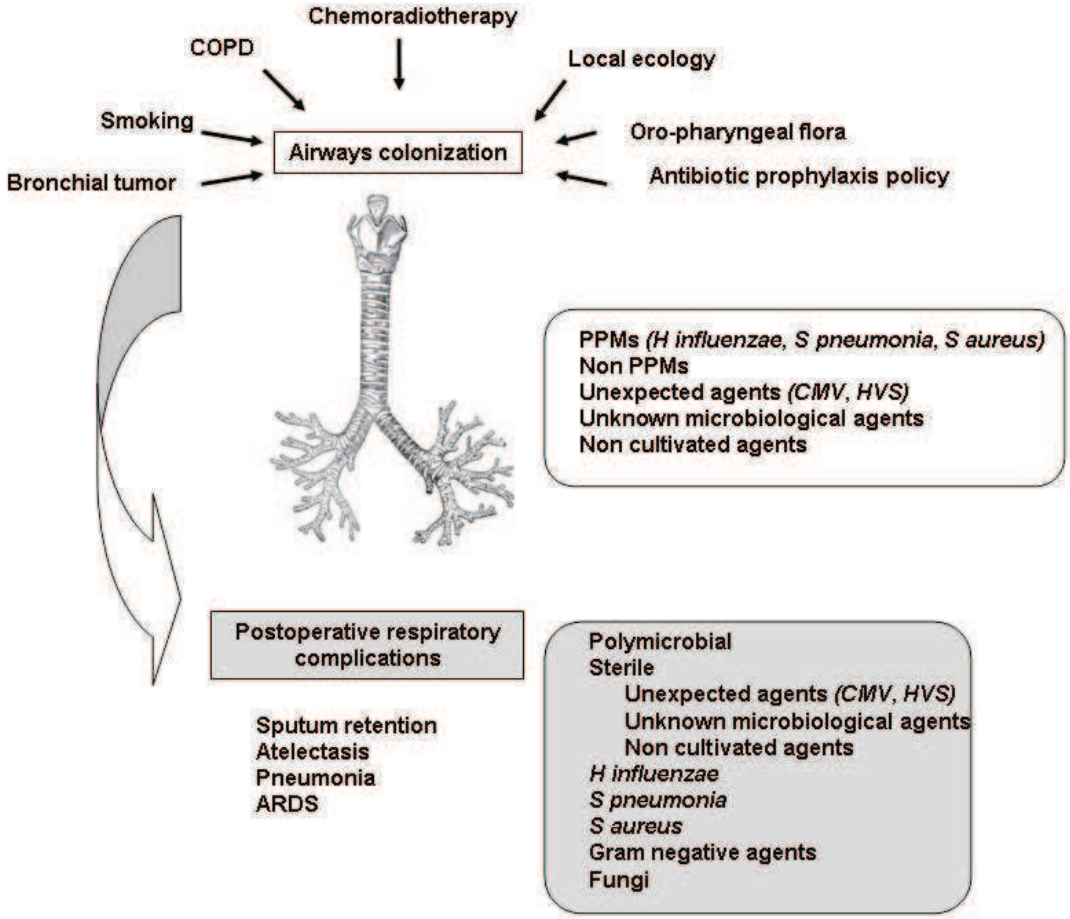

Figure 3: Meta-analysis of selected studies investigating antibiotics prophylaxis in lung cancer surgery.

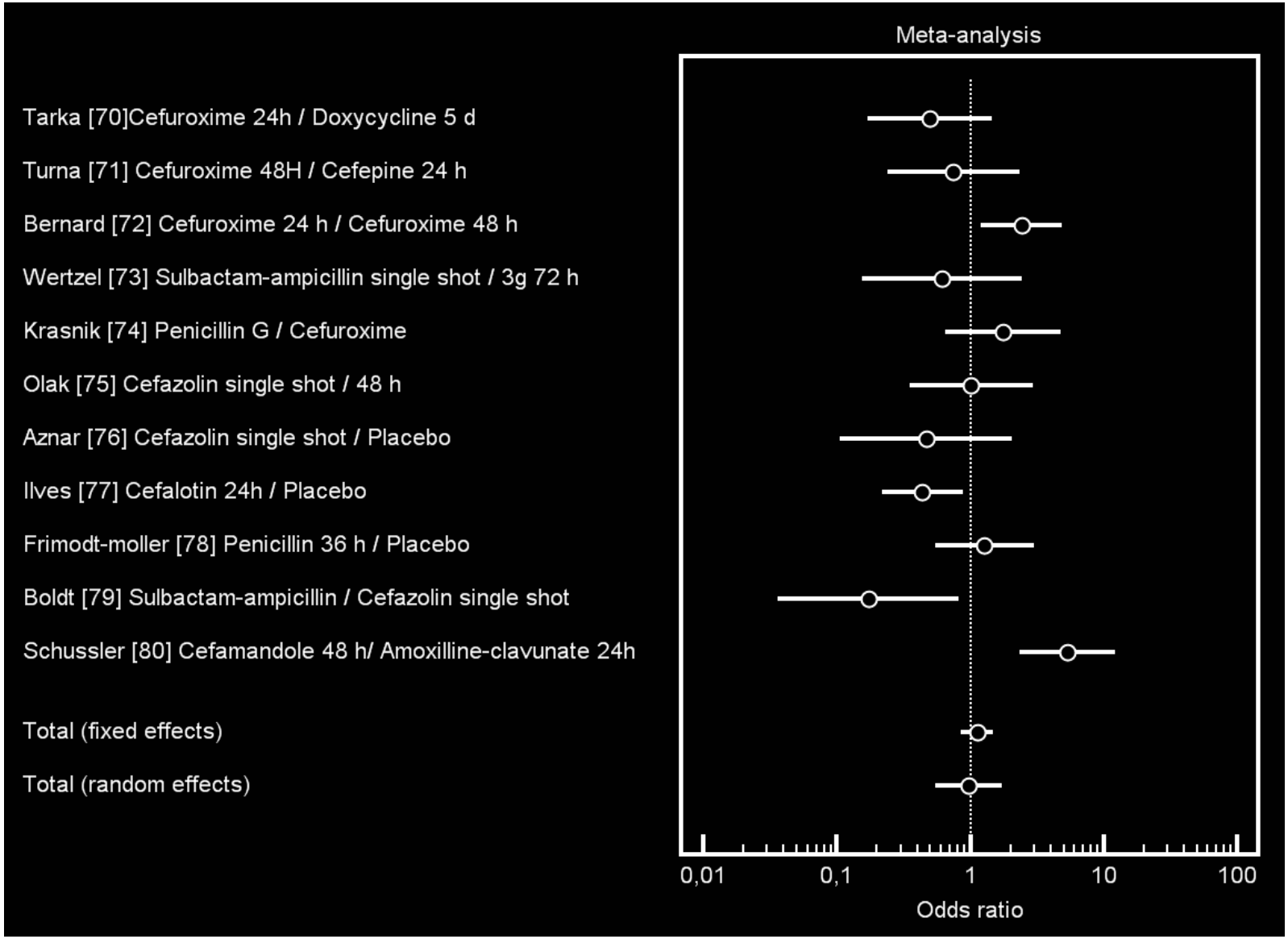

Table 1 : A systematic review of the literature of AWC. This summarizes 6 prospective studies (P) and 1 retrospective study (R).

Authors	Design	n	Samples	AWC rate	Postoperative documented microorganisms	Respiratory infections rate
Wansbrough-Jones [28]	P	54	BAL in resected specimen	12/54 (22%)	*H influenzae* 8 (66%) *S pneumoniae* 1 (8%) *S aureus 1* (8%)	8 (10, 7%)
Sok [29]	P	194	Sputum samples	23/126 (18%)	Gram negative spp 23 (18%)	34/194 (18%)
Ioanas [13]	P	41	PSB and samples in resected specimen	17/41 (41 %) - PPMs : 15 (36%) - non PPMs: 2 (5%)	*H influenzae* 8 (35%) *S pneumoniae* 3 (13%) Pseudomonas spp. 3 (13%)	5/12 (12%)
Belda [12]	P	78	Bronchoscopic aspirate and PSB	65/78 (83%) -PPMs: 28 (36%) -non PPMs: 56 (72%)	*S pneumoniae* 3 (5 %) *H influenzae* 2 (3 %) *S aureus* 2 (3%)	24/78 (31%)
Schussler [10]	P	168	Bronchoscopic aspirate	31/136 (22.8%)	*H influenzae* 19 (61%) *S pneumoniae* 9 (29%) Polymicrobial 9 (29%)	42/168 (25%)
Dancewicz [30]	P	44	Bronchoscopic BAL	26/44 (59%) -PPMs : 15(34%)	*S pneumoniae* 7 (26%) *S aureus* 4 (19%) *H influenzae* 3 (11%)	0
Yamada [31]	R	626	Sputum samples Aspirates	Non COPD : 50/475 (10.5%) COPD : 30/151 (20%)	*H influenzae* 21 (3.4 %) *S pneumoniae* 19 (3 %) *S aureus* 13 (2.1 %)	40/626 (6.4%) Non COPD : 17/475 (36%) COPD : 23/151 (14%)

Table 2: Meta-analysis of selected studies investigating airways colonizations (AWC) and postoperative respiratory complications (PCR). Test of heterogeneity: Chi=8.9, DF:6; p=0.179

Study	Postoperative respiratory complications in colonized group	Postoperative respiratory complications in non-colonized group	Odds Ratio	95% CI
Wansbrough-Jones [28]	5/12	2/42	14,286	2,3 to 88,69
Sok [29]	20/99	14/95	1,465	0,62 to 3,10
Ioanas [13]	2/17	3/24	0,933	0,13 to 6,29
Belda [12]	15/28	13/50	3,284	1,23 to 8,7
Schussler [10]	15/31	20/105	3,984	1,69 to 9,38
Dancewicz [30]	0/26	0/18	0,698	0,01 to 36,79
Yamada [31]	9/78	31/458	1,797	0,82 to 3,93
Total (fixed effects)	**66/291**	**83/792**	**2,330**	**1,58 to 3,42**
Total (random effects)	**66/291**	**83/792**	**2,447**	**1,45 to 4,11**

Table 3: Literature review of antibiotics prophylaxis in lung cancer surgery. There are 9 prospective randomized controlled trials (PRCT) and two large prospective studies tackling the problem with different antibiotic regimens. GTS: general thoracic surgery. POP: postoperative pneumonia. Test of heterogeneity: Chi=36.6, DF:10, p< 0.001.

Study	Design	Postoperative pulmonary complications with first antibiotic prophyalxis	Postoperative pulmonary complications with a second antibiotic prophylaxis	Odds Ratio	95% CI
Tarka [70] Cefuroxime 24h / Doxycycline 5 d	PRCT	6/60	11/60	0,495	0,17 to 1,43
Turna [71] Cefuroxime 48H / Cefepine 24 h	PRCT	6/50	8/52	0,750	0,24 to 2,34
Bernard [72] Cefuroxime 24 h / Cefuroxime 48 h	PRCT	30/100	15/100	2,429	1,21 to 4,87
Wertzel [73] Sulbactam-ampicillin single shot / 3g 72 h	Prospective	4/30	6/30	0,615	0,15 to 2,45
Krasnik [74] Penicillin G / Cefuroxime	PRCT	13/48	8/46	1,764	0,65 to 4,76
Olak [75] Cefazolin single shot / 48 h	PRCT	8/100	7/89	1,019	0,35 to 2,93
Aznar [76] Cefazolin single shot / Placebo	PRCT	3/70	5/57	0,466	0,10 to 2,03
Ilves [77] Cefalotin 24h / Placebo	PRCT	16/118	24/91	0,438	0,21 to 0,88
Frimodt-moller [78] Penicillin 36 h / Placebo	PRCT	18/45	15/47	1,422	0,60 to 3,34
Boldt [79] Sulbactam-ampicillin / Cefazolin single shot	PRCT	2/60	10/60	0,172	0,036 to 0,82
Schussler [80] Cefamandole 48 h/ Amoxilline-clavunate 24h	Prospective	23/168	8/277	5,334	2,32 to 12,22
Total (fixed effects)		**129/849**	**117/909**	**1,136**	**0,86 to 1,49**
Total (random effects)		**129/849**	**117/909**	**0,975**	**0,54 to 1,73**

II.1.3 Discussion

L'information disponible concernant les colonisations bronchiques chez des patients présentant un cancer du poumon et soumis à une chirurgie d'exérèse est bien documentée dans la littérature mais compte tenu de la grande variabilité de la méthodologie utilisée, les conclusions qui en résultent, demeurent controversées.

Les conclusions peuvent être résumées ainsi :

1) Les colonisations bronchiques proximales sont fréquentes de l'ordre de 20 à 41 % (avec des extrêmes de 10 à 83 %). Si leur existence ne fait cliniquement pas de doute, leur implication dans le développement d'une complication respiratoire postopératoire reste incertaine. L'identification d'une colonisation bronchique est étroitement dépendante des techniques de prélèvements au niveau des voies aériennes. Cette colonisation est bien documentée lorsque la recherche a été effectuée par des aspirations bronchiques ou des brossages bronchiques protégés. Peu de données existent sur la réalisation d'un lavage broncho-alvéolaire. L'essentiel des connaissances actuelles ne concernent que l'exploration des bronches proximales. L'exploration des bronches distales ou du parenchyme pulmonaire n'a été que peu évaluée. Les données existantes sont contradictoires. Partant du principe que ce sont ces territoires qui sont le siège de l'infection postopératoire (pneumopathie, SDRA), il en résulte qu'une recherche microbiologique à leur niveau reste indispensable. Si cette recherche s'avérait être concluante, ceci traduirait la possibilité d'une colonisation distale au moment de la chirurgie dans ce groupe de patients à risque et laisserait envisager la possibilité d'une recherche préopératoire. A l'inverse, si cette recherche s'avérait non concluante, ceci confirmerait l'hypothèse que les bronches distales et le parenchyme doivent être considérés comme stériles au moment de la chirurgie et que l'infection postopératoire ne se ferait que par une contamination des voies aériennes ou digestives supérieures dans les heures ou jours suivants la chirurgie.

2) Toute recherche sur les colonisations bronchiques n'a été limitée qu'aux seules bactéries. Ceci reste vraisemblablement l'élément le plus limitatif lorsque l'on considère que 99 % des espèces connus de la biosphère requièrent des techniques d'identification modernes pour être identifiées. Aucune donnée n'est disponible concernant les virus. Aucune étude n'évalue la possibilité de germes émergents compte tenu des limitations liées aux techniques de mise en culture spécifique avec la possibilité de germe difficilement ou incultivable sur milieu traditionnel.

3) Du point de vue statistique, notre méta-analyse basée sur une revue exhaustive de la littérature comprenant pour l'essentiel des études prospectives et non randomisées, suggère que la colonisation ait un effet potentiel sur le développement des complications respiratoires (Risque relatif : 2.447, Intervalle de confiance 95 % : 1.45-4.11). Du point de vue microbiologique, la concordance demeure difficile. Il existe que peu d'études ayant évalués un lien entre agents colonisants et agents pathogènes incriminés dans l'infection postopératoire. Encore une fois, les méthodes d'identification microbiologique et les techniques de prélèvements postopératoires limitent considérablement l'information potentielle associant colonisation et complication.

4) Il semble difficile de localiser l'endroit même de la colonisation. S'agit-il d'une colonisation des voies aériennes supérieures ou distales, du tractus digestif ou de la sphère oro-pharyngée ? Il apparait que la flore colonisante identifiée en préopératoire dépend de différents facteurs épidémiologiques étroitement dépendants du pays, de la flore hospitalière locale et de la politique nationale du pays concernant les antibiotiques. De notre revue de la littérature, même s'il apparait qu'une antibioprophylaxie plus adaptée aux infections respiratoires permette de réduire le taux de complications postopératoires, ce changement ne peut être réalisé qu'après avoir évalué la flore locale spécifique à chaque établissement. Les données d'un centre sont difficilement extrapolable à d'autres centres.

4) Enfin, les techniques d'analyse microbiologique par mise en culture traditionnelle demeurent vraisemblablement insuffisante pour détecter des germes difficilement cultivables, émergents ou viraux.

Malgré ces limitations, l'évaluation d'une éventuelle colonisation doit être encouragée pour documenter précisément la flore et l'écologie locale propre à chaque établissement. Les progrès dans l'identification de ces colonisations et dans la documentation des infections postopératoires devraient fournir une information fiable et reproductible dans les années à venir. Les progrès dans les techniques d'identification moléculaire pourraient permettre l'identification de germes difficilement cultivables ou émergents.

Les conclusions de cette première étude nous ont conduit dans un premier temps à évaluer la colonisation bronchique chez des patients opérés d'un cancer de l'œsophage et dans un deuxième temps, à identifier par des méthodes de biologie moléculaire les colonisations bronchiques distales et parenchymateuse des patients opérés pour cancer du poumon.

Article 2

II.2 Article 2

Airway colonisation and postoperative pulmonary complications after neoadjuvant therapy for oesophageal cancer

II.2.1 Introduction

La radio-chimiothérapie préopératoire suivie d'une chirurgie d'exérèse radicale basée sur l'oesophagectomie avec curage ganglionnaire, s'impose comme le traitement de référence des cancers de l'œsophage localement avancés *(Burmeister et al, 2005)*. La mortalité postopératoire de ce type d'approche demeure élevée *(Doty et al, 2002).* Parmi les causes de décès postopératoire, les complications respiratoires post-oesophagectomie arrivent au premier rang des complications (30 %) et surviennent dans 80 % des cas au cours des cinq premiers jours *(Ferguson et al 2002)* (*D'Journo et al, 2008)*. Ces complications sont proches de celles observées en chirurgie pulmonaire. Elles partagent un même continuum physiopathologique, une même sévérité et un taux de mortalité qui leur est associé qui reste élevé. Leurs mécanismes et leur prévention ne diffèrent donc pas de celles observées en cas d'exérèse pulmonaire.

En se basant sur l'analyse des facteurs de risques des complications respiratoires après exérèse pulmonaire, nous avons émis l'hypothèse qu'il existait une colonisation bronchique préopératoire notamment chez des patients traités par une radio-chimiothérapie préopératoire. L'évaluation microbiologique des voies aériennes des patients proposés pour une chirurgie œsophagienne n'a, dans la littérature médicale disponible, jamais été étudiée.

Cette étude rétrospective, basée sur un recueil prospectif des données, avait pour but une évaluation exhaustive préopératoire de la colonisation bronchique proximale par de multiples prélèvements réalisés par fibroscopie bronchique. L'analyse microbiologique était basée sur des méthodes d'identification phénotypique à la recherche de germes traditionnellement retrouvés dans les suites opératoires (bactérie et champignon), et d'autre part, sur des méthodes de mise en culture virale et de sérologie à la recherche spécifique de cytomégalovirus (CMV). Cette recherche était motivée par des observations cliniques réalisées par les équipes de réanimation de notre établissement, où avait été documentée une forte incidence de CMV chez des patients présentant une pneumopathie ou un SDRA après chirurgie thoracique oncologique *(Papazian et al, 1996 et 2007).* En fonction de

l'existence d'une colonisation bronchique, un traitement antibiotique ou antiviral préemptif était proposé avant la chirurgie. Un lien précis entre colonisation et complication était recherché, de même qu'une concordance entre germes préopératoires et germes postopératoires.

II.2.2 Article :

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Airway colonisation and postoperative pulmonary complications after neoadjuvant therapy for oesophageal cancer☆

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Abstract

Objective: To evaluate the clinical relevance of preoperative airway colonisation in patients undergoing oesophagectomy for cancer after a neoadjuvant chemoradiotherapy. **Methods:** From 1998 to 2005, 117 patients received neoadjuvant chemoradiotherapy for advanced stage oesophageal cancer. Among them, 45 non-randomised patients underwent a bronchoscopic bronchoalveolar lavage (BAL group) prior to surgery to assess airways colonisation. The remaining patients ($n = 72$) constituted the control group. The two groups were similar with respect to various clinical or pathological characteristics. **Results:** Thirteen of the 45 BAL patients (28%) had a preoperative bronchial colonisation by either potentially pathogenic micro-organisms (PPMs) ($n = 7$, 16%) or non-potentially pathogenic micro-organisms ($n = 6$, 13%). Cytomegalovirus (CMV) was cultured from BAL in four patients. Pre-emptive therapy was administrated in seven patients: four antiviral and three antibiotic prophylaxes. Postoperatively, 14 patients (19%) developed acute respiratory distress syndrome (ARDS) in the control group and three (7%) in the BAL group ($p = 0.064$). The cause of ARDS was attributed to CMV pneumonia in six control group patients on the basis of the results of open lung biopsies ($n = 3$) or BAL cultures ($n = 3$) versus none of the BAL group patients ($p = 0.08$). Timing for extubation was shorter in the BAL group (mean 13 ± 3 h) as compared with the control group (mean 19.5 ± 14 h; $p = 0.039$). In-hospital mortality was not significantly lower in BAL group patients when compared to that of control group patients (8% vs 12.5%). **Conclusions:** Airway colonisation by PPMs after neoadjuvant therapy is suggested as a possible cause of postoperative ARDS after oesophagectomy. Pre-emptive treatment of bacterial and viral (CMV) colonisation seems an effective option to prevent postoperative pneumonia.

Keywords: Oesophageal cancer; Oesophagectomy; Neoadjuvant therapy; Cytomegalovirus; Bronchoalveolar lavage; Bronchoscopy; Airways colonisation

1. Introduction

The best treatment option for patients with locally advanced oesophageal carcinoma has not yet been determined. Although surgery remains the cornerstone, there is established evidence supporting neoadjuvant chemoradiotherapy to increase survival in those patients [1]. Whether this benefit may be sufficient to warrant the considerable risks of multimodality treatments remains unclear. Indeed, chemoradiotherapy significantly increases postoperative mortality [2]. Pneumonia and adult respiratory distress syndrome (ARDS) are the most frequent precipitating events in this setting, eventually leading to mortality [3–7].

Reasons of this pulmonary morbidity are multifaceted, and those due specifically to the neoadjuvant treatment are probably very difficult to segregate from those due to the surgical procedure, to the perioperative anaesthetic management, and to the patient himself. Nevertheless, in the pathogenesis of nosocomial pneumonia occurring in hospitalised and chronically ill individuals, chronic airway colonisation seems to be an essential first step [8]. As well, colonisation may be supposed to facilitate the development

Abbreviations: ARDS, acute respiratory distress syndrome; BAL, bronchoalveolar lavage; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; FEV, forced expiratory volume in 1 s; PPMs, potentially pathogenic micro-organisms; Non-PPMs, non-potentially pathogenic micro-organisms.

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of pneumonia in the postoperative setting, when secretion clearance and cough reflex are impaired. Under immunosuppressive condition, these colonisations of the respiratory mucosal surface act in a manner that increases its ability to bind micro-organisms and lessen the risks of superimposed infections.

The aim of the present study was to identify preoperative bronchial colonisation in patients submitted to oesophagectomy after neoadjuvant chemoradiotherapy, with special emphasis on the possible benefit of a pre-emptive treatment on postoperative pulmonary complications.

2. Materials and methods

2.1. Study design

This retrospective study was conducted according to the current regulations for clinical research in France. As there was no intent of research at the time of data collection, all tests were proposed in the frame of medical care, with a presumed individual benefit for the patients. Patient charts were identified by screening a database into which data were entered prospectively for any patient undergoing surgery for thoracic malignancy at our department.

The study period ranged from January 1998 to July 2005. All patients underwent clinical examination, oesophagoscopy, full-body computerised tomography (CT), and endoscopic ultrasound (US). PET-scanning was selectively performed from 2003 when it became available at our centre in patients with equivocal results of the standard work-up. According to the guidelines of our multidisciplinary committee, all CT M0 fit patients with a locally advanced disease as defined as UST3N1 (predicted R0 resection) of the oesophagus or gastro-oesophageal junction were offered the option of a multimodal treatment.

Altogether, 117 patients with locally advanced oesophageal cancer received neoadjuvant chemoradiotherapy prior to a trans-thoracic oesophagectomy. Among them, 45 non-randomised patients accepted to have a bronchoscopic bronchoalveolar lavage (BAL group) before surgery to assess airways colonisation. The other 72 patients constituted the control group. The two groups were similar with respect to various biological, clinical or pathological characteristics (Tables 1 and 2).

Table 1
Characteristics of the two groups

	Control group, $N = 72$	(%)	BAL group, $N = 45$	(%)	p-Value
Adenocarcinoma	38	53	23	51	NS
Squamous cell	34	47	22	49	NS
Sex (male/female)	63/9	87	40/5	89	NS
Smokers	57	79	39	86	NS
Alcohol users	39	54	22	49	NS
COPD	13	18	9	20	NS
HTA	16	22	14	31	NS
Diabetes mellitus	5	7	4	9	NS
Mac Keown	20	28	15	33	NS
Ivor Lewis	52	72	30	67	NS
Peridural analgesia	30	42	23	51	NS

Statistical analysis included the Pearson χ^2 test, and Fisher's exact test as appropriate. Mean and standard deviations are presented.

Neoadjuvant chemotherapeutic regimens included an induction dose of continuous 5-fluorouracil in two cycles (800 mg/m^2) from D1 to D4 and from D28 to D32, associated with cisplatin (75 mg/m^2) on D1 and D28. Concurrent radiation was administered in daily fractions of 1.8 Gy using a three- or four-field technique during 5 weeks for a total dose of 45 Gy (±8 Gy). Surgery was planned 6 weeks after the end of the induction treatment (mean delay: 45.7 days ±20).

2.2. Bronchoalveolar lavage (BAL) and microbiological analysis

Fibre optic bronchoscopy was performed approximately 4 weeks after completion of the induction therapy. The tracheobronchial tree was fully examined and special attention was paid to the trachea and main bronchi. Bronchoalveolar lavage samples were obtained at the end of the procedure: 150 ml of physiological saline were instilled separately into the left and right main bronchus and washing for microbiologic examination was obtained by suction from all lung lobes.

All samples were sent for microbiological evaluation. Blood samples were obtained simultaneously to the bronchoscopy for blood cultures, for CMV viremia, antigenemia and serology. Routine staining of BAL included Grocott's stain for *Pneumocystis carinii* and Papanicolaou stain. *Legionella pneumoniae*, atypical mycobacteria and *Mycobacterium tuberculosis* were investigated by conventional techniques. Immunostaining for CMV was performed using a panel of commercially available antibodies. CMV culture was performed by shell via assay, spinning the BAL fluid onto human embryonic fibroblasts and determining of CMV immediate early antigens by immunofluorescence. CMV was investigated in blood by PCR and antigenemia assay was determined by CMV light kit. CMV serologic status was achieved by fluorescent antibody and anticomplement immunofluorescence.

2.3. Pre-emptive treatment

Micro-organisms were classified according to their potential pathogenic status (PPM and non-PPM) [9,10]. Agents classically recognised as causative of respiratory infections, whether or not belonging to the oropharyngeal flora, were considered as PPM (such as gram-negative rods, i.e. *Pseudomonas aeruginosa*, Enterobacteriaceae and *Haemophilus* spp.; gram-positive cocci, i.e. *Staphylococcus aureus*, *Streptococcus pneumoniae*; and gram-negative cocci, i.e. *Moraxella catarrhalis*). Non-PPMs were those micro-organisms belonging to the oropharyngeal or gastrointestinal flora that are not usually involved in respiratory infections in nonimmunocompromised patients (i.e. *Streptococcus viridians* group, *Neisseria* spp., *corynebacterium* spp., *Candida* spp., and others) [9,10]. Any CMV detection was considered as a CMV infection in accordance with the dedicated literature [11]. According to the BAL results, anti infectious specific treatment was administrated for PPM. Identification of non-PPMs was considered as a contamination and, in turn, was not treated.

Table 2
Preoperative characteristics of the two groups

	Control group, $N = 72$	(±)	BAL group, $N = 45$	(±)	p-Value
Chemotherapy (Nb cycles)	2.18	0.5	2.2	0.7	NS
Radiotherapy (Gy)	44.1	8	44	8	NS
ASA	2.2	0.5	2	0.7	NS
NYHA	2	0.6	2.07	0.7	NS
Age	59.6	9	60	8	NS
Weight (kg)	67	12	66	12	NS
Length (cm)	172	7	171	7	NS
VC (l)	3.9	1	3.8	0.6	NS
Fev1 (l)	2.9	0.9	3.1	0.7	NS
Fev1/VC (%)	92	22	91	16	NS
PaO_2 (mmHg)	82	8	84	9	NS
$PaCo_2$ (mmHg)	38	3	36	3	NS
White count cell (10^9/l)	5.2	2	4.9	3	NS
Platelets cell (10^9/l)	209	85	247	107	NS
Haemoglobin (g/dl)	12.4	1	11.8	2	NS

Statistical analysis included Student's t-test, the Pearson χ^2 test, and Fisher's exact test as appropriate. Mean and standard deviations are presented.

2.4. Surgery and postoperative course

All patients underwent a transthoracic en-bloc oesophagectomy with a 2-field lymphadenectomy. Anastomosis was performed at the top of the thorax (Ivor Lewis procedure, $n = 82$) or into the neck (Mac Keown procedure, $n = 35$). Medical and surgical complications were prospectively recorded. Respiratory complications were defined by all medical events concerning the lung parenchyma (i.e. pneumonia, sputum retention, atelectasis, acute lung injury, acute respiratory distress syndrome) in the absence of surgical complications requiring reoperation. Surgical complications included anastomotic leakage, laryngeal paralysis, chylothorax, pleural effusion, empyema and bleeding. Acute lung injury (ALI) and ARDS were defined according to the standardised ARDS criteria. ARDS was defined as PaO_2/FiO_2 less than 27 kPa. Additional criteria included the presence of bilateral infiltrations on plain chest radiograph, and a pulmonary artery occlusion pressure of less than 18 mmHg if measured or no clinical evidence of left atrial hypertension.

2.5. Statistical analysis

Statistical analysis included Student's t-test, the Mann–Whitney test, the Pearson χ^2 test, and Fisher's exact test as appropriate. Operative mortality consisted of either 30-day or in-hospital mortalities regardless of the length of the hospital stay. Descriptive analysis was expressed in terms of mean, median, standard deviation and frequency. Statistical differences between groups were determined by Student's t-test, the Mann–Whitney test, the χ^2 test and one-way analysis of variance (ANOVA). All statistical tests were performed using a 5% level of significance.

3. Results

3.1. General microbiological findings

Bronchoscopic BAL was positive in 13 of 45 patients (29%). Results are shown in Table 3. There were seven PPMs (15%) and six non-PPMs (13%). BAL samples allowed to detect six cases of fungal colonisation: *Candida albicans* and *famata* were grown in BAL fluid in two cases, *Aspergillus fumigatus and Niger* was detected in three patients and *Torulopsis glabrata* was found in one patient. BAL cultures disclosed *Escherichia coli* in one patient and *Haemophilus influenzae* in 2 ($>10^5$ cfu/ml). *S. aureus* was found in two patients but colonisation was not significant ($<10^3$ cfu/ml). *Pneumocystis carinii*, *Mycobacteria*, *Legionella pneumonia*, *Toxoplasmia* and *Amibia* have not been detected in any BAL fluid.

CMV was detected by shell vial assay and immunostaining in the BAL fluid in 4 out of 45 patients (9%). Viremia and antigenemia were negative in all patients. CMV serologic status was obtained in 29 patients: positive in 15 patients (51%), and negative in 13 (44%). Seroconversion was observed in one patient (3%), in whom CMV was also detected in his BAL fluid.

There were no significant differences in total volumes of retrieved BAL fluid in the different patient groups submitted to comparison. Results of BAL cytology are summarised in Table 3. BAL fluid cytology showed an increased percentage of alveolar macrophages in PPM patients when compared to that of non-PPM patients, but the difference did not reach statistical significance (Table 4).

3.2. Antiviral prophylaxis and pre-emptive therapy

Seven of 45 patients received pre-emptive therapy (16%) (Table 3). At that time, none presented with clinical or radiological signs of pneumonia. CMV prophylactic antiviral therapy using Ganciclovir was administered to 4 patients. Daily doses ranged from 200 to 350 mg. A second BAL was done before surgery to assess the absence of CMV in all cases. Pre-emptive antibiotic therapy was administered to three patients. Drugs and doses were based on the antibiogram and minimal inhibitory concentration. Fungal colonisations were considered as contaminations and were not treated.

3.3. Postoperative mortality and morbidity

All the 117 patients underwent oesophageal resection after a mean delay between chemoradiotherapy and surgery

Table 3
Microbiological findings in bronchoalveolar lavage (BAL) and pre-emptive treatment

Patient	Micro-organism	Delay RCT to BAL[a]	Delay BAL to surgery	Microbiological findings (BAL)[b]	Treatment	Length of treatment	Postoperative complications	Postoperative microbiological findings
1	PPM	28	32	CMV (Ig G+)	Ganciclovir	15	Pneumonia	*Staphylococcus*
8	PPM	33	30	CMV (Ig G+)	Ganciclovir	30	No	–
13	PPM	16	18	CMV (Ig G+)	Ganciclovir	15	No	–
20	PPM	33	8	CMV (Ig G–)	Ganciclovir	7	No	–
12	PPM	60	16	*Haemophilus*	Ciprofloxacin	16	Leakage	–
14	PPM	28	16	*E. coli*	Amoxicillin	8	Pneumonia	Unknown
35	PPM	30	25	*Haemophilus*	Amoxicillin	7	ARDS	*Haemophilus and Pseudomonas*
32	Non-PPM	33	14	*Candida famata*	No	–	Chylothorax	–
34	Non-PPM	69	13	*Torulopsis glabrata*	No	–	Pleural effusion	–
10	Non-PPM	33	16	*Aspergillus fumigatus*	No	–	No	–
39	Non-PPM	10	30	*Aspergillus niger*	No	–	No	–
28	Non-PPM	34	8	*Candida alb*	No	–	Cardiac failure	–
40	Non-PPM	30	11	*A. fumigatus*	No	–	Haemorrhage	–

[a] In days.
[b] CMV serologic status.

Table 4
Results of total BAL cytology

	Normal, $n = 32$	No PPM, $n = 6$	PPM, $n = 7$
Cell count (10^2/ml)	140	90	110
Macrophages (%)	80	73	52.5
Lymphocytes (%)	6	11	4
Neutrophils (%)	9	10	42

There were no significant differences in total volumes of retrieved BAL fluid in the different groups. There was a non-statistically significant increase in alveolar macrophages percentages in the three patient groups.

of 45.7 ± 9 days [11–99]. Intraoperative antimicrobial prophylaxis was cephalosporin-based. Timing for extubation was shorter in the BAL group (mean 13 ± 3 h) when compared to that of the control group (mean 19.5 ± 14 h; $p = 0.039$). Respiratory complications occurred in 37% in the control group and 40% in the BAL group (Table 5). Postoperative hospital mortality rates were similar in both groups (12.5 and 8%, respectively; NS). Pneumonia and ARDS were the most common complications leading to death. Among the seven patients who received pre-emptive therapy, three (43%) experienced postoperative respiratory events, with no mortality. In one patient, the same agent was detected pre- and postoperatively on BAL samples (*haemophilus*). Among those patients colonised with non-PPM, none developed fungal infection.

ARDS occurred in 14 patients (19%) of the control group and in three (7%) of the BAL group ($p = 0.064$). CMV pneumonitis occurred in six patients in the control group (8%) and was diagnosed on the basis of open lung biopsy (OLB) ($n = 3$) or BAL cultures ($n = 3$). Four of these six patients died (Table 6). None of the patients of the BAL group experienced CMV infection ($p = 0.08$).

Table 5
Clinical outcome of both groups

	Control group, $N = 72$	(%)	BAL group, $N = 45$	(%)
Operative mortality	9	12.5	4	8
90-Day mortality	11	15	6	13
Respiratory complications	27	37.5	18	40
Pneumonia	9	12.5	9	20
ARDS	14	19	3	7*
CMV pneumonia	6	8	0	0**
Cardiac failure	5	6	5	11
Prolonged ventilation	5	7	1	2
Reventilation	16	22	12	26
Surgical complications	14	19	11	24
Chylothorax	3	4	2	4
Leakage	5	7	4	8
Laryngeal paralysis	2	3	4	8

ARDS developed in 14 patients (19%) in the control group whereas three patients (7%) developed in BAL group ($p = 0.064$) (*). Cause of ARDS was attributed to CMV infection in six patients in control group (8%). CMV was detected in open lung biopsy (OLB) ($n = 3$) or from culture of BAL ($n = 3$). None of the patients from the BAL group developed CMV infection ($p = 0.08$) (**).

4. Discussion

4.1. Summary of main results

Our results suggest that after a neoadjuvant chemoradiotherapy for advanced oesophageal cancer, airway colonisation is a relatively frequent event (30%). They also suggest that colonisation by PPM may be a potential cause of postoperative respiratory complications. CMV pneumonitis was incriminated in 42% of the patients who developed ARDS, with an impressive two-third mortality rate. Pre-emptive treatment of bacterial and viral (CMV) colonisation seemed to be an effective option to prevent postoperative pneumonia and ARDS.

Table 6
CMV pneumonitis in the control group

Complications	CMV detection (days)	Delay between surgery and CMV pneumonitis	Postoperative outcome
ARDS	OLB	12	Recovery
ARDS	BAL	10	Death
ARDS	OLB	11	Death
ARDS	BAL	28	Death
ARDS	OLB	30	Death
ARDS	BAL	7	Recovery

Cause of ARDS was attributed to CMV infection in six patients in the control group (8%). CMV was detected in open lung biopsy (OLB) ($n = 3$) or from culture of BAL ($n = 3$). None of the patients from the BAL group developed CMV infection. Mortality rate of CMV pneumonitis was 66%.

4.2. Neoadjuvant therapy

Despite significant progress during the last decade, respiratory complications remain the major concern after oesophagectomy. Predictors of such complications include low FEV1, smoking status, advanced age, diabetes, low rate of albumin, poor performance status, use of transthoracic approaches, performance of extended lymphadenectomy, duration of one lung ventilation, timing of extubation, impaired postoperative pain management, and preoperative chemoradiotherapy [3–7]. Pathways by which neoadjuvant chemoradiation make postoperative respiratory complications happen are multiple.

Some authors have postulated that preoperative chemoradiation could lead to leucopoenia, anorexia, weight loss and interstitial pneumonitis [12]. It was shown recently that chemoradiotherapy leads to immunosuppression by severely impairing proliferative capacity of T lymphocytes [13]. Indeed, T lymphocytes play a key role in patient's defences against bacterial, viral and fungal infections. As well, it has been shown that surgery by itself could suppress cell-mediated immunity temporarily [14]. Defects in T cell proliferation and the resulting decline of IL-2 and IFN productions, expose the patients to an additional risk for sepsis [12–14]. Radiation-induced tissue damage could make the lung parenchyma more vulnerable to postoperative complications [15]. In patients treated with induction chemoradiotherapy, higher radiation doses result in increasing impairment of gas exchange by an alteration of post-cCRT Dlco [3]. Albeit the bronchial epithelium is somewhat radioresistant, radiation promotes metaplasia, alters mucus production, and results in focal necrosis and shedding of ciliated epithelial cells [12,16]. These modifications of the respiratory mucosal surface act in a manner that increases its ability to bind micro-organisms and increases the risks of superimposed infections.

4.3. Bacterial and viral airways colonisation

The lower bronchial tree is normally sterile in healthy people. A multitude of conditions may alter local defence mechanisms in the airways (e.g., impaired mucociliary clearance and expectoration), that may in itself lead to distal airway colonisation or infection. To the best of our knowledge, no data is available in the setting of oesophageal cancer. In contrast, prior airways colonisation has been suggested as a significant cause of pneumonia after neoadjuvant treatment before lung cancer resection. Patients with lung cancer often present with COPD, and consequently a roughly 40% rate of bacterial airway colonisation has been reported [9,10,16]. However, an association between previous bacterial colonisation and occurrence of postoperative respiratory infections could not be demonstrated firmly. One reason of the failure is that the process leading to infection is probably versatile.

One of the striking results of the present study is the 9% incidence of preoperative CMV detection in BAL group patients, and the 42% incidence of CMV infection in those patients who experienced ARDS postoperatively with a 66% mortality rate. CMV infection is a well-known problem in patients treated by high-dose chemotherapy for haematologic diseases [11], in HIV-infected patients, and in lung transplantation recipients [17]. Data on cancer patients are scarce, but CMV infection has been incriminated particularly if steroids were a component of the therapy. We have previously reported CMV as a possible cause of ventilator-associated pneumonia and ARDS [18]. In a prospective study, Heininger et al. [19] reported an incidence of 35.6% of active CMV infections in surgical ICU patients. These findings focus on two key points: diagnosis and treatment. In our study, we observed six cases of postoperative CMV pneumonia detected by BAL fluid cultures ($n = 3$) and/or open lung biopsies ($n = 3$). The diagnostic sensitivity of BAL using shell vial culture technique is low [20]. In contrast, OLB provides both microbiologic and pathological arguments, but is a rather invasive procedure, even if we reported recently its high benefit/risk ratio [21]. Routine blood determination of CMV pp65 antigen or PCR for the early detection CMV remains to be established in this setting. It has been demonstrated that pre-emptive Ganciclovir treatment reduces CMV end-organ disease and is accurately life-saving in bone marrow transplant recipients [22]. Ganciclovir treatment has also been associated successfully in symptomatic CMV diseases in immunocompromised patients [23]. A recent meta-analysis concluded that the use of Ganciclovir reduces the mortality of cytomegalovirus diseases in solid-organ transplant recipients [24]. These data suggest that pre-emptive antiviral therapy could prevent postoperative CMV infection or reactivation. As a matter of fact, none of the patients who received pre-emptive anti-viral therapy developed ARDS and/or CMV infection postoperatively.

4.4. Limitations

This exploratory observational study has several strong limitations. First, it was not designed as a randomised prospective trial, and possible bias may exist. The groups submitted to comparison, however, are contemporaneous, similar regarding main clinical characteristics, identical in fulfilling strict criteria for a multimodality treatment, and homogeneous regarding the invasiveness of surgery, i.e. transthoracic approach, two-field lymphadenectomy and temporary one-lung ventilation. Second, we did not assess airways colonisation in contemporaneous patients proposed to first-line oesophagectomy, thus the impact of neoadjuvant treatment remains speculative. Third, we acknowledge that we did not monitor longitudinally any inflammatory cytokine in BAL samples, which might have supported the working hypothesis of an accumulation of subsequent events in the genesis of respiratory complications (multiple hits hypothesis). Finally, our study did not include routine BAL investigations for the microbiological diagnosis of post-operative respiratory infections, and this limitation could have strongly biased the comparison between bronchoscopic perioperative colonisation and postoperative infection. However, this limitation is inherent to the clinical setting, in which the invasiveness of performing a bronchoscopy in a non-ventilated hypoxemic patient has to be weighted against the drawbacks of a probabilistic treatment.

Despite these limitations, we believe that the present study carries some new information. On the basis of our results, we elaborated the following comprehensive model.

Respiratory complications are likely triggered by either a single massive insult, such as a major surgical complication, or a series of less intense insults (multiple hit hypothesis). We recently demonstrated that inadequate one-lung ventilation could be one of these insults [25]. We hypothesise that preoperative radiochemotherapy and airways colonisation at the time of surgery may represent some additional 'hits'. Combination of these factors within a short period of time in a same patient may play a crucial role in initiating and/or propagating a compartmental inflammatory response leading to respiratory failure, then a systemic inflammatory process leading to multiple organ failure and death.

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Appendix A. Conference discussion

Dr J. Schirren (*Wiesbaden, Germany*): Thank you very much for this nice presentation. My question is: oesophageal cancer is correlated with alcoholism and smoking. How many smokers do you have in your group and did you have a sensation of smoking preoperatively?

Dr D'Journo: Smokers rate was comparable in both groups, in about 60% of cases. Before surgery we asked the patients to stop smoking three months beforehand.

Dr T. Grodzki (*Szczecin, Poland*): Thank you for the nice presentation. However I am a little bit confused with your data. Because you treated by antibiotics just seven patients, if I noticed properly, of more than 100. And you conclude very strong conclusions. First of all you don't know how many of the control group were colonised by PPM and second the majority of your RDS was caused by viral infection. What is the message of your presentation? Should we do bronchoscopy preoperatively, should we give antibiotics or what?

Dr D'Journo: In departments who perform routinely oesophageal surgery for cancer and who are already experienced on ARDS after oesophagectomy, assessment of microbiological colonisation of airway must provide conclusive results to explain postoperative development of respiratory infections. We acknowledge several limitations of our results but we believe that these data could provide new information for clinicians to avoid postoperative ARDS.

Dr T. Lerut (*Leuven, Belgium*): I am surprised by the high incidence of CMV, of course it may be so because you are searching for it, I just wanted to know whether you see the same incidence of CMV in your pulmonary operative material? What is the overall incidence or prevalence of CMV in your hospital, in your department?

Dr D'Journo: The idea of this study was made when we observed CMV pneumonia on open lung biopsy in the ICU of our hospital. In our institution, Professor Laurent Papazian, has demonstrated that for patients with mechanical ventilation there was a very high rate of CMV pneumonia. He described it as the so-called ventilator-associated pneumonia (VAP). The VAP

was related to CMV in half of the cases and might be due to a colonisation of the airway. We didn't assess the colonisation for lung resection and we focused our results on oesophageal resection.

Dr S. Mattioli (*Bologna, Italy*): You didn't randomise the study, how did you form the two groups?

Dr D'Journo: It is not a randomised study; it is just an observational prospective study. We proposed prospectively to the 117 patients who received neoadjuvant chemoradiotherapy to participate in this study with a preoperative fiberoptic BAL. Forty-five patients accepted to participate in this study and the others didn't. We prospectively compared the two groups.

Dr D. Van Raemdonck (*Leuven, Belgium*): Can you elaborate on the technique of the lavage that you did in these patients? What was the volume and what was the risk in doing a lavage in a patient immediately preoperatively?

Dr D'Journo: I didn't give the information into the slides. The pneumologist put about 100 ml of serum and retrieved some material to do microbiological studies and cytology. The most important thing is to perform a bronchial lavage into the alveoli space because otherwise you have a contamination from the main bronchus.

Dr D. Wood (*Seattle, WA*): Which surgery procedures underwent preoperative bronchial lavage and how long were patients treated before surgery?

Dr D'Journo: All positive patients with BAL were treated about 15 days before surgery. We accepted a delay of 1 month before surgery to do the pre-emptive treatment.

Dr Wood: Then perhaps the question is whether the results may be from actually a delay between induction therapy and surgery rather than the treatment itself.

Dr D'Journo: Yes, it is.

II.2.3 Discussion

Les résultats de cette deuxième étude démontrent qu'une colonisation bronchique proximale est fréquente chez les patients proposés pour une chirurgie d'exérèse œsophagienne après radio-chimiothérapie préopératoire. Leur incidence est évaluée à près de 30 %. Cette colonisation est polymorphe. Sept des patients présentaient une colonisation à germes potentiellement pathogènes (*S. aureus*, *E. coli*, *H. influenzae* et CMV) et 6 présentaient une colonisation à germes considérés comme non-pathogènes (Candida sp., Aspergillus sp., Toruloptis sp.). Une séroconversion IgM-IgG CMV était notée chez un patient avec un LBA positif.

Un traitement préemptif des colonisations, adapté aux germes potentiellement pathogènes, a permis une réduction significative du taux de complications respiratoires sévères (SDRA) et du taux de pneumopathies postopératoires à CMV documentées par biopsies pulmonaires. Un des résultats inattendu de notre étude est le taux élevé de colonisation potentielle à CMV (9%) dans le groupe des patients ayant reçu un LBA préopératoire. Ceci confirme les observations préalablement faites dans notre établissement où le CMV était incriminé comme agent potentiellement pathogène dans les complications respiratoires de plusieurs patients opérés en chirurgie thoracique *(Papazian et al, 1996)*. Nos résultats, documentés par biopsies pulmonaires, confirment l'étiologie virale à CMV de près de 49 % des SDRA postopératoires dans notre étude, avec une mortalité voisine de 66%.

Néanmoins, notre étude présente un certain nombre de biais qui ne permettent pas d'extrapoler nos résultats à l'ensemble de la chirurgie thoracique oncologique. La première limitation tient au design même de l'étude pour lequel deux groupes non randomisés ont été comparés rétrospectivement sur la base de données prospectives. Ceci induit indubitablement un biais de sélection. Néanmoins, les deux groupes comparés sont deux groupes homogènes soumis au même traitement préopératoire sur une même période d'étude. La deuxième limitation repose sur l'absence de groupe contrôle ne recevant pas de radio-chimiothérapie préopératoire. La troisième limitation repose sur les méthodes d'analyses microbiologiques utilisées. En effet, l'analyse microbiologique n'a été qu'une analyse uniquement phénotypique de l'ensemble des prélèvements réalisés en préopératoire. Par ailleurs, les prélèvements ne concernaient que l'évaluation des bronches proximales sans avoir de prélèvements des bronches distales ou du parenchyme pulmonaire. Enfin, l'analyse et le receuil de prélèvements postopératoires constituaient un

véritable problème dans l'identification des flores pathogènes et directement impliqués dans les infections respiratoires nosocomiales.

Malgré ces limitations inhérentes à la recherche clinique chez l'être humain, nos résultats documentent pour la première fois et de manière objective, une colonisation bronchique en chirurgie œsophagienne. Sur la base de nos résultats, nous nous sommes interrogés sur les moyens diagnostiques nécessaires à la mise en évidence de pathogènes potentiels. En effet, les méthodes de prélèvements et de mise en culture (méthode phénotypique) limitent considérablement l'information potentielle que l'on pourrait obtenir par une approche systématique et exhaustive. La participation du CMV en tant que germe colonisant préopératoire et pathogène postopératoire, souligne la part probable des germes émergents ou difficilement cultivables.

La conclusion de notre travail était d'envisager la mise en pratique d'outils moléculaires modernes et innovants dans l'identification des germes colonisants bactériens, viraux et émergents en chirurgie d'exérèse pulmonaire pour cancer.

Article 3

II.3 Article 3

Universal detection of bacteria and herpes viridae in distal airways of patients undergoing lung cancer surgery shows a link between CytoMegaloVirus and postoperative respiratory complications

II.3.1 Introduction

Comme nous l'avons vu précédemment, il existe un certain nombre de limites cliniques et microbiologiques à l'établissement d'un modèle reproductible et compréhensible dans la physiopathologie des infections respiratoires postopératoires en chirurgie thoracique.

Partant du principe que les bronches distales et le parenchyme pulmonaire sont le siège certain de l'infection postopératoire, nous nous sommes intéressés à leur éventuelle colonisation avant la chirurgie. En se basant sur le modèle de l'exérèse pulmonaire pour cancer, nous avons formulé l'hypothèse qu'il existait d'éventuels pathogènes bactériens, viraux ou émergents au niveau des bronches distales ou du parenchyme pulmonaire au moment de la chirurgie. Les données existantes dans la littérature sont controversées. Seules trois études ont mis en culture ce type de prélèvements. Leurs résultats sont contradictoires. Deux études documentent des cultures positives *(Wansbrouh-Jones et al, 1991) (Ioanas et al, 2002)*. Une seule considère ces sites comme stériles *(Schlusser et al, 2006)*. Les données issues de ces trois études sont criticables dans la mesure où leur méthodologie n'a été basée que sur l'analyse phénotypique de mise en culture traditionnelle.

Compte tenu des limites représentées par les techniques phénotypiques, nous avons formulé l'hypothèse que des techniques de biologie moléculaire d'amplification universelle des ADN présents dans les échantillons suivies du clonage des produits de PCR et du séquençage de ces clones, appliqués à des échantillons obtenus des bronches distales et de biopsies pulmonaires, permettraient l'identification de pathogènes bactériens, viraux ou émergents. Cette approche permettrait d'envisager un lien plus précis entre colonisations bronchiques et complications respiratoires, et permettrait l'identification de pathogènes difficilement cultivables ou émergents.

A partir d'une cohorte consécutive de 87 patients, opéré sur une période d'étude de 9 mois, nous avons effectué des prélèvements distaux sur pièce d'exérèse pulmonaire en

appliquant des techniques hautement spécifiques de biologie moléculaire basées sur la PCR (Polymerase Chain Reaction) en temps réel (rt-PCR), en ciblant l'ARN 16S ribosomique (universel), le CytoMégaloVirus (CMV) et l'Herpes Virus Simplex (HSV).

Comme nous l'avons mentionné, les méthodes phénotypiques peuvent se révéler infructueuses (Pace, 1997). Certaines souches bactériennes isolées en microbiologie clinique sont, en effet, difficiles à identifier par les tests phénotypiques habituellement réalisables au laboratoire et regroupant les caractères culturaux (atmosphère, température, milieux et durée), les caractères morphologiques (aspect après coloration de Gram et coloration de Ziehl) et les caractères biochimiques (activités enzymatiques simples : catalase et oxydase, activités enzymatiques métaboliques, métabolisme des sucres). Il peut s'agir d'espèces mal identifiées par les galeries d'identification manuelles ou automatiques parce que fastidieuses (bactéries à croissance lente), inertes (exprimant peu de caractères phénotypiquement discriminants) ou rares car nouvellement décrites ou récemment individualisées et absentes des thésaurus des galeries. D'autre part, un certain nombre de prélèvements reste stérile en culture conventionnelle, notamment dans le cas de bactéries non ou difficilement cultivables. L'identification par biologie moléculaire peut alors permettre de poser un diagnostic, dans des échantillons issus du corps humain.

La technique la plus couramment utilisée repose sur l'amplification puis le séquençage partiel du gène *rrs* codant l'ARN ribosomal 16 S, gène chromosomique d'une taille d'environ 1500 paires de bases, présent chez toutes les espèces bactériennes (gène universel), dont la séquence est spécifique de chaque espèce et dont les extrémités 5' et 3' sont conservées dans toutes les espèces bactériennes. Les prélèvements cliniques (LBA ou biopsie pulmonaire) doivent subir au préalable une étape d'extraction pour obtenir un ADN purifié. Le gène codant pour l'ARNr 16 S est amplifié par PCR en utilisant deux amorces universelles complémentaires des extrémités 5' et 3' conservées. Ce produit d'amplification est ensuite séquencé. Il existe actuellement des séquenceurs semi-automatiques qui ont banalisé cette étape dans les laboratoires de biologie moléculaire. Pour chaque séquence obtenue, des séquences homologues sont recherchées dans des banques de données via le réseau Internet. En fonction de son homologie, la souche bactérienne peut être positionnée parmi l'ensemble des espèces connues sous forme d'un arbre phylogénétique construit par un logiciel.

Nous avons évalué, sur les prélèvements de la pièce de résection, l'intérêt d'une recherche microbiologique innovante centrée sur les bactéries (recherche de l'ARN16s ribosomique dit universel) et les principaux virus rencontrés dans notre expérience clinique et sur la base de nos travaux précédents (CMV et HSV). Un lien entre une potentielle

colonisation bronchique distale (PCR positive) et le développement d'une complication respiratoire était recherché, de même qu'une corrélation entre agents colonisants et agents incriminés dans l'infection postopératoire.

II.3.2 Article

Soumis à l'***American Journal of Respiratory and Critical Care in Medicine***

Universal detection of bacteria and herpes viridae in distal airways of patients undergoing lung cancer surgery shows a link between CytoMegaloVirus and postoperative respiratory complications

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Words in abstract:

Words in text:

Objective: To assess with a molecular approach the incidence of airways colonizations in patients submitted to lung cancer surgery.

Methods: A prospective study of all patients undergoing major lung resections for cancer was performed over a 9-months period. Microbial assessments were obtained from broncho-alveolar lavage (BAL) or lung biopsies (LB) from the resected lung specimen during surgery. Potential pathogen micro-organisms were detected using real-time PCR (polymerase chain reaction) assays targeting bacterial 16S rRNA gene, CMV and HSV. All postoperative events were recorded and compared to the results of the preoperative microbiological assessment.

Results: 87 patients were included in the study after informed consent. A total of 240 samples were investigated by real-time PCR and 16 (6.3%) were positives in 13 patients. These 13 patients (15 %) constituted the positive-PCR group (6 in BAL, 5 in LB and 2 in both). The remaining 74 patients constituted the negative-PCR group. Colonizing agents were exclusively virus detected by positive real-time PCR (CMV, n=12 and CMV+HSV, n=1). All the 16s rRNA remained negatives. Of the 87 patients, 27 (31 %) developed postoperative respiratory complications, 18 (24 %) in negative-PCR group and 9 (69 %) in positive-PCR group (p=0.003). Pneumonia occurred in 6 (8%) in negative-PCR group and in 4 (31 %) in positive-PCR group (p=0.039). Among the 9 positive CMV-PCR patients with postoperative respiratory failures, 1 had a concordant CMV pneumonia and 8 had inconclusive concordance. On multivariate analysis, positive-PCR was the sole independent risk factor of postoperative respiratory complications (OR: 6.7: CI95 %: 1.35-33). Positive predictive value of positive-PCR in detection of postoperative respiratory complications was 0.70 (CI95%:0.5-0.9). and the surface under ROC curve was 0.72 (CI95%: 0.5-0.8).

Conclusions: When tested by molecular techniques, lung parenchyma and distal airways are free from bacteria but CMV was found in an unexpected high proportion. With a good positive predictive value, CMV-PCR should be seen as a reliable marker to identify patients at risk of postoperative respiratory complications.

INTRODUCTION

To date, postoperative respiratory complications remain the most frequent and serious complications, as well as being the primary cause of hospital death after lung cancer resection [1]. These complications are a heterogeneous group of diseases with various causes and pathogenic mechanisms. There is growing evidence that these different forms of respiratory failures should have a common substratum, notoriously infectious, with similar clinical presentations including atelectasis, tracheobronchitis, nosocomial pneumonia and acute respiratory distress syndrome (ARDS) [1-3]. Vast majority of these respiratory failures are mostly bacterial but part of them are frequently undetermined with inconclusive postoperative microbiological results [4,5]. Because infectious etiologies have been highly incriminated in the occurrence of these respiratory failures, airways colonizations are supposed to be an essential first step in the pathogenesis of nosocomial pneumonia and ARDS occurring in hospitalized and chronically ill individuals fulfilling all predisposing factors to bronchial colonization [5-9].

Few studies have addressed the problem of the airways colonization in patients submitted for lung cancer surgery [5-11]. From the previous published data, conclusions that can be reached remain however limited and can be summarized as follow: i) colonization is frequent in proximal airways but there is a huge variance in their incidence (18 to 41 %);ii) incriminated colonizing micro-organisms are notoriously bacterial (*H influenzae*, *S pneumoniae* and *S aureus)*. Data on viral colonization are not available; iii) identification of airways colonization is strongly dependant of sampling methods used. Airways colonization seems to be limited to proximal airways, in contrast to distal airways and to lung parenchyma, both considered as sterile by phenotypic methods of culture. iv) If an association between colonization and postoperative failures is clinically supposed, microbiological concordance between pre and postoperative agents remain unclear. Documented postoperative micro-organism is often different from the colonizing agents. v) All previous studies have investigated bronchial colonization using traditional phenotypic methods of cultures that precludes for definitive conclusion when considering that majority of

microbiological species required modern techniques of culture to be detected and identified. This is likely the strongest limitation of the current information concerning AWC in lung cancer patients.

Indeed, knowledge of microorganisms in the microbial diversity and in the biosphere has depended in the past mainly on studies of pure cultures in the laboratory. Recent data have demonstrated that 99% of organisms seen microscopically are not cultivated by routine techniques and required modern and innovative techniques to be identified [12,13]. Thus, development of microbiology has obviated the requirement to culture microbes to identify them. Instead of culture test, RNA genes amplification and sequencing of the bacterial 16S ribosomal RNA provide a new point of view of the problem. As a result, genetic and sequence-based methods have been successfully used in several infectious diseases with identification problem. This is the case in pleural infection [14], in community-acquired pneumonia [15] or in cystic fibrosis patients [16]. To our knowledge, there is no specific data on genetic and molecular assessment of airways colonization in patients submitted to lung cancer surgery.

In order to investigate with molecular techniques of identification the incidence and the characteristics of the colonization at the level of the distal airways, we have performed a 9-months prospective study including consecutive patients undergoing major lung resection for lung cancer in one-single institution.

MATERIAL AND METHODS

Study design

This study was conducted according to the current regulations for clinical research in France, and was financially supported by the University of the Mediterranean and the Assistance Publique - Hôpitaux de Marseille, after approval by the institutional review board. Informed consent was obtained from all patients before surgery.

All patients undergoing major lung resection between February 1, 2009, and October 30, 2009, for lung cancer and without signs of acute respiratory infections were eligible for this

study. Patients treated with antibiotics (because of respiratory or extrarespiratory infections) in the week preceding surgery were excluded from the study. All patients received a planned protocol for antibiotic prophylaxis according to the current French recommendations. Antibiotic prophylaxis consisted in a perioperative single dose of cefamandol 1.5 g repeated with 0.75 g when surgery exceeded 2 hours. Patients eligible for entry into the study but receiving explorative thoracotomy because of intraoperative findings were excluded from the study.

All the data concerning patient characteristics, results of microbiological studies, treatment procedures, and outcome were prospectively collected. Most patients were hospitalized the day before surgery for preoperative surgical and anaesthetic assessment. Surgery had been scheduled in all cases during a preoperative visit to the outpatient clinic. Information about age, sex, weight, lung function, indication for lung resection, and performans status was collected. White blood cell count (WBC), chest X-ray, and clinical examination were systematically performed to eliminate the possibility of underlying pneumonia or bronchitis. Nutritional status was assessed by determination of body mass index and evaluation of possible weight loss in the previous 6 months. Lung function was evaluated by spirometry.

All patients were intubated with a double-lumen endobronchial tube to perform single-lung ventilation. Lung resections were performed according to standard techniques. Side, type of resection, possible associated sleeve bronchial or chest wall resection, previous thoracotomy, and total procedure time were recorded. Postoperative outcome was collected prospectively.

Microbiological samples

During the surgical procedure and immediately after lung resection, a broncho-alveolar lavage (BAL) was performed in the resected specimen with a standardized protocol: 50 ml of physiological saline were instilled into the opened bronchus of the resected specimen and washing for microbiologic examination was obtained by suction from lung lobe. Endobronchial aspirate samples were obtained with a sterile suction catheter equipped with a mucus collection tube. When BAL has been performed, lung biopsies (LB) were taken from

lung resected specimen in healthy lung and in tumoral lung for microbiologic examination. After receiving the samples, approximately 3 mm^3 of lung biopsies were cut and conserved in RNAlater (QIAGEN, Courtaboeuf, France) at -80℃ unt il used. BALs were stored directly at -80℃ and 200 µL were used later for nucleic acid ex traction as described below.

Microbiological analysis

At the end of the study period, microbiological analysis was done between December 2009 and January 2010. Microbiological analysis was undertaken through a standardized protocol of DNA and RNA extraction for polymerase chain reaction (PCR) sequencing and amplification to rule out a possible colonization into the airways.

Before extraction, the lung biopsies were removed from RNAlater and were transferred into eppendorf tubes containing 400 µL of distilled water and 0.3g of acid-washed glass beads. The tubes were then vortexed for 2 minutes to achieve mechanical lysis and finally 200 µL of supernatants were collected for extraction. DNA and RNA were extracted from BAL and lung specimens using the instrument BioRobot EZ1 (QIAGEN) with the customized extraction kit (EZ1 Virus Mini Kit v2.0) following the manufacturer's instructions. The quantitative PCR assays were conducted in ABI 7900 HT Fast Real Time PCR system (Applied Biosystems, foster city, CA, USA). The sequences of the primers and probes used were as described previously targeting CMV [17], HSV [18], and 16S rRNA gene [16,19]. The quality of nucleic acid extraction was evaluated using primers and probe for albumine. Amplification mixture contained 10 µL of master mix (QuantiTect Probe PCR, QIAGEN), 2 µL (2 µmol/µL) of probe, 0.5 µL (10 pmol/µL) of each primer, 2 µL of distilled water, and 5 µL of DNA/RNA template. The PCR conditions involved an initial denaturation step at 95℃ for 15 min, followed by 45 cycles of denaturation at 95℃ for 20 s, and anneal ing/elongation at 60℃ for 1 min. PCR assays were done in two set of experiments for all samples to confirm the results.

Patients were considered as colonized if at least one of the two repeated PCR assays became positive to potential pathogenic micro-organisms. This constituted the "positive PCR group" whereas patients with negative PCR constituted the "negative PCR group". Association between positive PCR and postoperative respiratory failures was investigated

secondarily by screening all the patients' medical charts. Comparison between preoperative and postoperative samples was investigated to draw microbiological concordance between the two events. At last, all potential factors incriminated in the occurrence of postoperative respiratory failures were included in a logistic regression. Factors involved in the occurrence of positive PCR were also identified as well.

Postoperative assessment

A policy of early extubation was systematically employed. Decisions concerning intensive care unit (ICU) hospitalization after resection were established on the basis of type and extent of resection, predicted postoperative lung function, and associated comorbidities. Postoperative analgesia was achieved by one of the following methods: patient-controlled analgesia (morphine), thoracic epidural analgesia or thoracic paravertebral block. A regular program of physiotherapy was started on the day of operation. Oral alimentation was started on postoperative Day 1 after lobectomy and on the second day after pneumonectomy. In cases of previous head and neck surgery or if recurrent nerve paralysis was observed, a special program for realimentation was started. Patients were examined twice per day, and measurement of WBC was performed on Days 0, 1, 3, 5 and 7 if necessary. Chest roentgenograms were done postoperatively once per day during the period of chest drainage.

Postoperative complications

Respiratory complications were defined by all medical events concerning lung parenchyma (i.e. sputum retention, atelectasis, pneumonia, acute lung injury (ALI), acute respiratory distress syndrome (ARDS)) in the absence of early surgical complications. Sputum retention was defined by all respiratory events requiring bronchoscopic aspiration or non-invasive ventilation but without formal signs of infection (fever<38.5°, white blood cell count <12x10^3/ml). Pneumonia was defined as the occurrence of new and persistent lung infiltrates on chest radiograph with a body temperature exceeding 38.5℃, the evidence of purulent sputum and the existence of leukocytosis (> 12.10^9.L^{-1} or < 4. 10^9.L^{-1}). The infectious nature was deduced from the positivity of quantitative culture after protected brush specimen

or after bronchoalveolar lavage. Bacteriological culture of both lung biopsies and BALs and phenotypic identification were performed with standard methods as previously described [16]. In patients receiving mechanical ventilation, the diagnosis of pneumonia was confirmed by a culture of BAL > 10^4 cfu/ml. ALI and ARDS were defined according to the American European Consensus Conference on ARDS criteria [20]. ALI was defined as a PaO2/FiO2 level of less than 300 mmHg and ARDS as a PaO2/FiO2 level of less than 200 mmHg. Additional criteria included the presence of bilateral infiltrations on chest radiographs and no clinical evidence of left atrial hypertension. Surgical complications included bronchial fistula, recurrent nerve paralysis, chylothorax, pleural effusion requiring chest tube drainage, empyema and bleeding. Hospital mortality was defined as all death occurring during the in-hospital stay (< and > 30 days). Infections occurring within 1 month of surgery were recorded. Wound infection was defined as a reddened, painful, and indurated wound not necessarily associated with bacteria isolation. Empyema was defined as the presence of purulent fluid in the pleural drainage or as the isolation of pathogens from the pleural cavity. Other nosocomial infections were defined according to standard definitions. Need for antibiotics other than antibiotic prophylaxis was also recorded.

Postoperative microbiological assessment

Our general policy was to maintain a high clinical suspicion for postoperative respiratory infections and to try to document whenever it was possible the bacteria involved by sputum samples, quantitative fiberoptic bronchoscopy aspiration and/or protected specimen brush sampling. These conditions was achieved when there were evidence of: (*1*) abnormal radiographic findings (new or changing radiographic infiltrates that persisted after physiotherapy or bronchoaspiration), (*2*) fever greater than 38℃, and (*3*) one of the following criteria: a rise in C-reactive protein value or WBC count over the last 24 h (with WBC ≥ 12 x 10^9/L) or a new rise in procalcitonin value or an increase and modification of the expectorate, possibly with purulent aspect. Patients with bronchial aspirates (more than 10^5 cfu/ml), protected specimen brush samples (more than 10^3 cfu/ml), or positive blood cultures represented the “documented” group of respiratory complications. If the significant cutoff

values were not reached, but clinical and radiologic improvement occurred after the administration of antibiotics, patients were considered as having non-documented respiratory complications.

Statistical Analysis

Data were analyzed using the SPSS 17.0 package (SPSS Inc., Chicago, IL). Results are expressed as mean ± SD or median (range) for quantitative variables and as percentage for qualitative variables. The Mann–Whitney U test was used for quantitative variables. The Pearson chi-square or Fisher exact test was applied for qualitative variables. Predictive factors of AWC were obtained by univariate and multivariate analysis. Logistic regression was employed to determine variables to be included in the multivariate analysis using *P* values below 0.2. *P* values below 0.05 were considered to indicate statistical significance.

RESULTS

Over a 9-months period, 87 consecutive patients were included in the study after inform consent. There were 62 men and 25 women with a mean age of 63 ± 9 years. Table 1 summarizes the main patients' characteristics.

Airways colonizations detected by PCR

From the total cohort of 87 patients, a total of 241 samples were collected: 79 from BAL, 85 from healthy lung and 77 from lung tumor. These represent a mean of 2.7 samples per patient. All the samples were suitable for repeated real-time PCR amplification. Albumine test was performed to test quality of DNA extraction. Table 2 summarizes the results of double PCR extraction. Among the 241 samples, 1 sample was excluded from the analysis because of a lack of DNA material. From the remaining 240 samples, positive PCR was obtained in 16 samples (6.3 %) in 13 patients. These 13 patients (15 % of the total cohort of 87 patients) constituted the positive-PCR group (6 in BAL, 5 in lung biopsies and 2 in both). Pathogenic microorganisms were exclusively virus detected by positive PCR (CMV, n=12, CMV and HSV, n=1).

In the first part of the microbiological analysis, real-time PCR screening showed that 6 patients were positive to 16s rRNA. However, after standard PCR amplification and sequencing methods, these 6 amplicons were not from bacteria but from human origin. A second round of DNA extraction and 16S rRNA PCR amplification was done for these samples that were negative. These false-positive patients to PCR were considered as non-colonized. Accordingly, they were added to the 68 remaining patients with complete negative PCR to constitute the negative-PCR group.

Positive and negative-PCR group

There was no significant difference in term of clinical characteristics between the two groups according to the result of the PCR (Table 1). Patients with positive PCR had more alteration of diffusion lung capacity for carbon monoxide (DLCO), more alteration of global functional status (NYHA score), more diabetes melittus, more global tobacco consumption, existence of previous vascular diseases and more frequent previous thoracic surgery.

Postoperative outcome

Of the 87 patients, 27 (31 %) developed postoperative respiratory complications with a median delay of 4 days [range: 1 – 21 days]. According to PCR results, 18 patients (24 %) developed respiratory complications in negative-PCR group and 9 (69 %) in colonized group (p=0,003). Pneumonia occurred in 6 (8%) in negative-PCR group and in 4 (31%) in positive-PCR group (p=0,039). Table 3 summarizes the main postoperative outcome according to the PCR results. In-hospital mortality rate and length of stay in ICU were similar between groups. Length of hospital stay was significantly longer in the positive-PCR group (p=0.043).

We have adopted a policy to document microbiologically postoperative respiratory complications whenever it was possible by sputum samples, quantitative fiberoptic bronchoscopy aspiration and/or protected specimen brush sampling. Details of the 27 patients developing postoperative respiratory complications are provided in table 4. Postoperative cultures were positive in 18 patients (67 %) and negative in the remaining 9 patients (33%). Postoperative microbiological findings are exposed in Table 5. In the vast majority of cases, postoperative samples remained negative for traditional cultures or

indicated a Gram Negative Bacteria. Among the 9 positive-PCR patients with postoperative respiratory complications, 1 developed pneumonia with concordant pre and postoperative microorganisms (CMV). For the 8 remaining patients, concordance between pre and postoperative samples remained inconclusive because of negative cultures or polymicrobial nature (Table 6).

Multivariate analysis

We conducted two multivariate analyses in order to discriminate which factors were likely to influence occurrence of postoperative respiratory complications and the risk factors of a positive-PCR. Based on the two univariate analysis exposed in Table 1 and 7, we have included all variables with P values below 0.2 in the logistic regression.

In the first model dedicated to discriminate variables affecting postoperative respiratory complications (Table 8A), multivariate analysis revealed one independent variable: positive-PCR (p=0,02; OR:6,7, CI95%:1,35-33). In the second model dedicated to discriminate which factors were likely associated to a positive-PCR, multivariate analysis has identified an unique and independent factor: diabetes mellitus (p=0.031; OR:5,6; CI95%: 1.1-27) (Table 8B).

Sensitivity and specificity of positive-PCR on postoperative respiratory complications

We also conducted analyses to test reliability of positive-PCR in prediction of respiratory complications. Positive predictive value was 0.70 (CI95%: 0.44-0.94) and the positive likelihood ratio was 5 (CI95%: 1.68-14.82). Negative predictive value was 0.75 (CI95%: 0.52 – 0.99) and the negative likelihood was 0.714 (CI95% : 0.54-0.94). Sensitivity of positive-PCR was 33 % (CI95% : 15-51) with a specificity of 93 % (CI95% : 87-99). On ROC curve, area under the curve was 0.725 (p=0.01; CI95%: 0.56-0.88).

DISCUSSION

Our results suggest that, when investigated by molecular techniques, distal airways and lung parenchyma of patients operated for lung cancer resection are sterile from bacteria.

However, these sites present in high proportion virus and especially CMV. From the 240 collected samples obtained from our 87 included patients, positive CMV real-time PCR appears as frequent and should concern 15 % of those submitted to lung cancer resection. Of interest is the significant clinical association between the positive CMV real-time PCR and the risk to develop postoperative pulmonary complications. This assumption is also emphasized by the results of our logistic regression where positive-PCR was the sole independent factor incriminated in the postoperative respiratory complications occurrence. Moreover positive CMV-PCR should be seen as a reliable predictive marker of postoperative respiratory complications with a good positive predictive value.

In contrast to previous studies that have documented a bacterial colonization in proximal airways between 18 to 41 % [5-11], our series shows with modern and innovative techniques that lung parenchyma and distal airways have to be considered as completely sterile from bacteria. This is of paramount importance when considering that our patients fulfill all the predisposing factors of AWC such as smoking, cancer and chronic obstructive pulmonary disease. Our results differ to what has been previously reported with phenotypic methods of cultures of the resected specimen. In fact, previous studies have demonstrated that distal airways and lung parenchyma could present positive cultures between 7 to 22% [8, 21]. Wansbrough-Jones has documented bacteria cultured from 12 out of 54 lavage specimens from resected lung [21] and Ioanas has also reported 3 positives cultures from lung tissue biopsies from 41 resected specimens [8]. With a strong specificity, molecular techniques offer a modern approach where traditional techniques remain limited. This constitutes a strong scientific progress allowing microbiological limitations to be avoided.

Our observation provides substantial information regarding the mechanisms potentially implicated in the occurrence of postoperative respiratory infections. We have found that 16S rRNA PCR amplification from resected lung specimen as well as from BAL were negative. In fact these negative results are critical since positive predictive value of a positive PCR in this context will be very suggestive of an infection. Because, first, distal airways and parenchyma are notoriously sterile at the moment of the surgery, and, second that these locations are the

site of the nosocomial pneumonia, postoperative respiratory infections should be seen as the result of colonization by pathogens belonging from upper respiratory or digestive tract during the first postoperative days. Evaluation of the potential microbial diversity in such samples from upper respiratory tract could be useful in the future to better understand the link between colonization and postoperative complications using molecular techniques including 16S rRNA gene clonal library sequencing or 16S rRNA gene pyrosequencing [22,23]. Finally, recent metagenomic studies of the human microbiome using high-throughput sequencing analysis are currently ongoing in many clinical microbiology areas including respiratory tract infections [24-27] and may be indicated in the context of lung cancer surgery.

Among the total cohort of our study, 13 have presented positive CMV real time-PCR (15%) in lung parenchyma and in distal airways. This positive CMV PCR was associated with occurrence of postoperative pulmonary complications in 9 patients. Among these 9 patients, 1 patient had a documented postoperative CMV pneumonia and 8 remained with inconclusive postoperative samples. What remains unclear is if positive CMV real-time PCR in distal airways is the result of a viral replication of a potential pathogen or, conversely, the result of a reactivation from latency under specific local conditions or because of poor immunologic status. In others words, does positive CMV-PCR a cause of respiratory complications or a consequence of ongoing mechanisms favoring reactivation?

There are more arguments to believe that positive PCR is strongly associated to reactivation from latency. In critically ill patients, severe immunologic impairment is usually considered [28]. Active CMV infection is likely to occur in this context of “ICU-acquired immunosuppression” [29]. During recent years, CMV has been recognized as an emerging pathogen in critically ill patients who are not receiving immunosuppressive therapy [30-34]. However, the incidence of active CMV infection is debated and it has been studied essentially in surgical and trauma patients. Moreover, CMV incidence in strongly dependant of the country and the socioeconomic level. Sereoprevalence is near 30-70 % in developed countries whereas the rate can exceed 90 % in developed one [35]

Recently, a group from our institution has demonstrated from a prospective observational study that CMV infection was a frequent clinical problem entity in previously healthy medical ICU patients under mechanical ventilation. Indeed, among the 242 patients enrolled, 16% developed active CMV infection [36]. The incidence was in agreement with the results of a recent study conducted in a French surgical ICU which found CMV infection in 17% of the patients in whom the pathology was suspected [37]. This high CMV incidence probably reflects a national singularity in France and should not be extrapolated to others countries where seroprevalence of CMV strongly differs.

If positive CMV real-time PCR is considered as a marker of reactivation from latency, what remains unclear is the delay of appearance regarding the short delay between the onset of anesthesia and the lung resection (near 1 hour). This suggests that patients submitted to surgery probably present reactivation before surgery. Lung cancer, smoking, COPD, diabetes mellitus are so many factors that would explain this hypothesis. It is likely that a local or a general inflammatory immune response is the natural stimulus for reactivation of CMV. It has been demonstrated in mice that elevated TNF-α level in blood during systemic inflammatory response syndrome might promote CMV reactivation by direct stimulation of the CMV immediate-early enhancer / promoter region [38]. The role of TNF-α is illustrated by another mouse model of CMV reactivation induced by sepsis [39,40], with 100% of the preinfected mice developing active CMV infection. This should be crucial in this context when considering that causality between CMV reactivation and lung fibrosis is highly suggested [39,40]. Conditions which suppress the immune response may allow survival of cells which are truly latently infected and thus lead to the production of infectious virus [41]. Reactivation of CMV may therefore be secondary to other infections, and the ‘differentiation’ associated with reactivation of CMV may be a part of the host inflammatory response to that infection [42]. We acknowledge that we did not systematically investigate CMV infection during the postoperative period. CMV serology or assessment of pp65 antigenemia should have been the best appropriate methods of identification as it is reported [35].

If positive CMV-PCR is considered as an independent factor of postoperative respiratory complications, risk factors of positive CMV-PCR remain unclear. In fact, diabetes mellitus was considered as the sole independent factor in our multivariate analysis. We acknowledge that our study was not powered enough to individualize others potential risk factors of CMV reactivation. In fact, it seems speculative to conclude witch factors should be considered at risk because of the smaller numbers of patients. Previous thoracic surgery is an additional factor that came out in the multivariate analysis but without the statistical significance. This factor should be seen as a marker of prolonged hospital stay. However, length of preoperative stay is likely a surrogate for severity of illness and co-morbid conditions requiring inpatient work-up and/or therapy before the operation.

With a strong specificity and a good positive predictive value, regular CMV real-time PCR in distal airways at the moment of the surgery should be seen as a good marker to identify patients who are at high risks to develop respiratory complications. This provides for clinician an interesting tool to early identify a subgroup of patients where others predictive score remain limited and where objective techniques are missing. The cost of a CMV real-time PCR does not exceed one hundred euros and it is available in few hours. As a result, application of this technique in daily practice deserves further investigations to early detect patients presenting CMV reactivation in lung parenchyma and considered at high risk of respiratory complications.

CONCLUSIONS

When tested by molecular techniques, lung parenchyma and distal airways are free from bacteria but positive CMV real-time PCR was found in an unexpected high proportion. Positive CMV-PCR seems to be strongly associated with occurrence of respiratory complications. CMV in this setting should be seen as a witness of a poor immunologic status rather than a potential pathogen, in the context of an oncologic disease, favoring viral replication from latency. Whether or not this CMV reactivation is a cause of postoperative respiratory complications or a consequence of an ongoing inflammatory process, CMV real-

time PCR, with good positive predictive value should be seen as a reliable biologic marker to identify patients who are at high risks to develop respiratory complications.

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Table 1: Clinical characteristics among the 87 included patients and according to positive or negative-PCR status. BMI : body mass index; FEV : Forced expiratory volume in one second; VC: vital capacity; DLCO : Diffusion lung capacity for carbon monoxide; NSCLC : non small lung-cancer; COPD: chronic obstructive pulmonary disease; CRT: chemoradiotherapy; ASA: American Score of Anaesthesiologist; NYHA : New York Hear Association; TEA : thoracic epidural analgesia; PVB : paravertebral block; OCA: opiod controlled analgesia.

	All n=87	Negative-PCR n=74	Positive-PCR n=13	p
Preoperative clinical status				
Age (years, mean ± SD)	63,1 ± 9	62.7 ± 9	65.3 ± 9	0,359
Sex male, n (%)	62 (71)	52 (70)	10 (76)	0,452
Weight (kg, mean ± SD)	72,8 ± 21	71 ± 18	73 ± 29	0,785
Performans status (mean ± SD)	0,21± 0,4	0,19 ± 0,4	0,31± 0,4	0,396
BMI (mean ± SD)	24 ± 3	24± 4	25 ± 3	0,264
Charlson score (mean ± SD)	4,7 ± 1	4,8 ± 1	4,4 ± 1	0,611
FEV1 % of predicted (mean ± SD)	83 ± 18	84 ± 18	78 ± 18	0,238
DLCO % of predicted (mean ± SD)	72 ± 12	73 ± 11	66 ± 16	0,073
Preoperative risk factors for respiratory complications				
NSCLC, n (%)	74 (85)	63 (85)	11 (84)	0.619
Smoking history, n (%)	79 (90)	66 (90)	13 (100)	0.259
Smoking cessation (79 smokers), n (%)	57 (72)	48 (64)	9 (69)	0.72
Smoking PA (mean ± SD)	41 ± 25	40 ± 25	50 ± 24	0,178
Alcohol, n (%)	10 (1)	7 (9)	3 (23)	0.167
COPD, n (%)	33 (38)	27 (36)	6 (46)	0,357
Diabetes mellitus, n (%)	12 (13)	8 (11)	4 (30)	0,076
Previous cancer, n (%)	28 (32)	24 (32)	4 (31)	0,591
Previous CRT, n (%)	23 (26)	21 (29)	2 (15)	0,271
Neoadjuvant CRT, n (%)	9 (10)	7 (10)	2 (15)	0,403
ASA score (mean ± SD)	1,98 ± 0,5	1,9 ± 0,5	2,2 ± 0,5	0,323
NHYA score (mean ± SD)	1,6 ± 0,5	1,6 ± 0,5	1,8 ± 0,5	0,093
Previous cardiac disease, n (%)	12 (14)	9 (132)	3 (23)	0,253
Hypertension, n (%)	12 (14)	9 (12)	3 (23)	0.39
Peripheric vascular disease, n (%)	9 (10)	6 (8)	3 (23)	0,128
Details of operation				
Segmental resection, n (%)	4 (4)	3 (4)	1 (7)	
Lobectomy, n (%)	73 (83)	61(82)	12 (92)	
Sleeve lobectomy, n (%)	7 (8)	6 (8)	1 (6)	0.536
Pneumonectomy, n (%)	3 (3)	3 (4)	0	
Chest wall en-bloc resection, n (%)	2 (2)	2 (2)	0	
Previous thoracic surgery, n (%)	5 (5)	3 (4)	2 (16)	0,159
Side Right / Left	54 / 33	46 / 28	5 / 8	1
Analgesia TEA/PVB/ OCA	68 / 18 / 4	57 /13 / 4	8/5/0	_-

Table 2: Results of repeated real-time (rt) PCR assays performed on 241 samples obtained from 87 patients. Patients were considered as positive if at least one of the two repeated PCR became positive to potential pathogenic micro-organisms. BAL : bronchoalveolar lavage; LB: lung biopsy; HSV: Herpes Simplex Virus; CMV: Cytomegalovirus. * samples excluded from the analysis because of insufficient DNA material. ŦOne patient had a co-infection CMV and HVS.

		BAL	LB from healthy lung	LB from tumoral lung	Total numbers of patients with positive-PCR
Number of samples		79	85	77	
RNA extraction		79	85	77	
Number of samples analyzed		79	85	77	
Albumine	+	79	77	76	
	-	0	0	1*	
16S rRNA	*2 positive rt-PCR*	0	0	0	0
	1 positive rt-PCR	0	0	0	
	Negative	79	85	77	
HVS	*2 positive rt-PCR*	0	0	1	1
	1 positive rt-PCR	1	0	0	
	Negative	78	77	76	
CMV	*2 positive rt-PCR*	4	5	2	13Ŧ
	1 positive rt-PCR	3	0	0	
	Negative	72	72	75	

Table 3: Outcome of the 87 patients according to the PCR results.

	All	Negative-PCR	Positive-PCR	p
	n=87	n=74	n=13	
Overall complications, n (%)	43 (49,4)	32 (43)	11 (84)	0,006
Respiratory complications, n (%)	27 (31)	18 (24)	9 (69)	0,003
Atelectasis, n (%)	11 (13)	8 (10)	3 (23)	0,21
Pneumonia, n (%)	10 (11)	6 (8)	4 (31)	0,039
ARDS, n (%)	6 (7)	4 (5)	2 (15)	0,218
Reintubation, n (%)	8 (9)	5 (7)	3 (23)	0,094
Non-invasive Ventilation, n (%)	10 (11)	8 (11)	2 (15)	0.461
Bronchoscopic aspiration, n (%)	13 (14)	8 (11)	5 (38)	0.022
Cardiac arythmia, n (%)	9 (10)	8 (11)	1 (8)	0.784
Recurrential injury, n (%)	4 (5)	4 (5)	0	1
Pneumothorax, n (%)	1 (1)	1 (1)	0	1
Antibiotics, n (%)	20 (23)	12 (16)	8 (61)	<0.001
Hospital mortality, n (%)	3 (3)	3 (4)	0	1
Length of hospital stay, median (range)	9 (4-66)	9 (4-61)	14 (7-66)	0.043
Length of stay in ICU, median (range)	0 (0-49)	0 (0-45)	0 (0-49)	0.288

Table 4: Outcome of 27 patients with postoperative respiratory complications (18 in negative-PCR group and 9 in positive-PCR group).

N°Patients	PCR	Respiratory complications	Delay of respiratory complications (Days)	Postoperative sampling	Postoperative mortality	Documented respiratory complications	Antibiotics
28367417	Negative	Atelectasis	3	Bronchoscopic aspirate	No	Polymicrobial	No
28056097	Negative	ARDS	4	Bronchoscopic BAL	Yes	Pseudomonas species	Yes
28337216	Negative	Atelectasis	3	Bronchoscopic aspirate	Yes	Negative	No
28480756	Negative	Pneumonia	3	Bronchoscopic aspirate	No	*E coli*	Yes
28230459	Negative	Atelectasis	5	Sputum samples	No	Polymicrobial	No
27927584	Negative	Atelectasis	3	Bronchoscopic aspirate	No	*S aureus*	No
28487518	Negative	Atelectasis	7	Bronchoscopic aspirate	No	Polymicrobial and candida	No
28186810	Negative	Pneumonia	6	Bronchoscopic aspirate	No	*S pneumonia*	Yes
27868949	Negative	Pneumonia	2	Sputum samples	No	Negative	Yes
28264481	Negative	Atelectasis	4	Sputum samples	No	Pseudomonas species	Yes
28506867	Negative	ARDS	1	Sputum samples	No	Negative	Yes
28559449	Negative	Pneumonia	3	Bronchoscopic aspirate	No	Candida species	Yes
28271890	Negative	ARDS	21	Bronchoscopic BAL	Yes	Gemella species	Yes
28460304	Negative	ARDS	6	Bronchoscopic BAL	No	*K pneumonia*	Yes
28528607	Negative	Atelectasis	5	Bronchoscopic BAL	No	*K pneumonia* and *H influenzae*	No
28387200	Negative	Pneumonia	3	Bronchoscopic aspirate	No	Negative	Yes
28028505	Negative	Pneumonia	4	Sputum samples	No	Negative	Yes
28244321	Negative	Atelectasis	3	Sputum samples	No	*H infleunzae*	No
28138707	Positive	Pneumonia	5	Sputum samples	No	Negative	Yes
28460031	Positive	Atelectasis	7	Bronchoscopic aspirate	No	Enterobacter species	No
28204535	Positive	ARDS	15	Bronchoscopic BAL	No	CMV and *E coli*	Yes
28402133	Positive	Pneumonia	5	Sputum samples	No	Negative	Yes
28143925	Positive	Pneumonia	4	Bronchoscopic BAL	No	Negative	Yes
28001440	Positive	Atelectasis	6	Sputum samples	No	Polymicrobial	No
27993123	Positive	Atelectasis	1	Sputum samples	No	Polymicrobial	No
28508909	Positive	ARDS	5	Bronchoscopic BAL	No	Negative	Yes
27961020	Positive	Pneumonia	5	Bronchoscopic BAL	No	Polymicrobial and Candida species	Yes

Table 5: Postoperative samples in the 27 patients who developed respiratory complications (18 in negative-PCR group and 9 in positive-PCR group).

Microbiological agents	n=27	%
Negative cultures	9	33
Gram Negative Bacteria	9	33
E coli	2	7
Klebsiella sp	2	7
Pseudomonas sp	2	7
H influenzae	2	7
Enterobacter sp	1	4
Polymicrobial	6	22
Candida species	3	11
S pneumonia	1	4
S aureus	1	4
Others		
Gemella sp	1	4
Cytomegalovirus	1	4

Table 6: Outcome of the 13 positive-PCR patients and concordance with postoperative samples. BAL: broncho-alveolar lavage, LB: lung biopsy.

Patients	Positive PCR	Respiratory complications	Delay of respiratory complications (days)	Site of colonization	Postoperative samples	Postoperative microbiological agents
28067492	CMV	No	-	LB	No	
28181721	CMV	No	-	BAL	No	
28165369	CMV	No	-	BAL	No	
28656615	CMV	No	-	BAL	No	
28138707	CMV	Pneumonia	5	BAL + Tumor	Sputum samples	Negative
28460031	CMV	Atelectasis	7	LB + Tumor	Bronchoscopic aspirate	*E cloacae*
28204535	CMV	ARDS	15	LB	Bronchoscopic BAL	CMV + *E coli*
28402133	CMV	Pneumonia	5	BAL	Sputum samples	Negative
28143925	CMV	Pneumonia	4	BAL	Bronchoscopic BAL	Negative
28001440	CMV	Atelectasis	6	BAL	Sputum samples	Polymicrobial
27993123	HSV + CMV	Atelectasis	1	LB	Sputum samples	Polymicrobial
28508909	CMV	ARDS	5	Tumor	Bronchoscopic BAL	Negative
27961020	CMV	Pneumonia	5	Tumor	Bronchoscopic BAL	Polymicrobial + Candida

Table 7: Univariate analysis of factors of postoperative respiratory complications.

	No postoperative respiratory complications n=60	Postoperative respiratory complications n=27	p
Preoperative clinical status			
Age (years, mean ± SD)	63 ± 8	63 ± 11	0,971
Sex male, n (%)	42 (70)	20 (74)	0,801
Weight (kg, mean ± SD)	69 ± 19	72 ± 15	0,512
Performans status (mean ± SD)	0,12± 0,3	0,41± 0,5	0,003
BMI (mean ± SD)	24 ± 3	23± 4	0,463
Charlson score (mean ± SD)	4,8 ± 1	4,7 ± 1	0,918
FEV1 % of predicted (mean ± SD)	86 ± 18	78 ± 16	0,09
DLCO % of predicted (mean ± SD)	75 ± 11	65 ± 13	<0,001
Preoperative risk factors for respiratory complications			
NSCLC, n (%)	52 (86)	22 (81)	0,531
Smoking history, n (%)	53 (88)	26 (96)	0,426
Smoking cessation (n= 79 smokers), n (%)	40 (66)	17 (63)	0,8
Alcohol, n (%)	4 (7)	6 (22)	0,064
COPD, n (%)	17 (28)	16 (59)	0,009
Diabetes mellitus, n (%)	8 (13)	4 (15)	1
Previous cancer, n (%)	19 (32)	9 (34)	1
Previous CRT, n (%)	17 (29)	6 (23)	0,6
Neoadjuvant CRT, n (%)	5 (8)	4 (14)	0,45
ASA score (mean ± SD)	1,9 ± 0,5	2,15 ± 0,6	0,07
NHYA score (mean ± SD)	1,6 ± 0,5	1,8 ± 0,5	0,068
Previous cardiac disease, n (%)	9 (14)	3 (11)	1
Hypertension, n (%)	7 (12)	5 (18)	0,5
Peripheric vascular disease, n (%)	4 (7)	3 (1)	0,671
Positive PCR, n (%)	4 (6)	9 (33)	0,002
Details of operation			
Lobectomy, n (%)	55 (91)	25 (92)	1
Pneumonectomy, n (%)	3 (3)	0	1
Previous thoracic surgery, n (%)	3 (5)	2 (7)	0,64
Right side, n (%)	38 (63)	22 (59)	0,812
Epidural analgesia, n (%)	45 (75)	20 (74)	1
Hospital mortality, n (%)	0	3 (11)	0,028
Length of ICU stay, median (range)	0 (0-3)	0 (0-49)	<0,001
Length of hospital stay, median (range)	8 (4-15)	18 (5-66)	<0,001

Table 8: Multivariate analysis of factors of postoperative respiratory complications (a) and of positive-PCR (b).

A

Factors of postoperative respiratory complications	p	Odd Ratio	CI 95%	
			Low	High
ASA score	0,977	1,08	0,26	2,6
FEV1 % of predicted	0,287	0,98	0,95	1,01
TLCO % of predicted	0,172	0,96	0,90	1,01
Performans status	0,056	3,7	0,96	14,5
Alcohol	0,39	2,4	0,3	19
COPD	0,07	3,1	0,9	10
NYHA score	0,471	1,52	0,48	4,7
Positive-PCR	0,020	6,7	1,35	33,3

B

Factors of positive-PCR	p	Odd Ratio	CI 95%	
			Low	High
Previous thoracic surgery	0,087	6,9	0,75	63,5
Diabetes mellitus	0,031	5,6	1,16	27,6
NYHA	0,703	1,2	0,43	3,4
TLCO % of predicted	0,404	0,97	0,92	1,04
Previous vascular diseases	0,562	1,63	0,31	8,60
Smoking PA	0,446	1,01	0,98	1,03
Alcohol	0,422	2,25	0,03	16,44

II.3.3 Discussion

Contrairement aux bronches proximales qui présentent un taux de colonisation de l'ordre de 20 à 40 %, nos résultats confirment que les bronches distales et le parenchyme pulmonaire doivent être considérés comme stériles pour les bactéries. Ces résultats sont relativement inattendus compte tenu de l'ensemble des facteurs de risque des colonisations bactériennes retrouvés chez nos patients, tels que le tabagisme, l'insuffisance respiratoire obstructive et la présence d'un cancer bronchique. Ce résultat est d'ailleurs renforcé par l'emploie de techniques de biologie moléculaire permettant d'individualiser des germes difficilement cultivables ou émergents. A notre connaissance, il s'agit de la première utilisation de techniques de biologie moléculaire appliquées à la recherche d'une colonisation bronchique. Nos résultats apportent une information nouvelle concernant les mécanismes impliqués dans la survenue des infections respiratoires postopératoires. Compte tenu de la négativité de près de 240 PCR ARN 16S sur des biopsies de poumon sain, de tumeurs et sur des LBA obtenus sur pièce de résection, nous pouvons affirmer que ces sites de prélèvements ne démontrent aucune activité bactérienne. Il en résulte que les bronches distales et le parenchyme pulmonaire sont stériles au moment de la chirurgie. Tenant du compte du fait que la voie hématogène n'est qu'exceptionnellement la source de l'infection respiratoire et que l'infection survient avec une médiane de 4 jours, force et de conclure que l'infection postopératoire ne peut se développer qu'à partir d'une contamination des voies aériennes supérieures, des voies digestives ou de la flore oro-pharyngée. Cette observation est susceptible d'avoir des conséquences cliniques notamment dans une stratégie de prévention de ces complications. En effet une décontamination de la sphère oro-pharyngée préopératoire, à la chlorexhidine par exemple, serait une mesure de prévention à évaluer dans ce type de chirurgie. Ce type de protocole à d'ailleurs été utilisé avec succès dans la chirurgie cardiaque avec un effet démontré sur le taux de complications infectieuses postopératoires *(Segers P et al, 2007).*

Si les colonisations distales ou parenchymateuses n'existent à priori pas pour les bactéries, nos résultats retrouvent une PCR en temps réel positive pour les virus notamment pour le CMV. Nos résultats retrouvent un lien fort entre positivité d'une PCR-CMV et le développement d'une complication respiratoire. Ce lien est d'ailleurs retrouvé en analyse multivariée où l'existence d'une colonisation retrouvée par PCR demeure le seul facteur de risque indépendant de survenue d'une complication respiratoire postopératoire. Par ailleurs,

la détection par PCR du CMV permet avec bonne une valeur prédictive positive (0.70 ; IC95 % : 0.44-0.94) d'identifier un groupe de patients à risque de complications respiratoires.

La présence d'une PCR CMV positive chez près de 15 % de nos patients peut s'interpréter de plusieurs manières. Dans un premier cas, il pourrait s'agir d'une réplication virale lors d'une primo-infection. Cette hypothèse parait peu probable compte tenu de caractère asymptomatique de nos patients. Dans un deuxième cas, nettement plus probable, il pourrait s'agir d'une réactivation virale à partir d'une colonisation latente au niveau des cellules dendritiques, des monocytes ou de certains macrophages pulmonaires.

Les raisons de cette réactivation sont complexes. La réactivation CMV peut s'observer dans des contextes de fort stress médical ou chirurgical *(von Muller et al, 2006) (Heininger et al, 2001)(Cunha, 2010)(Chiche et al, 2009)*. Il a été rapporté que cette réactivation s'observait dans des conditions d'immunosupression acquise en réanimation *(Osawa et al, 2009)*. Une équipe de notre institution a documenté cette réplication pour les patients soumis à une ventilation mécanique avec un taux de 16 % (Chiche et al, 2009). Ces données sont conformes aux données françaises récemment publiées, où le taux de réactivation est de l'ordre de 17 % pour des patients en réanimation (Jabert et al, 2005). Puisque ces 13 PCR-CMV sont jugées comme des témoins d'une réactivation virale, ce qui reste incertain, c'est la rapidité de cette réactivation entre le moment de l'induction de l'anesthésie et celui du prélèvement dans le poumon réséqué. Ce délai est estimé à 1 heure. Ce délai est trop court pour expliquer son mécanisme. La réactivation est donc, vraisemblablement, présente avant même la chirurgie. Plusieurs facteurs d'ordre généraux, (existence d'un cancer, diabète…) ou d'ordre locaux (tabagisme, la bronchite chronique, tumeur bronchique) permettent de considérer cette éventualité. Il est vraisemblable qu'une défaillance dans la réponse immunitaire humorale ou cellulaire puisse expliquer une réactivation virale depuis des cellules où le CMV était à l'état quiescent *(Simon et al, 2005) (Cook et al, 2006)*. Cette réactivation CMV doit être considérée comme un marqueur d'un processus inflammatoire évolutif ou le témoin d'une défaillance immunitaire, plutôt que la résurgence d'un virus potentiellement pathogène. Il a été d'ailleurs documenté chez la souris qu'un état de choc septique ou qu'une élévation du TNF-α pouvaient être responsables d'une réactivation du CMV (Cook et al, 2006). Néanmoins, nous ne pouvons conclure à la lumière de nos données. Nous avons d'ailleurs documenté une concordance entre une PCR CMV positive préopératoire et une positivité postopératoire chez un patient présentant un SDRA. La recherche du CMV par rt-PCR reste néanmoins à encourager car ce moyen rapide (quelques heures) et peu couteux (moins de 100 euros) permettrait de d'identifier un groupe de patients à risque au moment de la chirurgie. Si cette réactivation virale est un marqueur d'immunosupression, son individualisation paraît pertinente lorsque l'on considère

son lien étroit avec la survenue de telles complications. Sa bonne valeur prédictive positive, sa grande spécificité et sa reproductibilité incite à l'évaluation de son intérêt en clinique.

Cette étude observationnelle présente un certain nombre de limites. Premièrement, il s'agit d'une observation prospective d'un groupe limité de patients pour lequel l'élément discriminant (complication respiratoire) reste un événement relativement peu fréquent en quantité qui ne concernait que 27 patients. Deuxièmement, nous admettons que toute question relative au CMV reste hypothétique dans la mesure où nous n'avons pas mesuré de manière longitudinale les sérologies IgG - IgM du CMV. L'évaluation de l'activité du lymphocyte *natural Killer* (LNK) dans les échantillons prélevés aurait eu un intérêt certain dans ce contexte pour approfondir cette réactivation. En effet, une sidération des LNK dans la résurgence du CMV est fortement suspectée *(Khan et al, 2007)*. Troisièmement, nous n'avons pas comparé entre-elles les méthodes phénotypiques et moléculaires. Nous sommes partis du principe que l'efficacité de la biologie moléculaire par rapport aux méthodes de culture traditionnelle était déjà démontrée *(Pace, 1997)*. Enfin, nous n'avons pas évalué la colonisation proximale en même temps que la colonisation distale. Néanmoins, cette limitation soulève de manière plus générale l'intérêt d'appliquer les techniques de détections moléculaires à la recherche d'une colonisation au niveau des voies aero-digestives supérieures. Ceci constitue la base d'une évaluation prospective dans un futur proche.

Discussion et Perspectives

III.1 Conclusions générales

A la lumière de nos travaux, nous pouvons conclure d'une manière générale, que les complications respiratoires représentent un véritable challenge en chirurgie thoracique oncologique. Qu'il s'agisse de la chirurgie pour cancer de l'œsophage ou pour cancer du poumon, une chirurgie incluant une thoracotomie associée à une exérèse d'un organe intrathoracique expose le patient à un risque de complications respiratoires élevé. Dans nos deux études cliniques, ces complications se placent d'ailleurs à la première place des causes de décès hospitalier. Nos travaux suggèrent que ces complications soient en réalité les manifestations à des degrés différents d'un même continuum physiopathologique, débutant par le stade d'encombrement bronchique jusqu'au stade de pneumopathie nosocomiale et de SDRA. Une fois constituées, ces complications, sont de manière fortement probable, de nature infectieuse et siègent au niveau des bronches distales et du parenchyme pulmonaire.

Cet état résulte-il d'une contamination à partir d'une colonisation bronchique ? Nos deux travaux originaux démontrent qu'une colonisation bronchique peut être considérée comme un élément constitutif de la physiopathologie des complications respiratoires postopératoires. Néanmoins les informations disponibles pour le démontrer de manière formelle sont faibles. Dans une première partie, nous avons démontré que, bien que la physiopathologie des complications respiratoires soit complexe et multifactorielle, l'existence d'une colonisation bronchique proximale apparaissait comme un élément récurrent dans de nombreuses études. Elle apparait dans notre méta-analyse comme un élément déterminant dans la survenue des complications respiratoires (Risque relatif : 2.47, Intervalle de confiance : 1.45- 4.11). Néanmoins, si un lien statistique et clinique parait évident, il n'en reste pas moins que la concordance microbiologique demeure non démontrée. Ceci témoigne des limites représentées par les méthodes de prélèvements utilisés et par les techniques d'identification microbiologique actuelles. Dans une deuxième partie nous avons documenté pour la première fois, qu'il existait une colonisation proximale au niveau des voies aériennes des patients proposés pour une chirurgie d'exérèse œsophagienne. Ceci reste très similaire à ce qui est observé en chirurgie d'exérèse pulmonaire. Cette étude a par ailleurs permis d'identifier d'autres pathogènes éventuels dans la liste des pathogènes potentiels. Le CMV apparait comme un élément déterminant dans le développement des complications respiratoires. Dans une troisième partie, en utilisant des techniques modernes et innovantes de biologie moléculaire, nous avons démontré que le parenchyme pulmonaire et les bronches distales des patients opérés d'un cancer du poumon étaient totalement

stériles pour les bactéries au moment de la chirurgie. Bien que cumulant les facteurs de risque des colonisations bronchiques proximales, la distalité bronchique et le parenchyme étaient à considérer comme vierge de toute colonisation bactérienne. Il en résulte que le mécanisme de l'infection postopératoire au niveau du parenchyme, proviendrait d'une colonisation des bronches proximales, puis distales par de germes potentiellement pathogènes dans les heures et jours suivants la chirurgie. L'origine de ces germes serait les voies aéro-digestives supérieures ou la sphère oro-pharyngée. En fonction de l'inoculum bactérien, de sa virulence et du terrain sous jacent, le germe colonisant deviendrait pathogène dans un délai classique de 3 à 4 jours après la chirurgie. Cette théorie expliquerait les observations cliniques où les complications respiratoires suivent un même continuum physiopathologique. La présence du CMV est à considérer comme un marqueur biologique d'une immunosupression globale. Rappelons que les patients issus de nos deux études présentent des cancers thoraciques sévères pour lesquels une baisse de l'immunité est connue. La réplication du CMV au niveau du parenchyme ou des bronches distales serait un marqueur précoce permettant de sélectionner un groupe spécifique de patients à risque. Son évaluation par PCR aurait un intérêt dans la sélection d'un groupe à risque.

Dans ce travail de thèse, il apparait qu'un des éléments des plus restrictifs pour l'établissement d'un modèle reproductible et compréhensible des mécanismes impliqués dans le développement des complications respiratoires, reste les méthodes de documentation et les techniques d'analyse microbiologique employées. Qu'il s'agisse de la période préopératoire ou de la période postopératoire, toute tentative de documentation microbiologique devrait être basée sur la pratique de techniques innovantes, modernes et capables d'identifier des germes pathogènes connus, difficilement cultivables ou émergentes. Les données issues de l'utilisation de ces outils en pathologie humaine ont permis de s'affranchir des limites inhérentes aux techniques traditionnelles. Pour le seul fait de la mucoviscidose, les techniques moléculaires ont permis par exemple l'identification d'un grand nombre de germes émergents *(Armogoum et 2009) (Bittar et al, 2009)*. Le développement de ces techniques (futurs séquenceurs, métagénomique) parait dans ce contexte, très prometteur.

III.2. Perspectives

Dans la mesure où, d'une part, le parenchyme pulmonaire et les bronches distales sont stériles de toutes bactéries au moment de la chirurgie, et que d'autre part, ce sont ces sites qui sont le siège de l'infection pendant la période postopératoire, il apparait extrêmement important de caractériser les mécanismes par lesquels, la flore aéro-digestive supérieure colonisante, se transforme en véritable flore pathogène dans les jours suivants la chirurgie.

Les données concernant la colonisation bronchique proximale, digestive supérieure ou oro-pharyngée sont nombreuses et bien documentées. Néanmoins, aucune étude ne les a évaluées par des techniques de biologie moléculaire comme précédemment décrites dans notre thèse. Il parait donc envisageable de caractériser chaque étage de la colonisation par des méthodes de prélèvements conventionnels et des les analyser en biologie moléculaire. La flore oro-pharyngée peut être évaluée par analyse de la salive ou par écouvillonnage oro-pharyngé. La flore bronchique proximale peut être évaluée par aspirations bronchiques protégées ou par brossage. La flore digestive supérieure peut être documentée par prélèvements obtenus à partir d'une sonde naso-gastrique. La principale limite demeure les prélèvements postopératoires pour lesquels une documentation microbiologique ne peut être obtenue que de manière invasive dans des circonstances cliniques défavorables. Un des progrès pourrait provenir de la mise en place de techniques moléculaires sur ces prélèvements (analyse des crachats, aspiration bronchique).

Si l'on admet que cette colonisation aéro-digestive est un élément pathologique déterminant dans la constitution des flores pathogènes, il convient d'évaluer des stratégies de prévention dans la réduction du risque opératoire. Ainsi, une décontamination oro-pharyngée semble la suite logique de nos travaux. Des efforts considérables ont été entrepris ces dernières décennies pour réduire le taux de complications nosocomiales. Une des stratégies proposées à des fins préventives reposent sur des décontaminations digestives et oro-phrayngée proposées dans différents domaines des soins intensifs. Qu'il s'agisse de la prise en charge en réanimation *(de Jonge et al, 2002),* en chirurgie cardiaque *(Segers et al, 2006) (DeRiso et al, 1996) (Houston et al, 2002)*, ou en chirurgie œsophagienne *(Akutsu et al, 2010)*, ces mesures s'avèrent être efficaces en réduisant le taux de complications infectieuses ou de complications respiratoires basses de manière significative. A notre connaissance aucune donnée n'est disponible en chirurgie thoracique oncologique et plus spécifiquement en chirurgie pulmonaire. Par ailleurs, dans toutes les études ayant évalué la décontamination oro-pharyngée, l'analyse microbiologique ne repose que une analyse phénotypique de mise en culture traditionnelle dont on connait les limites.

III.3.3 Projet de Programme Hospitalier de Recherche Clinique (PHRC)

Titre : Intérêt de la décontamination oro-pharyngée par Chlorhexidine Gluconate en chirurgie du cancer du poumon dans la réduction des infections postopératoires : étude multicentrique prospective randomisée.

Rationnel : Les complications respiratoires restent la première cause des complications postopératoires en chirurgie thoracique pour cancer du poumon. Le développement des ces complications sont le plus souvent de nature infectieuse. Ces complications respiratoires postopératoires doivent être considérées comme nosocomiales puisque survenant après 48 heures d'hospitalisation. Les pneumopathies nosocomiales (PN) figurent au second rang des infections acquises en milieu hospitalier (après les infections urinaires). La PN peut évoluer vers une pneumopathie acquise sous ventilation mécanique (PAVM) ou vers un syndrome de détresse respiratoire aigu (SDRA). La fréquence de ces complications respiratoires en chirurgie thoracique reste élevée et stable depuis de nombreuses années (30 % des patients). La mortalité qui est liée à ce type de complications peut atteindre près de 50 % dans sa forme la plus grave.

Des données récentes suggèrent que ces complications respiratoires sont liées à une colonisation préopératoire des voies aéro-digestives supérieures. La survenue d'une pneumopathie ou d'un SDRA semble le plus souvent la conséquence d'une inhalation microbienne à partir d'une colonisation plutôt que d'une invasion microbienne par voie systémique. Compte tenu de la stérilité au moment de la chirurgie au niveau des bronches distales et au niveau du parenchyme pulmonaire évaluée par technique de biologie moléculaire, il en résulte que les infections respiratoires postopératoires sont le résultat d'une contamination à partir des germes de la sphère oro-pharyngée ou aéro-digestive supérieure. Une colonisation précèderait quasiment toujours une PN. Il en résulte qu'une colonisation bronchique chez les patients atteints d'un cancer du poumon et proposés pour une chirurgie d'exérèse est retrouvée chez près de 41 % des malades lorsque les prélèvements sont réalisés sur les bronches proximales *(Schlusser et al, 2006) (Ioanas et al, 2002) (Cabello, 2005) (Belda 1997)*.

Une des stratégies proposées à des fins préventives reposent sur une décontamination oro-pharyngée proposée dans différents domaines des soins intensifs. Qu'il s'agisse de la prise en charge en réanimation *(de Jonge E et al, 2002),* de la chirurgie cardiaque *(Segers P et al, 2006) (DeRiso aj et al, 1996) (Houston S et al, 2002)* ou de la chirurgie œsophagienne, ces mesures s'avèrent être efficaces en permettant de réduire le taux de complications

infectieuses ou de complications respiratoires basses de manière significative. A notre connaissance aucune donnée n'est disponible en chirurgie thoracique pulmonaire. Par ailleurs, dans toutes les études ayant évalué la décontamination oro-pharyngée, l'analyse microbiologique ne repose que sur une analyse phénotypique de mise en culture traditionnelle dont on connait les limites.

L'utilisation de techniques de biologie moléculaire pour l'identification de colonisations bactériennes ou non bactériennes (virus, champignon) permettrait de pallier ces inconvénients. Des travaux récents utilisant des techniques de biologie moléculaire comme moyen d'identification des bactéries chez les patients atteints d'une pneumopathie communautaire ont pu être développés. Les limitations des différentes techniques d'identification microbiologique (analyse du polymorphisme du gène codant pour l'ARN 16S, hybridation) suggèrent que l'identification précise et exhaustive des colonisations des voies respiratoires ne peut être réalisée que par une approche systématique, en particulier l'amplification universelle des ADN présents dans les échantillons suivie du clonage des produits de PCR et du séquençage de ces clones. Cette approche a été rarement utilisée dans la littérature et seulement sur un nombre limité d'échantillons.

Indication / sujet :

Cohorte de sujets atteints de cancer du poumon, après consentement libre et éclairé. Le rapport bénéfices/risques de cette étude peut être considéré comme très favorable.

Objectifs :

Primaire :

- Réduction des événements infectieux postopératoires (respiratoire, profond, généraux)

Secondaire :

- Evaluer le lien entre colonisation et survenue de complications respiratoires postopératoires
- Procéder à une analyse systématique par PCR et clonage des produits de PCR de tous les agents présents dans les prélèvements réalisés (pathogènes émergents).
-Identifier une flore bactérienne ou non-bactérienne et d'évaluer l'efficacité de la décontamination vis à vis des agents infectieux rencontrés

Population de l'étude :

Population

- Sujets atteints d'un cancer du poumon proposés pour une chirurgie curatrice.
- Chirurgie d'exérèse anatomique : segmentectomie, lobectomie, pneumonectomie

Estimation du nombre de patients inclus/an :

- Compte tenu du volume opératoire du service de Chirurgie Thoracique, le nombre de patients qui pourraient être inclus pendant les deux premières années se répartit comme suit : 150 patients.
- Nécessité d'ouvrir l'étude à des centres collaborant : 150 patients

Lieu(x) de l'étude :

ASSISTANCE – PUBLIQUE HOPITAUX DE MARSEILLE (AP-HM)

- Fédération de Microbiologie Clinique – CHU Hôpital de la Timone
- Hôpital Nord, Service de Chirurgie Thoracique
- Hôpital Nord, Service d'Anesthésie- Réanimation
- Hôpital Nord, Service de Réanimation Médicale

AUTRES CENTRES PARTICIPANTS

Critères d'Inclusion/d'Exclusion :

Critères d'inclusion et fréquence des analyses

- Sujets atteints d'un cancer du poumon proposés pour une chirurgie curatrice.
- Chirurgie d'exérèse anatomique : segmentectomie, lobectomie, pneumonectomie

Critères de non inclusion

- Exérèse pour lésion infectieuse
- Tumeur bronchique proximale

Design de l'étude / Méthodologie :

Il s'agit d'une étude prospective randomisée en double aveugle contre placebo.

Afin d'évaluer les schémas prophylactiques préconisés, une analyse prospective sera réalisée chez des malades soumis à une résection pulmonaire majeure. Tous les malades recevront une antibioprophylaxie conforme aux recommandations de la SFAR.

Après avoir donné un consentement écrit, le groupe sera randomisé avant la chirurgie en deux groupes par tirage au sort informatisé. Le produit de décontamination sera anonymisé depuis sa délivrance jusqu'à l'obtention complète des données.

- Le produit décontaminant sera une solution de chlorhexidine gluconate à 0.12 % utilisé en rinçage oro-pharyngé et en une pommade nasale.
- Le placebo sera une solution comparable en tout point mais dépourvu d'activité antibactérienne.

Le produit sera appliqué sur la muqueuse oro-pharyngée, les dents et les gencives durant 30 secondes, 4 fois par jour, le jour avant l'opération, le jour de l'opération et le lendemain. La pommade nasale sera utilisée avec la même fréquence. L'infirmière pourra réalisée cette désinfection par application si le patient ne peut l'effectuer lui-même.

Prélèvements microbiologiques

Ils seront faits au moment de la chirurgie et dans la période postopératoire en fonction de l'état du patient en maintenant un haut niveau de suspicion infectieuse. La flore oro-pharyngée sera évaluée par analyse de la salive ou par écouvillonnage oro-pharyngé. La flore bronchique proximale sera être évaluée par aspirations bronchiques protégées ou par brossage. La flore digestive supérieure sera documentée par prélèvements obtenus à partir d'une sonde naso-gastrique.

Analyses microbiologiques

Les prélèvements seront traités au laboratoire d'une part à l'aide de techniques classiques de culture (géloses MacConkey, gélose au sang ANC et milieu de Sabouraud), d'identification (galeries API*, systèmes automatisés de type Vitek*) et de détermination des antibiogrammes et d'autre part un certain nombre de méthodes plus spécifiques et modernes seront réalisées sur ces prélèvements. Le séquençage de l'ARN 16S ribosomique sera effectué pour tout prélèvement. L'extraction de l'ADN est réalisée à l'aide de colonnes d'extraction d'ADN (QIAmp tissue kit) à partir de suspensions de souches préalablement cultivées sur géloses ou sur amibes puis purifiées pour les bactéries qui ne poussent pas sur milieu axénique. L'amplification est basée sur l'utilisation d'amorces universelles utilisées actuellement en routine, ces amorces étant également utilisées pour le séquençage. Les séquences seront comparées à celles actuellement disponibles dans GenBank.

Résultats attendus

Cet essai devra démontrer l'intérêt de la décontamination oro-pharyngée par la chlorhexidine gluconate dans la réduction des infections postopératoires, notamment respiratoires.

Durée étude

2 ans. Les sujets seront inclus après le recueil du consentement éclairé. Les inclusions seront réalisées pendant 24 mois. Les sujets sont revus tous les 3 mois.

Analyse des données et statistiques

Le nombre de patients à inclure doit être le plus grand possible pour permettre une représentation complète et exhaustive des différents colonisations. L'identification de facteurs de risque de cette colonisation sera recherchée par différentes variables cliniques (VEMS, CV, Tabagisme, âge, sexe…). Les comparaisons entre les variables numériques seront faites par un test U de Mann et Whitney. Les comparaisons entre les variables qualitatives sont faites par un test du Khi deux. Une régression logistique sera utilisée pour évaluer les facteurs de risques liés à une colonisation bronchique et les facteurs de risques d'une complication respiratoire infectieuse (encombrement, pneumopathie et SDRA). Les variables dont le p sera inférieur à 0.2 seront inclus dans l'analyse multivariée. Le risque de première espèce est de 5% et la puissance du test de 90%.

Faisabilité :

Les études préalables ont démontré la faisabilité de notre protocole.

Staff :

Investigateur principal : D'JOURNO Xavier Benoit

Rapport Bénéfice-Risque

Compte tenu, d'une part, de l'absence d'effet secondaire de la chlorhéxidine et du placebo et d'autre part du risque de mortalité en rapport avec une infection postopératoire, le rapport risque-bénéfice peut être considéré comme très favorable.

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