DEVELOPING STRATEGIES FOR THE PREVENTION OF POSTOPERATIVE PULMONARY COMPLICATIONS AFTER LUNG CANCER SURGERY

By

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ABSTRACT

Lung cancer is the leading cause of cancer death worldwide with curative intent surgery the most effective treatment. However, there is a risk of developing postoperative pulmonary complications (PPC) including pneumonia and clinically significant lung atelectasis. This thesis investigated the role of cigarette smoking in PPC development, including the impact of smoking cessation and the role of key immune cells in the lung, alveolar macrophage (AMs), with the aim to develop strategies to prevent PPCs in those who smoke before major lung surgery.

In a retrospective study of prospectively gathered data from a cohort of patients undergoing major lung surgery between April 2010 and April 2014, the risk factors and short and long-term outcomes of PPC development were investigated. In those patients with lung cancer diagnosis undergoing major lung surgery, the effect of self-reported smoking status on PPC development and short and long-term outcome after surgery were also investigated. In a translational cohort of patients undergoing lung resection for cancer recruited between August 2018 and August 2021, non-cancerous lung tissue was obtained to isolate AMs and smoking status determined using self-reported status and exhaled carbon monoxide (CO) measurements. AM phenotype was determined and function including efferocytosis, which is a process by which apoptotic neutrophils are removed. A protocol was also developed for a feasibility study of smoking cessation in the lung surgical pathway.

Development of a PPC after lung surgery was found to be associated with worse short-term and long-term outcomes after major thoracic surgery. Cigarette smoking was the biggest risk factor for PPC development, with current smokers having the highest risk of developing a PPC. Self-reported status alone, using a 6-week cut off to determine recent and long-term ex-smokers, the optimum timing for smoking cessation before lung surgery could not be defined. In the translational cohort of patients, the use of exhaled CO found that 1 in 5 patients continued to smoke immediately before surgery. AMs from smokers had increased expression of cell surface markers involved in inhibition of efferocytosis (SIRPα and CD33), and reduced expression of cell surface markers involved in promoting efferocytosis (CD206 and CD163) compared to long-term ex-smokers and never smokers. AMs from patients who were smoking had higher levels of SIRPα, with lower levels found in those who stopped after 4 weeks. Furthermore, AM SIRPα levels were higher and efferocytosis function reduced in those patients who developed PPC.

Smoking is the biggest risk factor for PPC development after major lung surgery, the feasibility of an integrated smoking cessation programme including the web-based application Quit4Surgery needs to be assessed. Modifying SIRPα has a potential role in restoring AM function in those who continue smoking before surgery: the mechanisms of cigarette smoking on SIRPα expression are an area for future research.

PUBLICATIONS ARISING FROM THIS THESIS

PAPERS

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Lugg ST Kerr A, Kadiri S, et al. Protocol for a feasibility study of smoking cessation in the surgical pathway before major lung surgery: Project MURRAY. BMJ Open. 2020. PMID: 331588219.

Lugg ST, Agostini PJ, Tikka T, et al. Smoking and timing of cessation on postoperative pulmonary complications after curative-intent lung cancer surgery. J of Cardiothorac Surg. 2017;12:52. PMID: 28629433

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ABSTRACTS

Lugg ST, Kerr A, Webb J, et al. The prevalence of smoking and e-cigarette use before lung cancer surgery. Poster Discussion European Respiratory Society (ERS) International Congress, Madrid, 30/09/19.

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Lugg ST, Agostini PJ, Tikka T, et al. Long-term impact of developing a postoperative pulmonary complication after lung surgery. Oral presentation: ERS International Congress, Amsterdam, 28/09/15.

Lugg ST, Tikka T, Agostini PJ, et al. Smoking at the time of curative-intent lung cancer surgery dramatically influences peri-operative complications. Poster: ERS International Congress, Amsterdam, 28/09/15.

DEDICATION

To my amazing wife Neha, who has been a rock and has always supported me in my studies ever since we met. Having recently been diagnosed with lymphoma and enduring 6 months of chemotherapy during a global pandemic, you have shown an unbelievable amount of courage. Always being motivated and determined to get through the hardest of times and seeing the best in things.

To my wonderful daughter Maya, who has grown up so quickly over the last few years. Initially arriving to the world slightly earlier than planned. Though it wasn't me presenting in the end, you still managed to get a special mention at ERS Milan. It has been such a joy to be around you. Here's to continuing to try everything and get back up even if we fail.

To my parents and grandparents who have always supported my academic endeavours. To my parents in-law and my brother in-law Raj who have supported us during my PhD.

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I am grateful for the collaborators at University College London including Professor Jamie Brown, Professor Robert West and Dr Olga Perski for their input in the development of the smoking cessation intervention for the feasibility study. I would also like to thank the team at Evolyst for their input in the software development of the web-based application 'Quit4Surgery' for use in the feasibility study.

COVID-19 STATEMENT

This project has been affected by the COVID-19 pandemic. During the 1st wave, recruitment stopped, thoracic surgery was taken off site, and I was shielding because of an extremely vulnerable family member. During the 2nd wave, I was working between acute medicine and the COVID-19 Respiratory Support Unit (RSU). Due to COVID-19, the thoracic surgical department and research team moved site to another hospital within the trust, with surgery and subsequent research study recruitment slow to take off between the first 2 waves. Furthermore, many thoracic surgical procedures had been cancelled/delayed in 2021 due to the backlog of medical admissions and pressure of intensive care services following the 2nd/3rd COVID-19 wave.

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CHAPTER 1: INTRODUCTION

My role in this chapter involved performing the literature review, critical appraisal and writing of the chapter including generation of figures. Section 1.4 of this chapter includes the peer reviewed and published state of the art review:

Lugg ST, Scott A, Parekh D, Naidu B, Thickett DR. Cigarette smoke exposure and alveolar macrophages: mechanisms for lung disease. Thorax. 2021. PMID: 33986144 (1)

1.1. Lung cancer

Lung cancer is the third most common cancer in the UK (2), but accounts for the most deaths. Worldwide lung cancer is the leading cause of cancer deaths, accounting for 1 in 5 (18%) of all cancer deaths (3), and impacts both transitioned and transitioning countries. Smoking can be linked to more than 80% of patients who are diagnosed with lung cancer (4). Other causes of lung cancer include exposure to radon gas, exposure to asbestos and other workplace substances known to cause cancer (such as arsenic, chromium and nickel), history of other lung disease such as tuberculosis (5) or idiopathic pulmonary fibrosis, a family history of lung cancer (6), and previous radiotherapy. Symptoms of lung cancer can range from a persistent or change in cough, hoarseness of the voice, shortness of breath, haemoptysis, anorexia or weight loss, chest pains and recurrent or persistent chest infections. In addition, patients may present with symptoms from metastatic disease, such as headaches, bone pain and pathological fracture, or symptoms of hypercalcaemia.

Lung cancer that begins in the lungs is termed as primary lung cancer, whereas cancer that spreads to the lung from a distant site is termed metastatic disease. There are two main types of primary lung cancer; non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC is the most common representing 87% of all lung cancer (7), and can be further divided according to the histology; adenocarcinoma, squamous cell cancer, large cell carcinoma and undifferentiated. Pulmonary carcinoid tumours are rare neuroendocrine neoplasms and represent 1-2% of all lung cancers (8).

Treatment decisions are based on the location of the cancer, the staging, the histology, and the patient's overall health and level of fitness. The main treatments available range from surgery, radiotherapy, chemotherapy, immunotherapy, combination therapies and symptom control.

1.1.1. Lung cancer pathway

Patients with suspected lung cancer can present either initially though the general practitioner (GP) or through secondary care via hospital referrals (e.g. accident and emergency or as an incidental finding). The UK National Screening Committee have only recently recommended an adult screening programme for lung cancer, with targeted screening for people aged 55-74 identified as being of high risk of lung cancer (9). This Targeted Lung Health Checks (TLHC) programme aims to introduce low dose computed tomography with integrated smoking cessation service provision. For those who are initially assessed in primary care with suspected lung cancer, usually an urgent or routine chest x-ray (CXR) shows suspicion of lung cancer. In the National Optimal Lung Cancer Pathway, such patients should have a computer tomography (CT) scan the same day or within 72 hours (10). Following this CT either from patients referred via GP or in-hospital, patients should be triaged by a respiratory physician to determine whether lung cancer is likely on basis of CT and clinical findings. Those of which lung cancer is likely should be fast tracked to lung cancer clinic and seen as part of the lung cancer pathway.

1.1.2. Curative intent pathway

In the lung cancer pathway, an early decision is made as to whether patient is potentially suitable for curative intent treatment, with a low threshold for a patient being in the curative intent pathway (10). Those patients being assessed in an outpatient setting with curative intent should have a positron emission tomography CT (PET-CT) and spirometry and as required detailed lung function including gas transfer (TLCO). Diagnostics and staging tests include percutaneous image-guided biopsy for those with peripheral lesions with normal hilar and mediastinal appearances on staging CT. Alternatively in those patients bronchoscopic guided biopsy could be considered. In those with mediastinal or hilar lymphadenopathy, staging endobronchial ultrasound (EBUS) plus or minus oesophageal ultrasound (EUS) are required for a systematic examination of mediastinal or hilar nodes. Patients with borderline fitness in the curative intent pathway (11) can be assess with shuttle walk test, cardiopulmonary exercise tests (CPEX) and echocardiogram (ECHO), with early cardiology assessment for cardiac co-morbidity. They may also be also recommended for preoperative rehabilitation (10). All patients should receive medical optimisation including smoking cessation (12).

1.1.3. Decision to treat

Full discussion of treatment option or further investigations is decided at a full multi-disciplinary team (MDT) meeting. There should be a rapid follow-up lung cancer clinic at the same time as a rapid referral to treatment specialist for curative intent. NICE

guidance state that diagnosis of lung cancer should be made within 31 days of the initial specialist referral (13). More recently NHS England have set out guidance for shortening the timeframe of diagnosis of lung cancer with both 28 day and 14 day timeframe pathways with an aim to diagnose more patients at an earlier stage of the disease who are suitable for curative intent treatment and thus improve overall survival (14).

1.1.4. Thoracic surgery

Surgery is the most effective strategy in treating patients with early-stage NSCLC who are fit enough to undergo the operation. Over 6800 patients in the UK undergo lung resection annually, which represents 17.5% of all lung cancer patients diagnosed (7). Surgery is rarely considered for SCLC (15), as in these cases the cancer has usually spread at that stage that surgery would not be effective at removing all the disease.

1.1.5. Thoracic surgical assessment

Within the lung cancer pathway, patients are referred to the surgical team from the MDT with staging and routine fitness testing available. They should be rapidly seen in clinic with advance tests requested, and research eligibility assessed. Patients are seen within a preoperative assessment clinic, which would typically involve nurse specialists, risk stratification for surgery and potential research trial entry. Risk stratification would direct patients straight to surgery or if a case is complex patients may require further testing, prehabilitation (16) or second opinion on surgery.

Comorbidities should be optimised including smoking, COPD, and nutritional state as early as possible in the pathway (12).

1.1.6. Thoracic surgical approach

The approach and type of surgery offered is dependent on the staging of the cancer, the patient's underlying lung function and the fitness and overall health of the patient. The approach of lung cancer surgery is either via open thoracotomy or video assisted thoracoscopic surgery (VATS). This involves 1 to 3 small incisions for insertion of a thoracoscope, which includes a fibre optic camera, and ports for the surgical instruments to resect the affected area of lung (17).

1.1.7. Lung resection

The most common resection is a lobectomy, where one of the lobes of the lung is removed including its surrounding visceral pleura, associated airways and pulmonary vasculature. Patients with NSCLC who are well enough and where treatment approach is curative should be offered a lobectomy (either open or VATS)(12). For patients with an earlier stage cancer, a sub-lobar resection in an alternative to lobectomy. A sub-lobar resection may include a wedge resection where an area of lung from one or two lobes is removed, a segmentectomy where a segment of the lung along with their veins arteries and airways are removed. More rarely a sleeve resection can occur, where cancer involving the airway is resected with the affected section of the bronchus and surrounding cancer of the lobe. For those patients with a central

tumour, or where the cancer is involving multiple lobes in one lung, a pneumonectomy may be performed where the whole lung is removed. In addition to the lung resection/removal, surrounding lymph nodes are also removed during the procedure to reduce the risk of cancer recurrence.

1.2. Postoperative pulmonary complications (PPC)

Postoperative pulmonary complications (PPC) are the most frequently observed complication following lung surgery. Anesthesia during surgery can reduce functional residual capacity and impair central respiratory drive, resulting in hypoxaemia (18). In addition, use of neuromuscular blocking drugs may impair respiratory function and impair swallowing increasing the risk of pulmonary aspiration (19, 20). Furthermore, ventilation during surgery can contribute to lung injury; this was initially recognised as barotrauma caused by pressure forces (21). Volutrauma has subsequently found to be the primary driver of ventilation induced lung injury (22) in which there are microfractures of the extracellular matrix, inflammatory signaling and vascular stress leading to inflammatory activation and wounding of tissue (23). Furthermore, repetitive opening and closing of atelectatic but recruitable lung units during ventilation, termed atelectotrauma, can also contribute to lung injury through surfactant dysfunction and increased shear forces (24, 25). This combined with baseline factors including smoking status and co-existing comorbidities such as respiratory disease, increases the risk of development of PPC.

Furthermore, thoracic surgery itself can damage respiratory muscles, alter respiratory anatomy, restrict ventilation and result in atelectasis. Therefore the incidence of PPC in thoracic surgery is much higher than in abdominal surgery (26), with upper abdominal surgery including oesophagectomy associated with higher risk than lower abdominal procedures (27). The incidence of postoperative pneumonia for example has been shown to be highest for abdominal aortic aneurysm repair (odds ratio (OR) 4.3, 95% confidence interval (CI) 3.3-5.5), followed by thoracic surgery (OR 3.9, 95%CI 3.4-4.6), upper abdominal surgery (OR 2.7 95%CI 2.4-3.0), neck surgery (OR 2.3 95%CI 1.7-3.1), neurosurgery (OR 2.1 95%CI 1.7-2.8) and major vascular surgery (1.3 95%CI 1.1-1.5) (28).

1.2.1. Common pathological mechanism for PPC

A broad definition for PPC is 'any pulmonary abnormality occurring in the postoperative period that produces identifiable disease or dysfunction which is clinically significant and adversely effects the clinical course' (29). This incorporates a wide range of complications including pneumonia, atelectasis, respiratory failure, pulmonary oedema, pulmonary embolism, pulmonary haemorrhage, pneumothorax and pleural effusion (29). More specifically, PPC is a composite of respiratory diagnosis following surgery that share a common pathological mechanism involving pulmonary collapse (atelectasis) and pulmonary infection (pneumonia or pulmonary aspiration), which can potentially lead to acute respiratory distress syndrome (ARDS) requiring re-

institution of mechanical ventilation and management in the intensive therapy unit (ITU).

1.2.2. Postoperative atelectasis

Postoperative atelectasis after lung surgery involves a range of lung collapse from a subsegmental, lobar or multilobar level. This often occurs due to impairment of mucociliary function and accumulation of secretions, with resultant lack of aeration to the remaining pulmonary parenchyma. Atelectasis is more common in sleeve resection due to local oedema in the anastomosis, disruption of the cilia clearing mucus and lobar denervation (30). Atelectasis can occur following right upper lobe lobectomy (31), in which there is right middle lobe collapse due to post-surgical oedema. Management of clinically significant lung atelectasis involves use of physiotherapy, nebulisers, positive end expiratory pressure (PEEP) support, and potentially bronchoscopy for evaluation and removal of impacted sputum.

1.2.3. Postoperative pneumonia

Postoperative pneumonia is common after lung resection and commonly presents as radiological findings as bronchopneumonia pattern with patchy consolidation on a CXR (32). Clinical findings such as elevated white cell count (WCC), fever and increase or change in respiratory secretions often precede these CXR findings. Hypoxia and/or the increase in oxygen requirements are also clinically important. Postoperative pneumonia is commonly caused by aspiration or bacterial colonisation within the

atelectatic lung (32). Aspirational pneumonias typically present with bilateral and basal opacification on the CXR. Treatment involves oral or intravenous antibiotics with concurrent respiratory support and physiotherapy, similar to the treatment of lung atelectasis.

1.2.4. Acute respiratory distress syndrome (ARDS)

Acute respiratory distress syndrome (ARDS) is respiratory failure within a week of a known insult or new/worsening respiratory symptoms with widespread bilateral pulmonary opacities that cannot be explained by another cause, e.g. pulmonary oedema. ARDS is defined using the Berlin criteria as acute onset hypoxaemia defined by the ratio of partial pressure of inspired oxygen (PaO_2) to the fraction of inspired FiO_2 (PaO_2:PaO_2 ratio) <300 mm Hg on at least positive end expiratory pressure (PEEP) ventilation of 5 mm H_2O (33). The pathology of ARDS in the acute 'exudative' phase (~7 days) includes diffuse alveolar damage, interstitial and alveolar oedema, alveolar haemorrhage, accumulation of inflammatory cells (mainly neutrophils), fibrin deposition and areas of atelectasis (34). Patients with sepsis-related ARDS have been shown to have impaired alveolar macrophage efferocytosis, which is the ability to clear dead neutrophils, and that reduced efferocytosis in these patients is associated with adverse clinical outcomes including mortality (35). The 'proliferative' phase then follows with alveolar type II cell hyperplasia, which can last >3 weeks and interstitial fibrosis can also occur in this phase. ARDS occurrence is higher in pneumonectomy cases, and lower in lobectomy and sub-lobar resections (7.9%, 2.96% and 0.88%

respectively), with a 40% mortality in these patients overall (36). There are multiple etiologies including pulmonary infection, mechanical ventilation and sepsis; of those, pneumonia is the most common clinically important presentation associated with ARDS after lung surgery (36).

1.2.5. Postoperative complications of differing biological mechanism

Other postoperative complications do not share the common biological mechanism as atelectasis, pneumonia, and ARDS. Pulmonary embolism and cardiogenic pulmonary oedema are cardiovascular complications. Pulmonary oedema more commonly occurs after pneumonectomy, particularly in right pneumonectomy due to the left lung receiving 45% of pulmonary blood flow, and in the short term reduced capacity to support increase increased fluid volume (37). Pulmonary embolism is a rare (~2%) and severe complication following lung resection (38).

Other pulmonary complications after lung surgery are more attributed to the surgery such as pneumothorax and pleural effusion, in addition to pulmonary haemorrhage/contusion as well as bronchial artery bleeds. Postoperative air leak into the pleural space is common and typically from direct injury to viscera and underlying lung during surgery, creating an alveolar-pleural fistula. Air leaks are considered to be a more minor complication and typically resolve within 24-48 hours though termed as prolonged after 5 days which is seen in approximately 5% of lung resections without anastomosis (39), with the vast majority (~80%) not requiring any intervention (40).

Bronchopleural fistula is an abnormal connection between a proximal bronchus and pleural space, which can occur early or late following surgery and is rare (≤ 1% for lobectomy/sub-lobar resections). Bronchopleural fistula incidence is higher in pneumonectomy and associated with need for reoperation and a higher mortality (41). Anastomotic dehiscence can occur after sleeve lobectomy or other procedures involving bronchial anastomosis and may require endobronchial intervention (42).

Pleural complications can occur including empyema, an infection into the pleural space, which has reduced following antimicrobial prophylaxis and improved surgical techniques; etiology includes prolonged air leak or inter-operative contamination (32). Haemothorax is bleeding into the pleural space which rarely occur intra-operatively or immediately postoperatively due to injury of the bronchial or chest wall vessels and may require embolization or reoperation. Chylothorax is leakage of lymphatic fluid into the pleural space, which can rarely occur due to damage to the thoracic duct or its tributaries (43).

1.2.6. Incidence and definition of PPC

The overall incidence of PPC in thoracic surgery varies from 15% to 37% depending on the type of pulmonary complications studied, the clinical criteria used, and the type of surgery included (29, 44). PPC are collected for audit and benchmarking purposes. One of the largest databases is from the United States Society of General Thoracic Surgery Database (45), of which pulmonary complications are collected as part of

major morbidity data alongside cardiac and renal complications; the PPC data collected includes pneumonia, ARDS, prolonged ventilation or re-intubation/respiratory failure, and rarer complications such as broncho-pleural fistula and pulmonary embolism. In the UK, postoperative pulmonary complications are not collected as part of the Royal College of Physicians National Lung Cancer Audit (NLCA) (46). Previous main reports with the Society of Thoracic Surgeons in the UK have reported 30-day and 1 year mortality data and 30-day readmission but no major postoperative complications such as PPC (47). The European Society of Cardiothoracic Surgery (ESCS) Annual Report (48) also includes mortality data and air leak as significant complication but no further PPC outcomes such as pneumonia, atelectasis or respiratory failure. Cardiopulmonary complications are collected through audit by The European Society of Thoracic Surgery (ESTS). In using the ESTS retrospective dataset of 47, 960 patients, a risk scoring for cardiopulmonary morbidity and 30-day mortality following surgery (Eurolung1 and Eurolung 2) has been developed (49), with the mortality scoring of Eurolung 2 shown to also to be associated with long-term survival (50). These scores can be calculated for individual risk in patients undergoing thoracic surgery to aid in shared decision making, however are not specific to the development of PPCs.

One such way of defining PPC is by use of the Melbourne Group Scale (MGS). The MGS is a standardised and validated scoring system within thoracic surgery to define the presence of a PPC such as pneumonia and clinically significant atelectasis which share

a common pathological mechanism (51). The MGS was initially developed and utilized in 2010 to assess PPCs in thoracic surgical patients in a randomised controlled trial (RCT) of physiotherapy intervention (52). The MGS defines PPC by the presence of four or more of the following eight factors: CXR findings of atelectasis or consolidation; purulent sputum differing from preoperative status; physician diagnosis of pneumonia; temperature >38°C; oxygen saturations of <90% on air; signs of infection on sputum microbiology; raised white cell count (WCC) >11.2x10^9/L; and prolonged high dependency unit stay (HDU) or readmission to HDU or ITU for respiratory complications.

In a cohort of patients undergoing thoracotomy for lung resection (n=129) (51), the incidence of PPC using the MGS was 13%, in comparison to other PPC assessment methods such as the Grosselink Score (GS; 6%) (53) and Brooks-Brunn Score (BBS; 40%) (54). The GS though developed for use in thoracic and oesophageal surgery, had a low sensitivity on detecting PPC with stringent criteria requiring major CXR infiltrates, increased WCC or use of antibiotics, and an elevated temperature of >38°C. Whilst the BBS designed for use in upper abdominal surgery, may over diagnose PPC as it required only 2 factors to score positive including altered breath sounds which was most frequently found in thoracic surgical patients due to the nature of their surgery. The MGS however, corresponded with the clinic outcome of PPC diagnosis in thoracic surgery (51), with a larger cohort (n=234) showing that PPC development (defined by MGS) had a dramatic effect on patient outcome by increasing hospital

mortality (0.5% to 12%), intensive therapy unit (ITU) admission rate (1.5% to 26%) and hospital length of stay (LOS) (5 to 14 days) (29). However, the long-term impact of developing a PPC after lung surgery had not been studied.

More recently an international collaborative of anaethesists and peri-operative medics have systematically reviewed the definitions of PPC for use as a standard outcome measure in research (55). The consensus the standardised endpoints in perioperative medicine (StEP) collaborative included a new definition of PPC (55), based on the US Centers for Disease Control definition for pneumonia (56), the Berlin classification of ARDS (33), definitions of aspiration pneumonia and atelectasis, and severity which is graded by FiO_2 requirement and/or ventilation. This new termed definition for PPC however has not been validated within a thoracic surgery cohort.

1.2.7. Risk factor for PPCs

There are several factors that have been associated with the increase the risk of patients developing PPC undergoing surgery. These can be categorised into pre-operative or intra-operative/post-operative risk factors and are potentially modifiable or non-modifiable.

With regards to pre-operative factors, non-modifiable factors include advanced age (57, 58), male sex (27), frailty (27, 59), increased American College of Anesthetists (ASA) score (27, 59, 60), previous cerebrovascular accident (28), underlying

malignancy (61), long-term steroids usage (28) and recent acute respiratory tract infection (57). Whereas modifiable factors include smoking (27, 28, 59, 61), COPD (27, 28, 59, 61), both low and elevated body mass index (BMI) (61), and other co-morbidities including hypertension (61), heart failure (60, 61), chronic liver disease (57), gastro-oesophageal reflux (62), renal failure (63) and diabetes mellitus (61). Pre-operative shock and sepsis in the non-elective setting have also been shown to be associated with increased risk of developing PPC (64). Blood laboratory testing of anaemia (57), low albumin (65), increased urea (28) and creatinine levels (64) have also been associated with increased risk.

With regards to intra-operative and post-operative factors, modifiable factors include the type of surgery (57, 58, 65) and the approach of surgery with laparoscopic approach associated with less complications (65, 66). Emergency surgery has been associated with increased risk of complications over elective (64, 65), as well as a longer duration of procedure (27, 58) and re-operation (67). Modifiable factors include the use of anaesthesia including neuromuscular agents (20, 68), use of general anaesthesia (28), and the type of mechanical ventilation strategy used (69). Intraoperative blood transfusion has also been associated with increased risk of PPC (28, 63). There is also increased risk of PPC use of nasogastric tube in the peri-operative period (70, 71).

Risk prediction models have been developed to try and identify those individuals at high risk of developing a PPC after surgery. However, many are limited by use of retrospective databases and investigating only one type of pulmonary complication such as postoperative pneumonia (28, 59), and not a composite outcome. An example of a prospective multi-centre study included the risk prediction model to assess respiratory risk in surgical patients in Catalonia (ARISCAT) (57), which used the European Perioperative Clinical Outcome (EPCO) definition for PPC development. Independent risk factors associated with PPC in the model included low oxygen saturations (SpO2 <96%), respiratory infection within the last month, age, pre-operative anaemia (<100 g/dl), duration of procedure (>2 hours), intrathoracic/upper abdominal surgery and emergency surgery. The prospective evaluation of a risk score for PPCs in Europe (PERISCOPE) (58) externally validated the ARISCAT with good predictive power (0.80, 95%CI 0.78-0.82). These risk models however are not focused specifically on patients undergoing thoracic surgery.

Independent risk factors associated with PPC development in patients undergoing lung surgery have previously been identified as cigarette smoking, advanced age, reduced mobility, increased ASA score, elevated BMI and chronic obstructive pulmonary disease (COPD) (29). Open thoracotomy over VATS approach has also shown to be associated with the increased incidence of PPC in a propensity matched cohort (44).

Of the risk factors, current cigarette smoking has been demonstrated to be associated with a higher risk of developing a PPC. In oesophagectomy involving surgery with open thoracotomy and single lung ventilation, current smokers had greater post-operative systemic inflammation, higher inflammatory alveolar oedema and increased development of ARDS compared to non-smokers (72); current smokers had a poorer prognosis after oesophagectomy compared to non-smokers. Cigarette smoking is a potential modifiable risk factor for the development of PPC, thus smoking cessation would have an important role in patients who smoke and are undergoing lung surgery.

1.3. Smoking cessation

Within the UK, 14% of the adult population continue to smoke; including 14% in England and 16% in Wales (73). Those who wish to stop smoking should be given targeted Very Brief Advice (VBA), be offered medication and then referred to local stop smoking services. In the UK, the English Stop Smoking Services offer free evidence-based behavioural and pharmacotherapy for those who wish to quit (74). The smoking cessation service provided should be in line with that of the National Centre for Smoking Cessation Training (NCSCT) (75). Combination behavioural support and pharmacotherapy for smoking cessation is the most effective and cost-effective evidence based approach and has been shown to increase sustained and validated quit rates at least 3-fold compared to an unaided quit attempt (76). Options for licensed pharmacotherapy in the UK include combination nicotine replacement therapy (NRT), bupropion (Zyban), varenicline (Champix) and cytisine.

1.3.1. Nicotine replacement therapy (NRT)

Dual nicotine replacement therapy (NRT) (77) is a combination of the long-acting transdermal patch with one of the other short-acting forms such as the gum, inhalation cartridges, sublingual tablets and nasal spray. Dual NRT is advised over single-form NRT as it has been shown to increase the chance of a successful quit attempt (78). Furthermore, NRT bought over the counter outside of specialist smoking cessation services or brief advice has not been shown to increase the chance of successful quit attempt (79); demonstrating the importance of behavioral support and healthcare lead smoking cessation. Use of NRT carries minimal risk to users. In a Cochrane review of over 50,000 participants comparing any type of NRT to a placebo or non-NRT control group (78), adverse events related to NRT were product related, including skin irritation from patches and mouth irritation from tablets or gum, and importantly there was no evidence that NRT increases the risk of cardiovascular events.

1.3.2. Buproprion, varenicline and cytisine

Bupropion (Zyban) is an atypical antidepressant that increases the chance of quit attempt success at a similar level to NRT, as demonstrated by a Cochrane review including 10 studies and 8230 participants finding the the relative risk (RR) of smoking cessation between bupropion and NRT was 0.99 (95% CI 0.91 to 1.09) (80). The action is probably through reducing the urge to smoke but comes with side effects of disturbance in sleep and increase seizure threshold and is contraindicated in certain

groups (81). Varenicline (Champix) is a nicotinic-acetylcholine receptor partial agonist and has been shown to increase the success of a quit attempt by about 50% more than NRT or bupropion (82); with action to both reduce cravings but also reduce reward effect of smoking during a quit attempt (83). Common side effects include nausea and sleep disturbances. There were initial concerns particularly over neuropsychiatric effects of varenicline, which have since been shown not to be elevated over placebo; a Cochrane review included studies from 27 trials in 12,625 participants showed the chances of successful smoking cessation was three-fold compared to placebo and varenicline use was not associated with an increase of neuropsychiatric events including depression and suicidal ideation. (82). Unfortunately since 2021, the company that manufactures varenicline recalled the medication as a precautionary measure due to the presence of levels of the impurity N-nitroso-varenicline above the acceptable level of intake set by both the European Medicines Agency (EMA) and the Medicines and Healthcare products Regulatory Agency (MHRA) (84). It is not clear whether varenicline will return to the UK, though there are alternative medications such as cytisine, which is also a nicotinic-acetylcholine receptor partial agonist and has shown to be effective for smoking cessation (85, 86), with similar side effects to varenicline (87), and could be used in the UK in the future for smoking cessation.

1.3.3. Smoking cessation behavioural therapy

Incorporation of behavioural therapy provides an increased chance of a successful quit attempt, with pharmacotherapy having additive effect (88). As part of the stop

smoking service, patients are seen initially face-to-face or phone contact every week for 4 weeks with a counsellor trained in smoking cessation and then followed up less frequently for 12 weeks in total (77). At that appointment, they would have carbon monoxide (CO) measurement. Exhaled CO is an easy, rapid, non-invasive objective verification of smoking status. Exhaled CO issued primarily as a motivational aid to show the recovery following cessation, with levels less than 10 parts per million (ppm) at 4 weeks after the quit date used as a performance target and outcome in research (77, 89).

1.3.4. Very brief advice

Very Brief Advice (VBA) should be offered to all patients who smoke. VBA typical includes asking about current/past smoking behaviour, providing information on consequence of smoking, and options for support as detailed above (77). Even 30 seconds of brief advice has been shown to increase chance of smoking rates by 1-3% (90).

1.3.5. Digital interventions in smoking cessation

There is increasing interest in the use of digital interventions to aid quit attempt in smokers. Internet interventions have been shown to provide benefit in comparison to broad based interventions such as print materials (91). Mobile phone-based interventions, predominantly text-based interventions have been shown to have a beneficial impact on quit rates and were higher in those studies using biochemical

verification (92). There is little evidence to date of smoking cessation applications, with very few available applications having any scientific support (93). With health-related applications becoming increasing used for modern healthcare, even small individual effects can be rapidly scaled for larger public health effects (94), thus digital intervention in smoking cessation is a much needed area of research.

1.3.6. Electronic cigarettes

More recently e-cigarettes are being used for off-license smoking cessation. E-cigarettes are devices containing a battery powered heating element that produces a vapour from heating a liquid containing propylene glycol and/or glycerin, water and the addition of nicotine and flavorings (95). The vapour does not contain CO, though may contain potentially harmful chemicals, which are much less and at lower levels than that of cigarette smoke. For that reason, e-cigarettes are regarded as less dangerous in comparison to cigarettes by the Royal College of Physicians (96) and the British Thoracic Society (BTS), who both support a harm reduction strategy of e-cigarette usage over smoking (97); this is currently recommended by NICE guidelines in the UK (98). The European Respiratory Society (ERS), however, have been more cautious in its approach and currently do not advocate e-cigarettes as a smoking cessation aid when evidence based non-harmful smoking cessation aids already exist and the long-term impact of e-cigarettes are unknown (99). As a smoking cessation aid, a large RCT of 886 participants showed that e-cigarettes used within stop smoking services showed superior abstinence rates to NRT with (RR of 1.98, 95%CI 1.30 to 2.58)

(100); though 80% of those using an e-cigarette continued to use at 1-year compared to less than 10% of those on NRT. In addition, it is worth noting that NRT had failed in 75% of the participants in the trial, which could have influenced the study outcome. With regards to the harms of e-cigarettes, in 2019 there was great concern over a national outbreak of cases in the US from e-cigarette or vaping, product use-associated lung injury (EVALI) (101), with such cases rarely seen in the UK. These have since been widely attributed to the addition of vitamin E acetate in the supply of THC-containing products which has been detected in the broncho-alveolar lavage (BAL) of these patients (102), and supported further in an animal model of EVALI-like lung injury (103). With regards to the long-term harm of e-cigarette use, little is known clinically, and the effect of usage in lung cancer patients and in those undergoing surgery. There is a growing body of evidence from *in vivo* and *in vitro* models that indicate long-term changes to the pulmonary environment (104). This includes dysregulated inflammatory cytokine output reflecting some elements of immunopathogenesis of COPD, and changes to lipid homeostasis that are likely to result in irreversible parenchymal lung tissue damage, contributing to chronic lung disease in long-term vapers (104).

1.3.7. Smoking cessation benefits on lung health

After stopping smoking CO levels in the blood will have halved within 8 hours (105). After 24 hours, carbon monoxide levels will become that of a non-smoker. Improvement of pulmonary gas transfer (DLCO) has been shown to occur after 24

hours of cessation with no further benefits after 1 month (106); pulmonary epithelial permeability improves after 24 hours and furthermore at one week post cessation (107). Sensitivity of the upper airway reflexes improves after several days of smoking cessation (108); some patients may develop a worse cough after stopping smoking but this nearly always improves after 6-8 weeks. Nasal mucociliary clearance has been shown to improve after 2 weeks of smoking cessation (109). Symptoms of cough, sputum expectoration, wheeze and breathlessness have been shown to improve following smoking cessation and can occur within a few months of cessation (110, 111). Smoking cessation has a beneficial effect in reducing long-term lung function decline (FEV_1) at any age but is more pronounced in earlier quitters (112). In COPD smoking cessation reduces rate of FEV_1 decline by half (113); smoking cessation also reduces hospitalization from COPD in those who quit, which wasn't seen in those who had reduced tobacco consumption (114). Smoking cessation halves the risk of developing lung cancer (115); people who stop even in their later ages have risk reduction within a few years compared to continued smoking (116). Over the age of 35, quitting smoking recovers 2-3 months of life expectancy for each year of smoking avoided (117). In those with diagnosed early-stage NSCLC, lifetime modeling estimates that a 65-year-old current smoker has a 5-year survival of 33% after compared to 70% in non-smokers (118). Furthermore, in those with newly diagnosed NSCLC of all stages, smokers had a lower 12-month survival than never/ex-smokers and quitting smoking was associated with 25% reduction in mortality, which may be clinically important, although not statistically significant after adjustment for other factors (119).

1.3.8. Optimal timing for preoperative smoking cessation to reduce PPC

PPC following thoracic surgery reduces with increasing smoking cessation time, but no optimal time can be defined (120). There were 2 of 7 relevant studies that showed PPC risk increased in the first few weeks after smoking cessation (121, 122), although these were both retrospective in design and relied only on self-reported smoking status. The mean doubling time for NSCLC is 5 months; however every millimeter matters with regards to staging of lung cancer as the smallest increase in tumour size and/or lymph node involvement can impact survival, and shorter lung cancer pathways are associated with improved survival (123). Therefore, current NICE guidance states that lung cancer surgery should not be delayed for smoking cessation and National cancer targets mandate that surgery should be performed within 31 days of decision to operate at the MDT (12). However, in curative-intent lung cancer surgery, PPC development also significantly impacts on short-term outcomes including morbidity and in-hospital mortality after surgery. Effective smoking cessation strategies are urgently needed in these patients. Smoking cessation may need to be integrated within the thoracic surgical pathway to be delivered in a timely manner. However, it is also important to determine the biological effects of smoking and the relationship with PPC development, as there may be other ways to reduce the risk of PPC in patients who smoke prior to surgery.

1.4. Alveolar macrophage (AM)

Macrophages are present in almost all tissues of the body (124). They derive their name from *makros* and *phagein,* Greek for 'big eater' after their primary bacterial killing mechanism, phagocytosis. In the lung they are the most abundant immune cell present under homeostatic conditions, representing over 90% of the alveolar immune cells. As sentinel cells, AMs play an important gate-keeping role in innate immunity within the respiratory tract. AMs classically exert regulatory effects via non-specific immune-defence mechanisms such as phagocytosis, the production of inflammatory mediators such as reactive oxygen species (ROS), and the expression of inflammatory cytokines such as interleukin (IL)-1, -2, -4, -6, -8, tumour necrosis factor-α (TNFα) and interferon gamma (IFNγ). AMs also resolve inflammation via the release of anti-inflammatory mediators and clearance of apoptotic bodies (efferocytosis). Therefore, AMs both initiate and resolve the immune the response, as well as having a role in surfactant/lipid processing and iron homeostasis.

A major factor that likely contributes to the effect of cigarette smoke exposure on lung surgery outcomes is the effect on alveolar macrophages (AMs). Cigarette smoke has multiple mechanisms on AMs to influence lung disease, as summarised in **figure 1.1**.

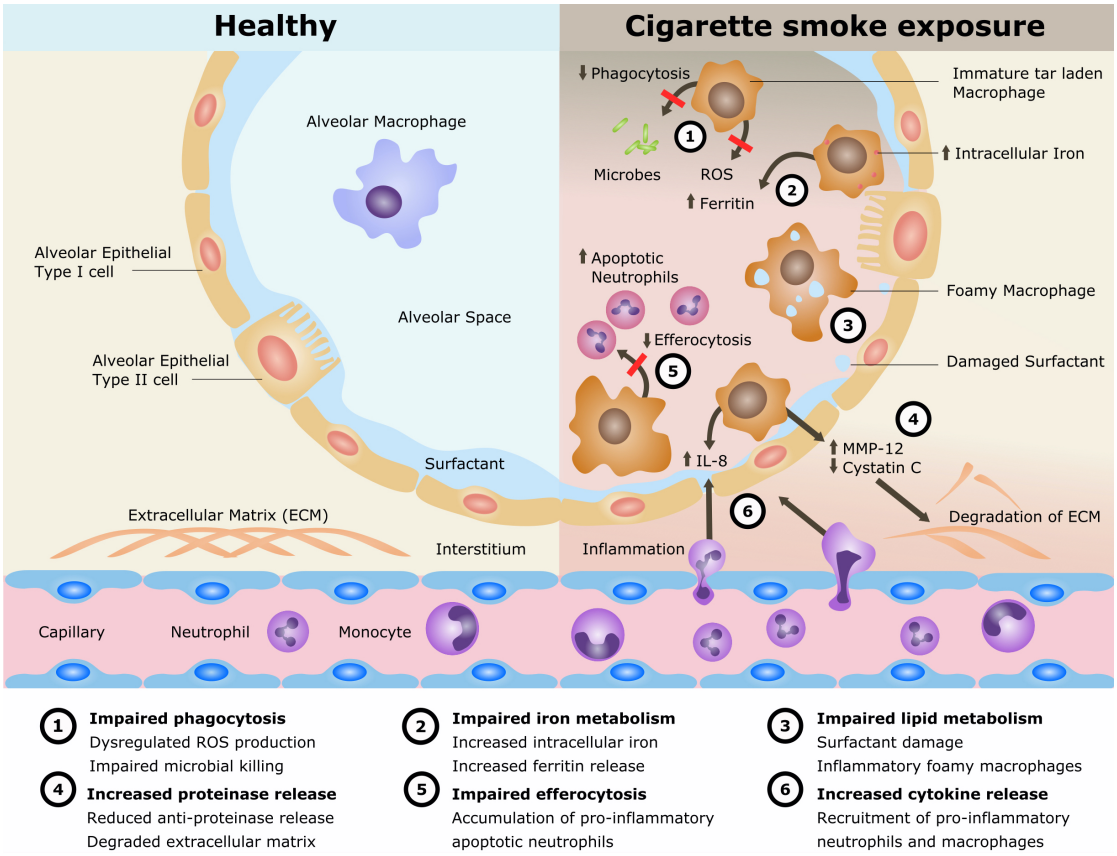


Figure 1.1 Mechanisms for disease in cigarette smoke exposure of alveolar macrophages. (1)

1.4.1. Otogeny/lifespan of the AM

Once thought to originate from circulating monocytes from the bone haematopoietic stem cells (HSC) (125), mechanistic murine work has indicated that AMs are initially derived from the yolk sac in foetal development and are independent of the HSC under homeostatic conditions. It is becoming clearer that resident lung-resident AMs maintain their population during homeostasis by proliferation *in situ* rather than having a reliance on the macrophage precursors in the blood (126, 127). Whereas

during ongoing inflammation, monocytes are recruited to the lung and develop into AM like cells (128, 129). Studies looking at chimerism in transplant patients have shown evidence of donor persistence of AMs (130), that can persist up to 2 years (131). However, in the inflammatory state in transplant patients and in healthy older volunteers, the majority of human AM originate from circulating monocytes (132).

1.4.2. Lung monocyte/macrophage populations

Distinct monocyte/macrophage populations in the lung can be defined as AMs, interstitial macrophages (IMs) and monocyte-derived cells according to their cell surface phenotype (see **figure 1.2**). Lung macrophages express the mannose receptor CD206, which has a role in recognition of microbial carbohydrates and mediating phagocytosis. This receptor is not detected in monocytes within the blood or intravascular compartment (133). AMs reside within the airway lumen and alveolar space, in contact with both the airway environment and epithelium. They are recognised by their distinctively high side scatter, high expression of CD206 and relatively low expression of monocyte marker CD14. IMs are located between alveolar epithelial cells, and unlike AMs, are negative for CD169 (Siglec-1 or sialoadhesin), a cell adhesion molecule shown to discriminate phenotype between AMs (CD206+CD169+) and IMs (CD206+CD169-) (134, 135). AMs can be further sub-divided by levels of expression of the high affinity-scavenger receptor CD163 (CD163++ or CD163+++ sub-populations) (135). Within the lung there is also a population of immature, infiltrating CD14+ pulmonary monocyte-derived cells, thought to be

recently recruited into the extravascular space. These can be distinguished by their expression of CD206 as well as other markers CD141, CD11c, HLA-DR, and CCR7 compared with the intravascular $CD14^{+}$ monocytes, with CD1a and CD1c allowing further categorisation of these cells (133).

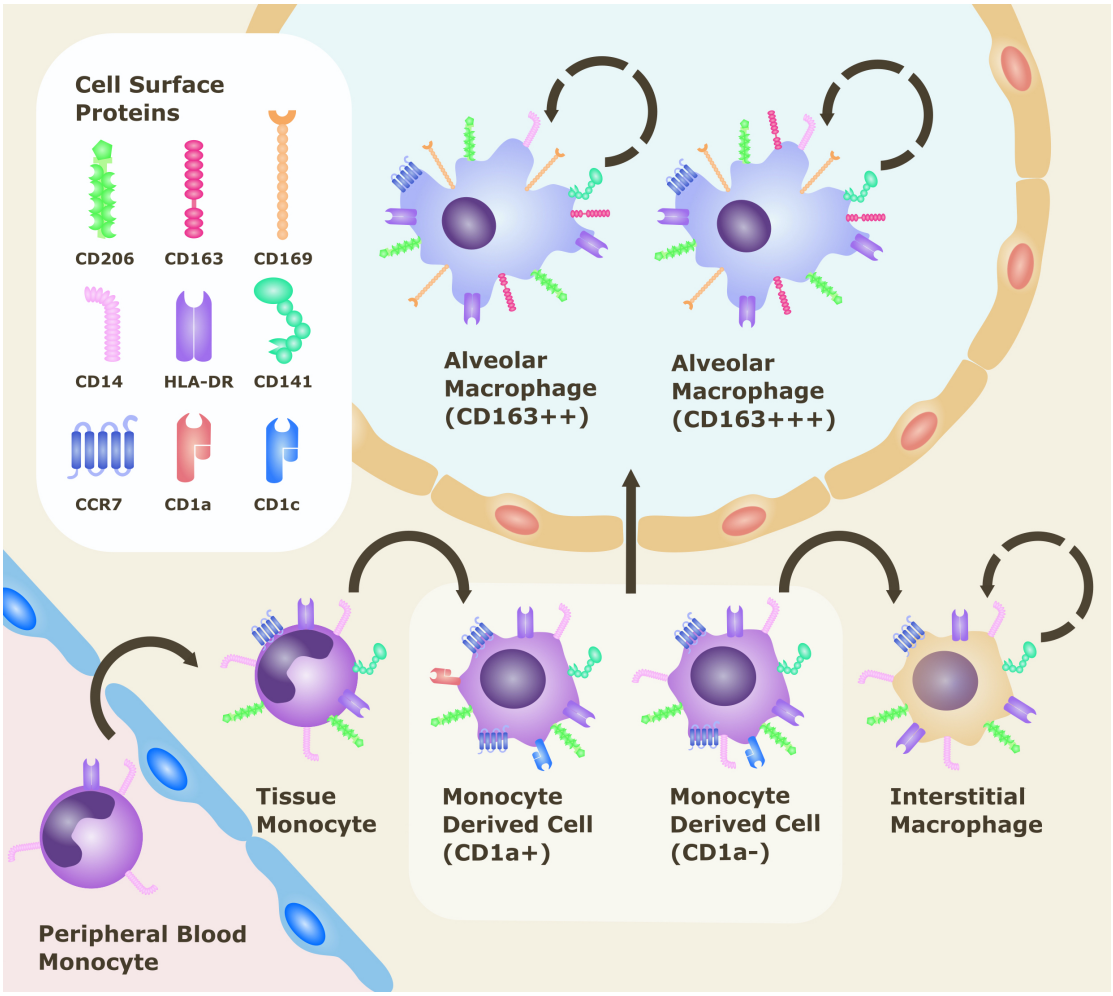


Figure 1.2 Cell surface markers of monocyte/macrophage populations within the lung. (1)

1.4.3. AM phenotype and normal function in airways

Macrophages have been shown to exhibit plasticity and can change their phenotype depending on the local environment. Macrophages have traditionally been described to form two distinct polarized phenotypes *in vitro* following cytokine exposure of monocyte-derived macrophages (MDMs). 'M1 macrophages' are classically activated, typically by lipopolysaccharide (LPS), IFNγ, and/or TNFα, and are involved direct destruction of intracellular pathogens, and produce pro-inflammatory cytokines, as well as a T helper 1 (Th1)-cell environment. 'M2 macrophages' are alternatively activated, typically by interleukin (IL)-4 and IL-10 and are a more heterogeneous group and involved in down-regulating inflammation or pro-resolution and tissue repair, producing a Th2 environment. M1 and M2 have been largely unaltered since their initial classification (136); M2 roles have been further categorised into M2a (parasite destruction) 2b (immune regulation) and 2c (tissue remodelling and extracellular matrix deposition) (137). With regards to cell surface markers, M1 are typically characterised by expression of HLA-DR, CD14 and CD38, whilst M2 are characterised by CD36, CD206 and CD163. Many studies have looked at M1/M2 with relevance to disease. However, more recently studies have moved away from this model; *in vivo* these phenotypes exist as part of a spectrum and many of the M1/M2 markers are co-expressed on the same macrophages, such as CD206 ubiquitously expressed by all pulmonary macrophages (133).

1.4.4. Cigarette smoke constituents

Cigarette smoke is an aerosol consisting of solids and liquid droplets (the particulate ("tar") phase) in a gaseous phase, and contains approximately 4,500 different substances (138), which have various toxic, mutagenic and carcinogenic effects. These include nicotine, tar, ammonia, carbon monoxide, carbon dioxide, formaldehyde, acrolein, acetone, benzopyrenes, hydroxyquinone, nitrogen oxides and cadmium (139). Inhaled particulate matter is deposited in the respiratory tract depending on the size, with larger particulates in the upper airways and smaller particulates deposited in the alveoli, which result in a chronic low-grade inflammation. Smoking results in chronic inflammation and recruitment of inflammatory cells to the airways. Cigarette smoke also causes oxidative stress which triggers local lung inflammation by activation of epithelial cells, alveolar macrophages, neutrophils, and T lymphocytes (140). Furthermore, cigarette smoke also increases the virulence of pathogens, increasing the risk of pulmonary infections (141).

1.4.5. CS exposure and AM recruitment

Cigarette smoking induces an increase in immune cells within the lung (4-5 fold), as assessed by BAL (142) with most of the increase attributed to the AM cellularity (143). The increased cellularity of both AM and neutrophils may be explained by chemotactic factors generated in the lung. CS exposure activates the pro-inflammatory transcription factor nuclear factor kappa B (NF-κB) and increases messenger RNA

(mRNA) expression of IL-8 (one of the mediators controlled by NF-κB) within the macrophages (144, 145). This process is at least partially an autologous effect, with other cells such as bronchial epithelial cells shown to release increased IL-8 in response to CS that would contribute to stimulate macrophage and neutrophil influx (146). The increased BALF cellularity may take over 1 month to significantly reduce and up to 6 months to fully normalise (147).

1.4.6. Accumulation of autofluorescent bodies

AMs from smokers contain autofluorescent bodies (148) due to the uptake of CS particles (i.e. 'tar'). Even short-term ex-vivo AM exposure to tar results in an increase in autofluorescence (149). These smoking related inclusions can continue for up to 270 days after cessation (150) and have been found on autopsy of patients who had stopped smoking 2 years prior (151). This has been shown to be the case in other studies that have investigated AMs from BAL between healthy smokers and non-smokers (148). This may be relevant to understanding the prolonged effects of smoking on AMs following smoking cessation. Furthermore, the autofluorescence is not related to amount of exposure, such as pack year consumption (152). This suggests a maximum possible capacity of tar uptake, anything beyond this point will not be retained, destroying any dose response seen in earlier stage of exposure. There is also the factor of AM flux and turnover that may contribute to lack of dose effect at very high CS exposure levels. In addition, it could mean there is a fixed response within the AM following exposure that never goes within the lifespan of the cell.

1.4.7. CS exposure and AM phenotype

Smoking alters the phenotype of AMs, with observed differences in cell surface marker expression compared to non-smokers (**table 1.1**). Smoking increased expression of CD14 and reduced expression of CD71 in a monocyte/macrophage population from BAL (153). Absence of the transferrin receptor CD71 in AMs is characterised by reduced expression of mature markers, impaired phagocytosis and expression of profibrotic genes (154). In COPD, lung macrophages from smokers expressed lower levels of the high affinity-scavenger receptor CD163 and lower levels of the non-opsonic receptor CD36 compared to ex-smokers (155). CD163 and CD36 both have an important role in efferocytosis, which is the clearance of apoptotic cells (156). CD91 has a role in phagocytosis as part of the CD91-calrecticulin mannose receptor complex. It has been shown to be reduced in expression in AMs from healthy smokers and COPD smokers when compared to never smokers (153). CD31 (PECAM) is a surface marker that has been reported to promote tethering of apoptotic cells and is reduced in AMs from smokers (153). Blocking of CD91 and CD31 has shown to reduce AM efferocytosis.(153) CD44 is involved in clearing of hyaluronan, lipid metabolism, surfactant processing and efferocytosis, all which have a role in regulating inflammation; CD44 is reduced in AM from smokers when compared to non-smokers (153, 157). CD11c is an integrin that mediates cell-cell and cell to matrix interactions and is expressed at higher levels in healthy smokers compared to non-smokers (158-160). CD54, also known as intercellular adhesion molecule-1 (ICAM-1), has a major role in immune and inflammatory regulation and has been shown to be expressed in

lower levels in AMs from smokers compared to non-smokers (159, 161). CD169 is a cell marker used to characterise AMs/IMs, however little is known about the affects of CS exposure on expression. CD169 has been shown to be involved in phagocytosis of non-typeable *Haemophilus influenzae* and levels are reduced in COPD ex-smokers compared to non-COPD ex-smokers, however current and recent ex-smokers (2 months) were not assessed (162). Abnormal AM expression in COPD smokers may also be related to other factors. Ageing has been shown to alter airway monocyte populations with an increase in CD14+CD16- classical monocytes from BAL in younger adults compared to older adults (>50 years old) (132). Medications such as corticosteroids can also impact AM phenotype, with an increase CD163 and CD206 expression on COPD lung macrophages (163). Altered AM phenotype can also be associated with other co-morbidities such as the reduction in CD71 expression seen in patients with idiopathic pulmonary fibrosis (154) or an increase in 'M2' CD206 expression AMs in patients with asthma (164).

Cell surface marker	Function	Level of expression in smokers	Reference
CD11c	Integrin alpha X chain; adherence to stimulated endothelium and phagocytosis of complement coated particles	↑	(158-160)
CD14	Co-receptor working with toll-like receptors (TLRs); facilitates response to lipopolysaccharide (LPS)	↑	(153)
CD16	Type III Fc gamma binds immunoglobulin, participates in signal transduction and phagocytosis	↓	(159)
CD31	Platelet endothelial cell adhesion molecule (PECAM), promotes tethering of apoptotic cells in efferocytosis	↓	(153)
CD36	Scavenger receptor, regulates efferocytosis of apoptotic neutrophils and role in fatty acid uptake and lipid metabolism	↓	(155)
CD44	Cell surface glycoprotein involved in cell-cell interactions, adhesion & migration; receptor of efferocytosis	↓	(153, 157)
CD54	Intercellular adhesion molecule-1 (ICAM-1); involved in cell-cell interactions and leucocyte migration	↓	(159, 161)
CD71	Transferrin receptor, role in iron metabolism and iron import into cells through endocytosis	↓	(153, 161)

CD91	LDL receptor-related protein; binds to collagen like region on lung collectins and role in phagocytosis	↓	(153)
CD163	Scavenger receptor for haemoglobin-haptoglobin complex; innate immune receptor for bacteria; can be influenced by corticosteroids	↓	(155)

Table 1.1 Cigarette smoking related phenotypic changes of alveolar macrophages.

Therefore, smoking influences the phenotype of pulmonary macrophages, resulting in more undifferentiated immature monocyte-like macrophages with a reduction in the mature AM surface markers needed for phagocytosis, efferocytosis, cell-cell and cell to matrix interactions. There is also an increase in the immature markers of CD14 and CD11c, which are expressed more in blood/tissue monocytes and monocyte derived cells (133, 165). The increase in the immature macrophages seen in smokers may be due to the influx of monocyte-derived cells from the peripheral blood, with the additional increase due to AM proliferation within the lung.(153)

1.4.8. CS exposure and AM immune function

Detection and phagocytosis of foreign particulates and microbial matter is key to AM function and, in many cases, is the first step in orchestrating an immune response to infection. More frequently, within the airway, non-opsonised phagocytosis occurs via

scavenger receptors, leading to cytoskeletal rearrangements and particle engulfment (166). This process results in a phagosome, which fuses with the lysosome to form the phagolysosome, with subsequent acidification to destroy the foreign particle/organism.

In patients undergoing surgery, anesthesia has also been shown to detrimentally affect AM phagocytic and antimicrobial function (167). During surgery and anesthesia, smoking further reduces AM phagocytosis of non-opsonised and opsonised (pre-incubated with rabbit immunoglobulin G anti-bovine serum albumin) (167) albumin coated fluorescent particles and killing of *L. monocytogenes*, which were halved compared to non-smokers (168). The expression of pro-inflammatory cytokines (TNFα, IFNγ, IL-1β) was increased only half as much in smokers (168). This supports the observed increased risk in postoperative pulmonary complications in patients who smoke before surgery. The effect of smoking cessation has been investigated (169); 4h after induction of anaesthesia, the decreases in antimicrobial function against *L. monocytogenes* were 1.5-3 times greater in AMs from current and recent ex-smokers (2 months) compared to never smokers. AMs from current and mid-term ex-smokers (2–5 months) mounted a blunted inflammatory response with only a 50% to 20% increased expression of pro-inflammatory (TNFα, IFNγ, IL-1β) and anti-inflammatory (IL-10, IL-4) cytokines compared to never smokers. Therefore, even stopping smoking for 2-5 months may not be enough time to fully restore AM function.

1.4.9. CS exposure and AM surfactant/lipid processing

The AM has a role in regulating the surfactant layer coating the alveolar epithelium by clearing damaged and oxidised surfactant produced by the alveolar epithelial type II (AT2) cell ensuring low surface tension to prevent alveolar collapse (170). AMs contribute to around 50% of surfactant turnover through degradation, with the remaining surfactant uptake and recycling by AT2 cells (171). Granulocyte macrophage colony-stimulating factor (GM-CSF) is required for the differentiation and functional activity of the AM in mice (172) and has an important role in surfactant homeostasis. CS exposure is associated with damaged pulmonary surfactant, inflammation and dysfunctional processing and lipid accumulation in AMs (a defining feature of foam cells); IL-1α-dependent inflammation following exposure is required to maintain surfactant homeostasis in the lungs through production of GM-CSF (173). In another murine study, CS exposure increased pulmonary GM-CSF and AM accumulation.

1.4.10. CS exposure and AM efferocytosis/resolution of inflammation

Efferocytosis is the clearance of primarily apoptotic neutrophils in the setting of acute lung inflammation. During efferocytosis the cell membrane of the AM engulfs the apoptotic cell, forming a vesicle called the efferosome, preventing the apoptotic cell from breaching its membrane and leaking toxic elements such as enzymes, oxidants and proteinases into the surrounding tissue. Thus, efferocytosis requires the AM to

adopt an anti-inflammatory state in order to prevent inflammatory responses to self-proteins (174).

Efferocytosis is reduced in AMs from smokers compared to never-smokers (153). AMs recognise apoptotic cells using cell surface receptors including CD44 (hyaluronan receptor), which is expressed at lower levels in smokers (153) and is important for clearance of apoptotic neutrophils and the release of anti-inflammatory and pro-repair mediators such as TGF-β (175). CS has been shown to reduce efferocytosis in AMs through the interaction of AM with CS modified ECM proteins and aberrant surfactant processing (176). Defective efferocytosis of AMs may also be driven by the significantly increased levels of CS induced oxidative stress (177). Another mechanism for reduced efferocytosis may be due to CS-induced defects in phagosome and the lysosome fusion, demonstrated in rat models (178).

CS reduced AM efferocytosis was restored through the delivery of GM-CSF to the alveolar space and was associated with reduced morbidity following influenza infection (179). In COPD patients, AM efferocytosis was higher than in those who stopped smoking compared to those who continued to smoke, suggesting a CS-related effect on AM in COPD that may be partially resolved upon smoking cessation (153). Following community acquired pneumonia, smokers had reduced AM efferocytosis, as were those who were not on a statin; suggesting that smoking and statins may have antagonistic effects on the Rac1 and RhoA pathway in influencing efferocytosis and

inflammation resolution (180). CS is known to affect pathways leading to the activation and membrane localisation of the enzyme Rac, which facilitates the cytoskeletal rearrangements needed for efferocytosis (181). Statins are a potential therapy to improve AM efferocytosis, as demonstrated in AMs from mice and COPD patients (182). There is some observational evidence (183) and randomised controlled trial data (184) to show statins may improve outcomes in COPD including time to exacerbation, exacerbation frequency and hospitalisation. Statins are also likely to play an anti-tumour role in lung cancer, through inducing tumour cell apoptosis and reducing tumour angiogenesis (185). Macrolides including azithromycin, are another potential therapy to rescue defective efferocytosis as shown in patients with COPD (186), possibly due to an up regulation of CD206 but the mechanism remains to be fully elucidated. Glucocorticoids improve AM efferocytosis but decrease phagocytosis in murine models of *S. pneumoniae* (187). Thus, resolving efferocytosis may leave the AMs that have ingested apoptotic cells less able to recognize and kill bacteria (188). Therefore, a balance must be struck between microbial clearance and pro-resolution.

1.4.11. Signal regulatory protein α (SIRPα)

SIRPα is a membrane glycoprotein expressed in monocytes/macrophages. SIRPα is activated through binding of CD47, a transmembrane protein widely expressed on human cells. SIRPα activation results in phosphorylation of cytoplasmic tyrosine kinase-based inhibitory motifs, which results in downstream signaling of protein tyrosine phosphatases SHP-1/2 associated with inhibition of phagocytosis of the cell

(189). Thus SIRPα:CD47 provides a 'don't eat me' signal; CD47 expression can be upregulated in cancer cells and thus to date most research to the SIRPα:CD47 axis is the context of cancer (190). Surfactant proteins A and D (SP-A and SP-D) also bind to SIRPα with downstream inhibitory pathway of reduced efferocytosis (191). Thus SIRPα can act as an inhibitory regulator of efferocytosis (**figure 1.3**). SIRPα may play an important role in the setting of acute inflammation and pulmonary infection and a potential therapeutic target. Nothing is yet known on the effect of CS exposure and SIRPα in AMs, and whether changes in phenotype relate to dysregulated function in the context of disease.

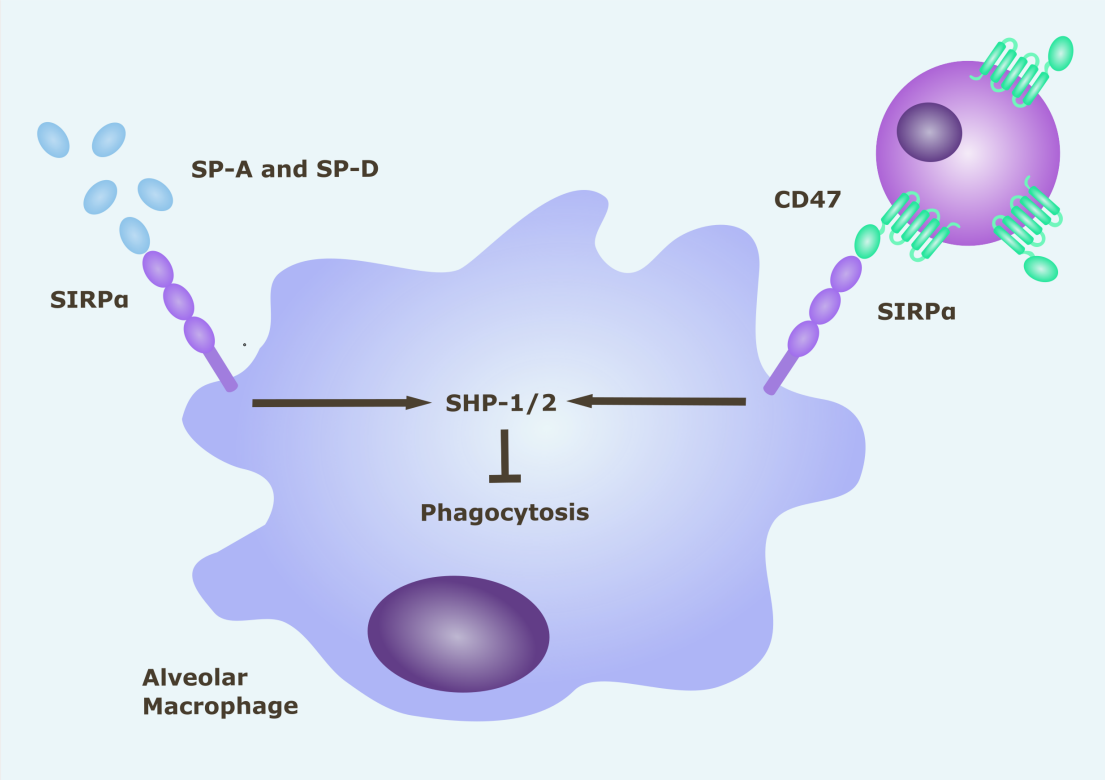


Figure 1.3 The role of SIRP-alpha and its ligands in phagocytosis.

1.5. Summary

For those diagnosed with early-stage lung cancer, curative-intent surgery is the mainstay of treatment. PPCs are a common complication in patients undergoing lung surgery and are associated with an increase in morbidity and mortality in the short-term. Smoking has been recognised as a risk factor for PPC development and is potentially modifiable. Smoking cessation within the UK is delivered locally with a combination of pharmacotherapy and behavioural support having the most evidence base for improving quit rates. Smoking cessation has long-term benefits including reducing symptom burden and treatment complications even in those with late-stage lung cancer, however the timing of cessation required to reduce PPC incidence is uncertain. Effective smoking cessation strategies in these patients are needed, and can be provided with an integrated smoking cessation model. However, the timing to allow longer duration of smoking cessation prior to surgery is outweighed by the need for shorter duration within the lung cancer pathway to avoid upstaging of the cancer, with national mandates in place. Cigarette smoking has a multitude of effects on AM phenotype and function. Of particular interest is the ability to restore the efferocytosis capacity of the AM, to be able to clear apoptotic neutrophils and resolve inflammation following lung injury. However, the duration of cessation needed for partial or full resolution of phenotype and function is largely unknown. Furthermore, the role of SIRPα in this process is of particular interest. The AM is a potential therapeutic target to ameliorate the risk of development of PPC in those high-risk patients who continue to smoke prior to curative-intent lung cancer surgery.

CHAPTER 2: HYPOTHESES AND AIMS

2.1. Hypotheses

Smoking before major lung surgery is a considerable risk factor in the development of PPCs, of which the AM has a role and may present a future therapeutic target to improve outcomes in those at risk. Strategies to help patients quit smoking before lung surgery would involve a smoking cessation programme integrated into the thoracic surgical pathway.

2.2. Aims

1) To determine the impact of postoperative pulmonary complications in patients undergoing major thoracic surgery including smoking as a potential risk factor.

2) To determine the impact of timing of smoking cessation in patients undergoing lung cancer surgery as determined by self-reported status.

3) To determine the role of the alveolar macrophage and SIRPα in cigarette smoking and postoperative pulmonary complications after lung cancer surgery.

4) To develop an web-based smoking cessation application for use in a feasibility study of an integrated smoking cessation programme in the thoracic surgical pathway before major lung surgery.

CHAPTER 3: LONG-TERM IMPACT OF DEVELOPING A POSTOPERATIVE PULMONARY COMPLICATION AFTER LUNG SURGERY

My role in this chapter involved clinical data collection, data analysis with statistical support, literature review, critical appraisal, and scientific writing. This chapter includes the peer reviewed and published original research article:

Lugg ST, Agostini PJ, Tikka T, Kerr A, Adams K, Bishay E, Kalkat MS, Steyn RS, Rajesh PB, Thickett DR, Naidu B. Long-term impact of developing a postoperative pulmonary complication after lung surgery. Thorax. 2016 Feb;71(2):171-6. PMID: 26769017. (192)

3.1. ABSTRACT

Introduction: Postoperative pulmonary complications (PPC) such as atelectasis and pneumonia are common following lung resection. PPCs have a significant clinical impact on postoperative morbidity and mortality. We studied the long-term effects of PPCs and sought to identify independent risk factors.

Methods: This retrospective study of prospectively gathered data involved all patients following lung resection in a regional thoracic centre over 4 years. PPCs were assessed daily in hospital using the Melbourne group scale based on chest x-ray, white cell count, fever, purulent sputum, microbiology, oxygen saturations, physician diagnosis and intensive therapy unit (ITU)/high dependency unit readmission. Follow up included hospital length of stay (LOS), 30-day readmissions, and mortality.

Results: 86 of 670 patients (13%) who had undergone a lung resection developed a PPC. Those patients had a significantly longer hospital LOS in days (13, 95%CI 10.5-14.9 vs. 6.3, 95%CI 5.9-6.7; $p<0.001$) and higher rates of both ITU admissions (28% vs. 1.9%; $p<0.001$) and 30-day hospital readmissions (20.7% vs. 11.9%; $p<0.05$). Significant independent risk factors for development of PPC were COPD and smoking ($p<0.05$), not age. Excluding early postoperative deaths, developing a PPC resulted a significantly reduced overall survival in months (40 95%CI 34-44 vs. 46 95%CI 44-47; $p=0.006$). Those who developed a PPC had a higher rate of non-cancer related deaths

(11 vs. 5%; p=0.020). PPC is a significant independent risk factor for late deaths in non-small cell lung cancer patients (HR 2.0, 95%CI 1.9-3.2; p=0.006).

Conclusion: Developing a PPC after thoracic surgery is common and is associated with a poorer long-term outcome.

3.2. INTRODUCTION

Lung cancer is the most common cause of cancer death within the UK (193). For those patients diagnosed with non-small cell lung cancer (NSCLC) potentially curative surgery is generally accepted as the most effective treatment (194). There is some evidence that patients with lung cancer in the UK present at a later stage and have a higher comorbidity than patients in some other European countries (195); this also might be due to poor organisation of resources and not just due to patient characteristics. Thus, surgical resection rates amongst those with proven NSCLC are lower in the UK (14%) compared to central Europe (24%) (196, 197). To address this most recent European and UK guidelines has widened the selection criteria for lung cancer surgery (198), which has helped improve resection rates. The 5-year survival rates for lung cancer in the UK is poor (9%) compared to central Europe (13%) as demonstrated in the 1999-2007 EUROCARE-5 report (199). A more recent study has shown that cancer survival in England is improving, however continued investment is needed to close this international gap (200). Furthermore, whilst in hospital mortality has fallen over the last 3 decades, mortality rates within the first 90 days of surgery are considerable (201). We need to understand the causes of these late complications if we are to improve long-term outcomes.

Lung cancer resection is also associated with a considerable risk of postoperative pulmonary complications (PPCs), of which pneumonia and atelectasis are the most common (29). PPCs have a significant health and economic impact on patients and

health care services. Indeed, as less fit patients are undergoing surgery, the incidence of PPCs is likely to increase further. The longer-term effects of developing a PPC in hospital following lung cancer surgery have not been defined. The identification of modifiable risk factors for PPCs has a crucial role in the development of innovative strategies to reduce the impact and incidence of PPCs. We hypothesise that PPCs are associated with the late postoperative mortality and morbidity observed following lung resection.

This study aims to assess both immediate and long-term impact of PPCs, and to identify potentially modifiable independent risk factors.

3.3. METHODS

This retrospective study of prospectively gathered data was conducted at a single centre large regional thoracic unit serving 6 million people. Consecutive patients who underwent open thoracotomy or video-assisted thoracoscopic surgery (VATS) for lung resection/removal in a regional thoracic centre between April 2010 and April 2014 were observed. Data was prospectively gathered as part of a single centre ethics number with the approval of the National Research Ethics Service (NRES) Committee West Midlands. This retrospective study was internally registered with the Birmingham Heartlands Hospital audit department (audit code 1672) to evaluate our service. Decisions regarding patient operability and resectability were informed by the British Thoracic Society guidelines for lung cancer resection (198).

Patients were admitted to hospital on the day of surgery. All operations were performed with single lung ventilation under general anaesthesia, and patients were subsequently scheduled for extubation in the operating room. Postoperatively, patients were managed in a dedicated thoracic high dependency unit HDU (level 2) and ward unless complications required their admission to the ITU. Postoperative pain control was achieved by continuous thoracic epidural analgesia, paravertebral infusion, intrathecal morphine and/or intercostal blocks or systemic opioids (parenteral administration or intravenous patient-controlled administration). The choice of analgesic technique was made by the anaesthetist after discussion with the patient. From the first postoperative day, all patients had a daily physiotherapy programme comprising deep breathing exercises, incentive spirometry, supported coughing and mobilisation.

The Melbourne Group Scale (MGS) is a standardised scoring system validated by our group to define the presence of a PPC such as pneumonia or clinically significant atelectasis, which are likely to adversely affect the patient's clinical course (29, 51).
In a cohort of patients undergoing thoracotomy for lung resection (n=129) (51), the incidence of PPC using the MGS was 13% and corresponded to the clinical outcomes of PPC diagnosis in comparison to other PPC assessment methods which either had a low sensitivity on detecting PPC due to stringent criteria (6% using Grosselink Score (53)), or may over diagnose PPC due to inclusion of factors such as abnormal breath

sounds (40% using Brooks-Brunn Score (54)). In a larger cohort (n=234), PPC development (defined by MGS) had a dramatic effect on increasing hospital mortality (0.5% to 12%), intensive therapy unit (ITU) admission rate (1.5% to 26%) and hospital length of stay (LOS) (5 to 14 days) (29). Using this score, PPC is defined in those patients presenting with four or more of the following eight dichotomous factors: chest x-ray (CXR) findings of atelectasis or consolidation; raised white cell count (WCC) (>11.2x10^9/l); temperature >38°C; signs of infection on sputum microbiology; purulent sputum differing from preoperative status; oxygen saturations <90% on room air; physician diagnosis of pneumonia; and prolonged HDU stay or readmission to HDU or ITU for respiratory complications. The MGS was used daily by senior physiotherapists who were performing their routine respiratory assessments. Patients were discharged once they were medically fit and discharged from physiotherapy.

Data collected included demographics and pre-operative record of smoking status, body mass index (BMI), percentage predicted forced expiratory volume in one second (FEV_1), American Society of Anesthetist (ASA) score, subjective preoperative activity level and comorbidities including chronic obstructive pulmonary disease (COPD) defined by clinical diagnosis of the referring clinician and staged according to percentage predicted FEV_1. Postoperative data included type of analgesia used and underlying pathology (including lung cancer staging if applicable). Total length of stay (LOS) was defined as the LOS in hospital after the date of surgery. The HDU and ITU

LOS were also recorded, as well as ITU admission and 30-day readmission to hospital secondary to surgical or pulmonary complications. All patients were followed up for overall survival (OS) and the cause of death was obtained from both the death certificate and hospital records. Deaths were classified as postoperative complication for patients who died within initial hospital admission or within 30 days of surgery, cancer related for those patients who died of disease progression/recurrence, and non-cancer related, or cause of death uncertain where records were not available or unclear.

3.3.1. Statistical analysis

Results are expressed as mean (SD or 95% CI) for continuous variables and as a percentage for categorical variables. As PPC was the outcome variable of the study, it was treated as a random variable rather than a fixed effect (i.e. group variable). Therefore, instead of using chi-squared/fisher's exact test and t-test/Mann-Whitney/Kruskal Wallis tests, univariate analyses of risk factors for development of PPC were assessed by performing individual un-adjusted logistic regression analysis and inclusion of one covariate at a time. Risk ratios with 95%CI were generated for variables found to have statistically significant association with PPC on univariate analysis. As PPC score is dichotomised (PPC or non-PPC) a backward multivariate binary logistic regression analysis was performed to identify the independent predictors of PPC within this dataset. Potential interactions between the variables were assessed by adding the variable interaction into the binary logistic regression

analysis and were deemed non-significant if $p>0.05$. As LOS was found to be positively skewed (right skewed) and not normally distributed, the effect of PPC on hospital, HDU and ITU LOS were assessed using the Mann-Whitney non-parametric test. After excluding those who died of early postoperative complication, the impact of PPC on long-term overall survival was assessed using Kaplan Meier plots and the log-rank test. In those with NSCLC diagnosis, cox regression was assessed including all risk factors in a model for long-term survival. All analyses were performed using the IBM SPSS version 20 and SAS 9.3 statistical package version (SAS Institute, Inc, Carry NC).

3.4. RESULTS

There were 670 patients who underwent pulmonary resections during the study period; 377 of who were male (56%). The mean±SD age of the group was 66.4±10.8 years. Mean predicted FEV_1 was 80.2±20.8%, mean BMI 26.9±8.1 kg/m^2, 328 patients (49%) had an ASA score ≥3, 176 patients (26%) had COPD, 149 patients (22%) were current smokers.

The most frequent procedure was lobectomy (n=497, 74%) followed by wedge or segmentectomy (n=111, 16%) and pneumonectomy (n=37, 5.5%). VATS lobectomy and segmentectomy were also performed (n=54, 8% and n=8, 1% respectively). Most common histological diagnosis was NSCLC (n=477, 71.2%) followed by benign disease (n=73, 11%) and metastatic disease of non-lung primary (n=69, 10.3%). Eighty-eight

patients (13%) had clinical evidence of PPC using the MGS. The median day of this occurring was day 2 postoperative. The four most common positive factors to trigger a score of 4 were raised WCC (n=75, 85%), purulent sputum (n=72, 82%), CXR findings (n=69, 78%) and reduced saturations (n=68, 77%).

On univariate analysis (**table 3.1**) the significant risk factors associated with developing a PPC were percentage predicted FEV_1, ASA >3 (Risk Ratio (RR) (4 vs. 1) 4.11, 95%CI 1.53-11), self-reported preoperative activity level ≤400m (RR 1.8, 95%CI 1.20-1.68), COPD diagnosis (RR 2.13, 95%CI 2.45-15.01) and current smoking (RR (current vs. never) 6.06, 95%CI 2.57-3.40) ($p<0.05$). Age, BMI, type of surgery (individually or collectively), VATS approach, cancer diagnosis or staging and cardiovascular disease was not significantly associated with development of PPC.

Variables	**Value**	**Total (n=670)**	**PPC (n=88)**	**Non-PPC (n=582)**	**P-value**
Age	Mean (±SD)	66.4 (±10.8)	67.1 (±9.6)	66.4 (±11)	0.547
FEV_1% predicted	Mean (±SD)	80.2 (±20.8)	75.8 (±20.7)	80.94 (±20.7)	0.034
BMI	Mean (±SD)	26.9 (±8.1)	27.2 (±5.6)	26.9 (±8.4)	0.757
Gender	Male Female	377 (66%) 293 (44%)	57 (65%) 31 (35%)	320 (55%) 262 (45%)	0.086

ASA Score	1 2 3 4	44 (7%) 298 (44%) 313 (47%) 15 (2%)	5 (6%) 32 (36%) 44 (50%) 7 (8%)	39 (7%) 266 (46%) 269 (46%) 8 (1%)	0.004
Pre-operative activity level	≤ 400 m > 400 m	185 (27%) 465 (73%)	35 (42%) 49 (58%)	150 (27%) 416 (73%)	0.005
Type of surgery	Lobectomy Subsegmentectomy/ wedge Pneumonectomy Exploratory/biopsy Sleeve Chest wall	497 (74%) 111 (16%) 37 (6%) 13 (2%) 8 (1%) 4 (1%)	64 (73%) 15 (17%) 3 (3.5%) 3 (3.5%) 2 (2%) 1 (1%)	433 (74%) 96 (16.5%) 34 (6%) 10 (2%) 6 (1%) 3 (0.5%)	0.651
VATS	Yes No	62 (9%) 608 (91%)	9 (10%) 79 (90%)	53 (9%) 529 (91%)	0.735
Analgesia	Epidural Intrathecal morphine Morphine Infusion PCA Paravertebral Other	254 (38%) 146 (22%) 63 (9.5%) 50 (7.5%) 146 (22%) 5 (1%)	27 (31%) 23 (26%) 9 (10%) 4 (5%) 24 (27%) 1 (1%)	227 (39.4%) 123 (21.4%) 54 (9.3%) 46 (8%) 122 (21.2%) 4 (0.7%)	0.443

Pathology	NSCLC Small cell LC Carcinoid Metastatic disease Benign Other	477 (71.2%) 10 (1.5%) 27 (4%) 69 (10.3%) 73 (11%) 14 (2%)	64 (73%) 3 (3%) 4 (4.5%) 6 (7%) 11 (12.5%) 0	413 (71%) 7 (1%) 23 (2%) 63 (11%) 62 (11%) 14 (4%)	0.598
NSCLC Staging	0 IA IB IIA IIB IIIA IIIB IV	5 (1%) 120 (26%) 112(24%) 94 (20%) 53 (11%) 77 (16%) 5 (1%) 4 (1%)	0 13 (22%) 10 (17%) 14 (23.3%) 9 (15%) 13 (22%) 0 1 (1.7%)	5 (1%) 107 (26%) 102 (25%) 80 (20%) 44 (11%) 64 (16%) 5 (1.3%) 3 (0.7%)	0.762
COPD	Yes No	176 (26%) 494 (74%)	38 (43%) 50 (57%)	138 (24%) 444 (76%)	<0.001
COPD Severity (FEV_1% predicted)	Mild (<80) Moderate (50-80) Severe (30-50)	51 (29%) 95 (54%) 27 (16%)	12 (32%) 19 (61%) 6 (16%)	39 (29%) 76 (56%) 21 (15%)	0.904
IHD	Yes No	85 (13%) 585 (87%)	15 (17%) 73 (83%)	70 (12%) 512 (88%)	0.665

HTN	Yes No	279 (42%) 391 (58%)	37 (42%) 51 (58%)	242 (42%) 340 (58%)	0.934
Diabetes	Yes No	80 (12%) 590 (88%)	14 (16%) 74 (84%)	66 (11%) 516 (89%)	0.220
Smoking status	Current Ex Never	149 (22%) 391 (68.5%) 129 (22.5%)	35 (40%) 48 (54%) 5 (6%)	114 (20%) 343 (59%) 124 (21%)	<0.001

Table 3.1 Baseline characteristics in PPC and non-PPC groups including univariate analysis of risk factors associated with developing PPC.

PPC, postoperative pulmonary complication; FEV_1, forced expiratory volume in 1 second; BMI, body mass index; ASA, American College of Anaesthetists; VATS, Video-assisted thoracoscopic surgery; PCA, patient controlled analgesia; COPD, chronic obstructive pulmonary disease; HTN, hypertension; IHD, ischaemic heart disease, NSLC, non-small cell lung cancer.

Using backward logistic regression to identify perioperative variables independently associated with PPCs our multivariate analysis included all variables. No significant interactions across any of the variables were found. Only COPD diagnosis and smoking status were associated with the development of a PPC (**table 3.2**). Patients who had COPD were 1.81 times more likely to develop PPC compared to non-COPD patients (95%CI 1.11–2.95; p=0.017). Current smokers were 5.42 times more likely to develop PPC that never smokers (95%CI 1.99-14.76; p<0.001), and ex-smokers were 2.8 times

more likely to develop PPC than never smokers (95%CI 1.08-7.28; p=0.035). The goodness-of-fit test of this model remained non-significant during these steps with a p value very close to 1 showing a very good model fit (Hosmer and Lemeshow p=0.975). The overall predictive power of the model was 66.3% indicated by the area under the curve (ROC). The resulting logistic model had a sensitivity of 60.2% and specificity of 65.9% with the cut-off probability point set at 0.12.

So in our model, a probability more than 0.12 means that a patients has 60.2% possibility to have a PPC. When the yielded probability is less than 0.12, patients have 65.9% chance to not develop PPC. The sensitivity of the model is the percentage of the group accurately identified by the model as having a PPC and the specificity is the percentage correctly identified as not having one.

Variables	**Estimate**	**SE**	**P-value**	**OR**	**95% CI**
Constant	-3.18	0.45	<0.001	-	-
COPD	0.59	0.25	0.017	1.81	1.11 - 2.95
Current smoker (vs. never)	1.69	0.51	<0.001	5.42	1.99 - 14.76
Ex smoker (vs. never)	1.03	0.49	0.035	2.80	1.08 - 7.28

Table 3.2 Preoperative risk factors associated with PPC on multivariate analysis.

COPD, chronic obstructive pulmonary disease.

Patients in the PPC group had a significantly longer (days) hospital LOS (12.7, 95%CI 10.5-14.9 vs. 6.3, 95%CI 5.9-6.7; p<0.001), longer HDU LOS (4.2, 95%CI 3.4-4.9 vs. 1.9 95%CI 1.8-2.1, p<0.001) and longer ITU LOS (2.1, 95%CI 1.0-3.3 vs. 0.2, 95%CI 0.1-0.4, p<0.001).

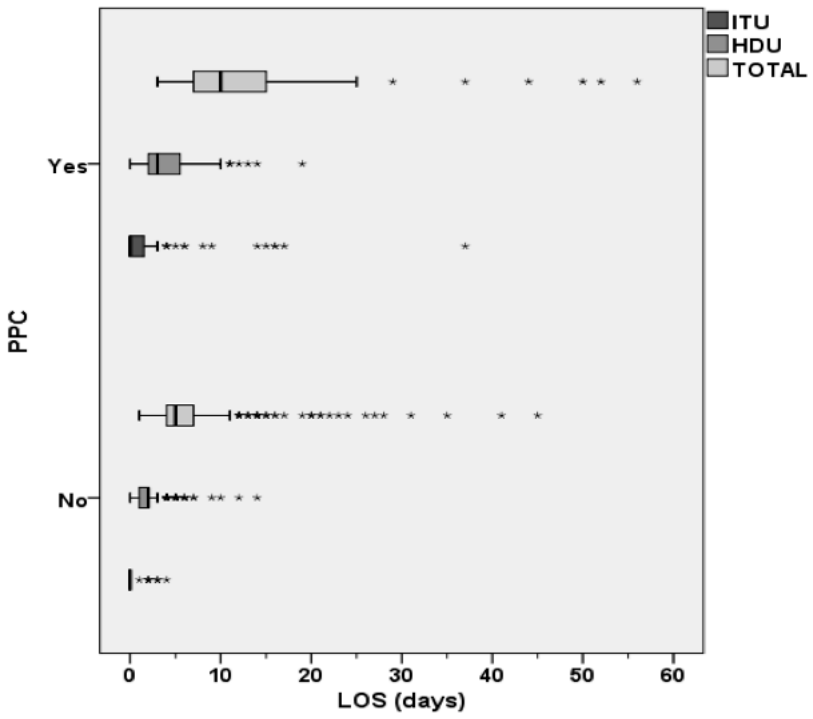


Figure 3.1 Effect of PPC on hospital length of stay (days). (192)

PPC, postoperative pulmonary complication; LOS, Length of stay; ITU, Intensive treatment unit; HDU High dependency unit, TOTAL; Total length of hospital stay.

In the PPC group there were higher rates of ITU admissions (28% vs. 1.9%; p<0.001) (**figure 3.1, table 3.3**). The 30-day hospital readmission was higher in the PPC group (21% vs. 12%; p=0.023). Patients who developed a PPC in hospital had higher rate of readmission secondary to pneumonia and lower respiratory tract infections (9.1% vs. 3.8%; p=0.046).

Of all patients there were 176 (26%) deaths with a median follow up of 12 months (95%CI 9.4-14.6). Causes of death included early postoperative complications (8.5%), cancer related deaths (65%), non-cancer related deaths (21%) and cause of death uncertain (5%). Patients with a PPC had a significantly higher 30-day (9% vs. 0.7%; p<0.001) and 90-day mortality (17% vs. 2.9%; p<0.001).

Variables		**PPC (n=88)**	**Non-PPC (n=582)**	**P-value**
Number of ITU admissions		25 (28%)	11 (1.9%)	<0.001
Mean LOS (95% CI) (days)	Hospital	12.7 (10.5-14.9)	6.3 (5.9-6.7)	<0.001
	HDU	4.2 (3.4-4.9)	1.9 (1.8-2.1)	<0.001
	ITU	2.1 (1.0-3.3)	0.2 (0.1-0.4)	<0.001
	Total	18 (21%)	67 (12%)	0.023
	Pneumonia/LRTI	8 (9%)	22 (4%)	0.046

Number of 30-day hospital readmissions	Postoperative complication		10 (11%)	43 (7%)	0.198
	Uncertain		0	2 (0.3%)	0.754
Number of deaths	Total		37 (42%)	139 (24%)	<0.001
	30 day-mortality		8 (9%)	4 (0.7%)	<0.001
	90 day-mortality		15 (17%)	16 (3%)	<0.001
Cause of death	Postoperative complication		9 (10%)	6 (1%)	<0.001
	Excluding postoperative complication		28 (32%)	133 (22%)	0.017
	Cancer related		18 (20%)	97 (17%)	0.380
	Non-cancer related	Total	10 (11%)	27 (5%)	0.020
		Respiratory	6 (6.8%)	13 (2.2%)	0.028
		Cardiovascular	3 (3.4%)	10 (1.7%)	0.395
		Other	1 (1.1%)	4 (0.7%)	0.507
	Uncertain		0	9 (1.5%)	0.615

Table 3.3 Morbidity and mortality following the development of a PPC.

PPC, postoperative pulmonary complication; ITU, intensive treatment unit; LOS, length of stay; HDU, high dependency unit; LRTI, lower respiratory tract infection.

Excluding patients who died of a postoperative complication (15/670, 2%), those who develop a PPC have a reduced OS with a mean follow up of 40 months (95%CI 34.1-43.8 vs. 45.8, 95%CI 44.3-47.3; p=0.006) (**figure 3.2**).

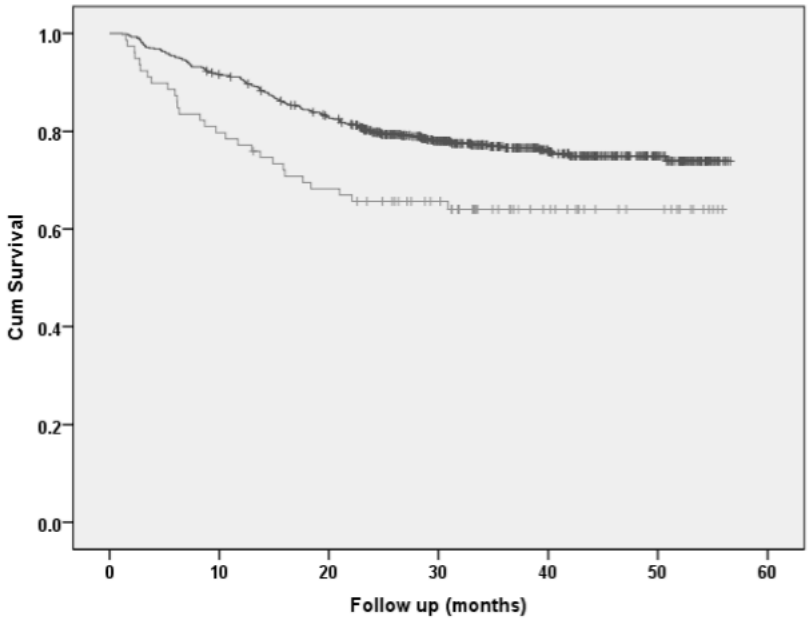


Figure 3.2 Overall survival of patients with and without a PPC excluding early postoperative deaths. (192)

PPC, Postoperative pulmonary complication; Black line: no PPC, Grey line: PPC.

There were a significantly increased number of non-cancer related deaths in those who develop PPC (11% vs. 5%; p=0.020). Of which, patients who developed a PPC were significantly more like to die of non-malignant respiratory disease (6.8% vs. 2.2%; p=0.028). Significant independent risk factors for late deaths in patients with NSCLC were PPC (Cox regression: HR 2.00, 95%CI 1.19-3.20; p=0.005), cancer stage, age and 30-day readmission to hospital (table 4).

Variables	Estimate	SE	P-value	HR	95% CI
PPC	0.69	0.25	0.006	2.00	1.19 – 3.20
Staging IIA/B	1.07	0.22	<0.001	2.92	1.89 – 4.56
Staging IIIA	1.28	0.25	<0.001	3.60	2.20 – 5.85
Staging IIIB	1.91	0.61	0.002	6.72	1.61 –18.90
Age	0.03	0.01	0.003	1.04	1.01 - 1.06
Readmission (30-days)	0.62	0.23	0.008	1.86	1.15- 2.89

Table 3.4 Significant Independent risk factors for late deaths in patients with non-small cell lung cancer. PPC, Postoperative pulmonary complication.

3.5. DISCUSSION

Our study has confirmed that PPCs are common in thoracic surgery. The short-term morbidity of developing a PPC following thoracic surgery is striking, with significantly longer hospital, ITU and HDU LOS, higher frequency of ITU admissions and higher frequency of hospital readmissions secondary to pulmonary infections. Furthermore, patients who develop a PPC have a significantly increased mortality, both in the early and late stages following surgery. After excluding immediate post-operative deaths, developing a PPC is a significant independent risk factor for late deaths and these patients have a worse long-term survival. Furthermore, we observed a high rate of non-cancer related deaths in this cohort.

The frequency of PPCs observed at this regional thoracic surgery unit (13%) is concurrent with other studies (29, 51, 202-205). Earlier observational cohorts from a single centre of thoracic surgical patients undergoing all resection types and with PPC defined using the MGS in 2007 and 2008 found an incidence of 13% (n=17/129) (51) and 14.5% (n=34/234) (29). A retrospective single site study of patients undergoing lung resection found a higher PPC incidence of 25% (n=68/268), though this was an older study (2000) with over 30% of patients having pneumonectomy and a higher 30-day mortality of 7.5% compared to 1.7% in our cohort (202). Similarly, an earlier study of 276 pneumonectomy cases for malignant disease from 1986 to 1988 classified pulmonary complications and cardiac complications separately, without using composite scoring found higher rates of pneumonia (10.2%) and retained secretions/atelectasis (13.5%), and a high incidence of respiratory failure (16%) with mortality of 7%, which is likely due to the age of the study and including pneumonectomy only cases (204). This is further supported by a single institutional study that demonstrated in patients undergoing lung resection for cancer that there was a reduction in PPC incidence (18.7% verses 15.2%) and perioperative mortality (3.7% verses 2.4%) over the time periods (1990-1994 to 2000-2004), which was associated with lesser resection types (205), and PPC incidence similar to our study. We did not find a higher rate of PPC in pneumonectomy or sleeve lobectomy cases in our study, though the numbers were very small with 6.9% and 1.3% respectively, which limits the capacity to find any signal in both univariate and multivariate analysis.

The incidence of PPC following thoracic surgery ranges between studies predominantly because there is no standard; it is dependent on the type of complications included, the definition of pulmonary complications, and the type of surgery. Use of the objective MGS to define PPC does not include rare but serious postoperative complications such as broncho-pleural fistulas, which required re-operation in 0.4% of our patients, and also pulmonary embolism, which has been described in 2% of patients after thoracic surgery (206). However, the fact that those more frequent, and probably less severe PPCs detected by the MGS were still associated with a higher short-term and long-term mortality rate is an important message.

Patients who develop a PPC are more than twice as likely to be readmitted within 30-days of surgery, and nearly 3 times more likely to re-present with respiratory tract infections. Hospital readmission is not only a serious morbidity; we demonstrate it also to be an independent risk factor for the late deaths observed in our study. Patients who are readmitted within 30-days of lung cancer surgery have been shown to have a 6-fold increase in 90-day postoperative mortality (207). In our study the 90-day mortality was 2.6 times that of 30-day mortality, compatible with other study findings (201, 208). Thus, this increased mortality following thoracic surgery is not explained by immediate postoperative deaths alone; we hypothesised that PPCs have a role in these later deaths observed.

Our study has shown that PPCs are significantly associated with reduced OS following thoracic surgery for lung resection. There is a paucity of data on the impact of PPCs on long-term outcome in thoracic surgery. A retrospective study which included patients aged 66-80 who have undergone a lobectomy for stage 1 NSCLC found that development of a PPC was associated with reduced 5-year OS (52.7 vs. 65.9%, $p<0.001$) and was an independent risk factor for mortality (HR 1.46, 95% CI, 1.24-1.73) (209). We have also shown that developing a PPC is a independent risk factor for late deaths, but in comparison our study is not limited by any inclusion criteria other than national guidelines (198). This includes those patients who are older and have more advanced staging of cancer, which we and other authors have found to be additional independent risk factors for late deaths (210, 211).

We demonstrate for the first time that after excluding immediate postoperative deaths, patients with a PPC are more likely to die of late non-cancer related deaths. This compares to the outcome of patients who are admitted to hospital secondary to community-acquired pneumonia (CAP). These patients have an increased risk of subsequent pneumonia and mortality after discharge, and a substantial proportion of these deaths are due to non-malignant respiratory disease (212). Some of the late deaths in patients who developed a PPC were secondary to cardiovascular disease, indeed there is increasing evidence that pneumonia is associated with increased risk of cardiovascular complications, which may be due to residual inflammation having a

role in triggering procoagulant pathways in these individuals (213).

COPD and current smoking were found to be independent risk factors in the development of PPCs in ours and other studies (29, 211). Our multivariate model for assessing of risk factors has a c-index of 0.66, which is low and therefore it cannot be used as a diagnostic/highly predictive tool. We would ideally like a predictive power ideally more than 80-90%, with 70-80% to be acceptable (214). This is likely due to the variables included in the model being limited to what was collected. Inclusion of other factors previously found to be independently associated with PPC development such as pre-operative anaemia and duration of surgery (57) may further aid in predicting PPC development in patients undergoing lung cancer surgery.

Nevertheless, our analysis identified factors having significant association with PPC. Patients diagnosed with COPD have increased risk of both pneumonia and atelectasis after surgery (215, 216). We found on univariate analysis that a lower preoperative FEV_1 percentage predicted was associated with the development of a PPC, though on multivariate analysis, FEV_1 is not an independent risk factor, as previously demonstrated (29). In our cohort carbon monoxide lung diffusion capacity (DLCO) was performed only in patients with limited exercise tolerance or lung volumes so this data was not collected. However, studies have shown DLCO not to be an independent risk factor for long-term survival following thoracic surgery for lung cancer (217, 218).

Regarding COPD diagnosis, there several reasons why a diagnosis of COPD is important in PPC development. Firstly, COPD is a multimorbid condition and associated with numerous comorbidities such as cardiovascular disease and metabolic disease which have previously showed to be associated with PPC development (60, 61). There are other associated co-morbidities which have been shown to be associated with worse outcomes in COPD and could contribute to PPC development such skeletal muscle dysfunction and nutritional abnormalities (219), and the presence of gastro-oesophageal reflux disease (62, 220). Oral corticosteroid usage is common in COPD, and has been shown to increase the risk of PPC (28). In addition, recent acute respiratory tract infections prior to surgery are associated with an increase in PPC (57), and are more common in patients with COPD diagnosis (221). Patients with COPD may also have reduced exercise tolerance and increased frailty (222), which are shown to be associated with PPC development (27, 59). Risk modification for patients with COPD would be through a pulmonary rehabilitation programme (223), which in thoracic surgery has shown encouraging results in reducing PPCs (224), but more robust studies are needed in this field.

Smoking is the biggest independent risk factor for developing PPCs; indeed our study has shown PPCs to be up to five times more common in current smokers when compared to never smokers, similar to other study findings (225). Key mechanisms are likely to involve the effect smoking has on impairing the mucociliary escalator (109), and also both the antimicrobial and pro-inflammatory functions of alveolar

macrophages, which decreases further during anaesthesia and surgery (168). Risk modification would be in the form of smoking cessation; however the duration needed in order to reduce postoperative risk remains a debated topic and is a much-needed area of future research.

Independent risk factors for developing PPCs have previously been identified as smoking, advanced age (≥75 years old), reduced mobility, ASA ≥3, cardiovascular comorbidity, COPD and BMI >/ 30kg/m^2 (29, 51, 202-204). Interestingly BMI, age and ASA were not significant risk factors for the development of PPCs in our study. Other investigators have found that obesity does not confer greater morbidity and mortality after lung resection (226). We have shown that age is not a predictive factor for development of PPC. This is an important finding for the clinical community and is supported by other studies that show no significant difference in postoperative complications in patients over the age of 75 (211, 227).

3.5.1. Study strengths and limitations

This is a real-life study involving consecutive patients undergoing thoracic surgery in a tertiary centre; our patient demographics and staging of cancer is comparable to national data (201). The main limitation of our study is the low number of VATS cases recorded. VATS lobectomy has since increased to 1/3 of all lobectomies carried out in the UK due to the perceived minimally invasive nature of the procedure with reduced complications. We have previously described that the PPC incidence in VATS cases

seems to be lower (228). The data of this study precedes the growth of VATS lobectomies; therefore, the majority of our lung resections over the time frame were done via thoracotomy. Within the latter half of the study 2 of the 5 surgeons had started to perform an increasing number of VATS lobectomies (35%) compared to the first half of the study period (3.4%). The finding of VATS approach not to show a significant reduction in incidence of PPC is most likely because of under powering due to the low number of VATS cases, and may also be in part due to the earlier adoption and learning of VATS procedures by the thoracic surgeons. Further studies into the effects of transition of thoracotomy to VATS on PPC frequency and long-term outcome will need to be conducted. There is limitation with regards to the patient follow up data; as information on mortality and cause of death were obtained from hospital records and death certificates in a retrospective manner, in a few patients cause of death could not be ascertained (5%). Additionally, we did not follow up for cancer recurrence and therefore could not assess the effect of PPC on disease free survival in patients with lung cancer, which would be an interesting area for further investigation.

There are limitations to be aware of using multivariate analysis. Backward elimination in the multivariate analysis allowed for all variables to be included and each step knocks out a variable at a time, with only significant variables contributing to the model in the final step. However, including all variables in the multivariate analysis can dilute true associations and lead to larger standard errors (229), which may in part explain why the standard errors of the significant independent factors associated with

PPC development in the model were relatively high (COPD diagnosis 0.25, smoker verses ex-smoker 0.49 and smoker verses never 0.51). This study did include more variables in the analysis than the ratio of one predictive variable for every 10 events as suggested by some (230), however this rule may be too conservative and 5-9 events per predictive variable may be enough (231). Multivariate analysis can include variables which are related such as COPD and FEV_1, however COPD and FEV_1 interaction was added into the logistic regression and was not significant and dropped out during the backwards stepwise elimination process. Therefore, COPD and FEV_1 could be assessed as independent variables in the analysis, with only COPD diagnosis being the significant independent risk factor in the model. Finally, regarding survival analysis there are non-linear associations, so hazard ratio may not be the same over time, such as the experience in VATS approach going up between years of 2010-2014.

3.5.2. Conclusion

In summary, developing a PPC after thoracic surgery is associated with a poorer long-term outcome. We have found COPD and smoking are independent risk factors for developing PPCs, whilst age was not a predictive factor. Further research is required into the effect of risk modification on the development of PPCs and subsequent long-term outcome following thoracic surgery.

3.6. Introduction to Chapter 4

Following on from the results of Chapter 3, having recognised that smoking was the biggest risk factor for PPC development. We sought to investigate the effect of smoking cessation on the development of postoperative pulmonary complications and observe the timing of smoking cessation on short and long-term outcomes after lung cancer surgery.

CHAPTER 4: SMOKING AND TIMING OF CESSATION ON POSTOPERATIVE PULMONARY COMPLICATIONS AFTER CURATIVE-INTENT LUNG CANCER SURGERY

My role in this chapter involved clinical data collection, data analysis with statistical support, literature review, critical appraisal, and scientific writing. This chapter includes the peer reviewed and published original research article:

Lugg ST, Tikka T, Agostini PJ, Kerr A, Adams K, Bishay E, Kalkat MS, Steyn RS, Rajesh PB, Thickett DR, Naidu B. Smoking and timing of cessation on postoperative pulmonary complications after curative-intent lung cancer surgery. J of Cardiothorac Surg. 2017;12:52. PMID: 28629433. (232)

4.1. ABSTRACT

Introduction: Smoking is a risk factor for postoperative pulmonary complications (PPC) following non-small cell lung cancer (NSCLC) surgery. The optimal timing for preoperative smoking cessation has not been identified. Our study aimed to observe the impact of pre-operative smoking cessation on PPC incidence and other postoperative outcomes including long-term survival.

Methods: This retrospective study of prospectively gathered data included consecutive patients following resection for NSCLC in a regional thoracic centre over a 4-year period (2010 - 2014). Patients were stratified according to self-reported preoperative smoking status. The primary endpoint was PPC incidence, which was assessed from postoperative day one onwards using the Melbourne Group Scale. Secondary endpoints included short-term outcomes (hospital length of stay (LOS), intensive therapy care unit (ITU) admission, 30-day hospital readmission rate) and long-term survival.

Results: 462 patients included 111 (24%) current smokers, 55 (12%) ex-smokers <6 weeks, 245 (53%) ex-smokers ≥6 weeks and 51 (11%) never smokers. PPC occurred in 60 (13%) patients in total. Compared to never smokers, current smokers had a higher frequency of PPC (22% vs. 2%, $p = 0.004$), higher frequency of ITU admission (14% vs. 0%; $p = 0.001$) and longer median (IQR) hospital LOS (6 (5) vs. 5 (2); $p = 0.001$). In the

ex-smokers there was a trend for a lower frequency of PPC (<6 weeks, 10.9% vs. ≥6 weeks, 11.8%) and ITU admission (<6 weeks, 5.5% vs. ≥6 weeks, 4.5%), but there was no difference between the <6 weeks or ≥6 weeks ex-smoking groups prior to surgery. There were no significant differences in long-term survival found between the groups of differing smoking status (median follow-up 29.8 months, 95%CI 28.4 – 31.1).

Conclusion: Current smokers have higher postoperative morbidity; this risk reduces following smoking cessation but 6 weeks does not appear to identify a time-point where differences in outcomes are noted.

4.2. INTRODUCTION

Non-small-cell lung cancer (NSCLC) represents approximately 85% of all lung cancer cases. In early-stages, surgery remains the corner stone of treatment, but is complicated by a high risk of developing a postoperative pulmonary complication (PPC), of which atelectasis and pneumonia are most common. Developing a PPC has been shown to be associated with an increase in short-term complications including increased hospital mortality (0.5% to 12%), ITU admission rate (1.5% to 26%) and hospital length of stay (LOS) (5 to 14 days) (29). Patients who develop a PPC also have a poorer long-term outcome with a 6-month reduction in mean overall survival (192). We have previously demonstrated that smoking is the strongest independent risk factor for developing a PPC after thoracic surgery (29, 192). Furthermore, smoking has been shown to have a negative impact on long-term overall survival and disease progression in lung cancer patients following curative-intent surgery (233).

Risk modification is via preoperative smoking cessation. Few studies have suggested that smoking abstinence of at least 4 weeks may be necessary for patients undergoing thoracic surgery in order to reduce the incidence of PPC and hospital mortality (121, 234). Despite this, an optimal duration of preoperative smoking cessation has not been identified. Many studies addressing this question are retrospective in nature with small sample sizes, the definition and criteria of PPC varies between studies and there is a paucity of published studies demonstrating the effects of preoperative smoking cessation on long-term survival.

To address these issues, this study primary aim was to prospectively observe the timing of preoperative smoking cessation on PPC incidence following curative-intent lung cancer surgery. The study secondary aim was to follow up these patients to ascertain if an optimal interval of preoperative smoking cessation is identifiable to improve the short-term postoperative outcome and long-term survival.

4.3. METHODS

4.3.1. Patient selection

This retrospective study of prospectively gathered data included all patients who underwent curative-intent lung resection for NSCLC via open thoracotomy or video-assisted thoracoscopic surgery (VATS) in a large UK thoracic centre between April 2010 and April 2014. Data was prospectively gathered as part of a single centre ethics number by the National Research Ethics Service Committee West Midlands, Edgbaston. Decisions regarding patient selection and operability were informed by the British Thoracic Society guidelines (198). Preoperative smoking status was categorised into current smokers who continued up to the date of surgery; ex-smokers who stopped smoking <6 weeks prior to surgery, ex-smokers who stopped smoking ≥6 weeks prior to surgery, and never smokers. Six weeks was chosen as the reporting period as previous evidence suggested that preoperative smoking cessation interventions might be more beneficial when offered within this timeframe, rather than immediately before surgery (235). Smoking data was collected by patients self-

reporting to the specialist thoracic research team (including nurses and physicians) at the pre-operative assessment and the hospital admission using a paper-based case report form, which was then uploaded onto the electronic database.

All operations were performed with single lung ventilation under general anaesthesia. Patients were subsequently scheduled for extubation in the operating room and postoperatively managed in a thoracic high-dependency unit (HDU) (level 2) and specialist ward unless complications required their admission to the ITU. The choice of postoperative analgesia was agreed between the anaesthetist and patient. From postoperative day one, all patients received daily physiotherapy comprising of deep breathing exercises, incentive spirometry, supported coughing and mobilisation.

All patients included in the study had a confirmed pathologically staged diagnosis of NSCLC (TNM 7^{th} edition). Baseline data included demographics, body mass index (BMI), percentage predicted forced expiratory volume in one second (FEV_1), American Society of Anaethesiologists (ASA) score and subjective preoperative physical activity level. Comorbidities including cardiovascular disease COPD diagnosis were both defined by the referring physician, with COPD severity staged according to percentage predicted FEV_1.

4.3.2. Primary end point

The Melbourne Group Scale (MGS) is a standardised scoring system previously validated by our group to define the presence/absence of a PPC (29). The MGS defines PPC when patients present with four or more of the following eight factors: chest x-ray (CXR) findings of atelectasis or consolidation, raised white blood cell count (WBC) (>11.2^{9}/l) or administration of antibiotics (in addition to prophylactic antibiotics), temperature >38°C; signs of infection on sputum microbiology, purulent sputum differing from preoperative status, oxygen saturations <90% on room air, physician diagnosis of chest infection/pneumonia, and prolonged HDU stay or readmission to HDU or ITU for respiratory complications. The MGS was used daily from postoperative day one onwards by senior physiotherapists who were performing their routine respiratory assessments. CXR results and physician diagnoses of chest infection/pneumonia were confirmed by a senior thoracic surgeon.

4.3.3. Secondary end points

Postoperative data included hospital LOS, ITU LOS, ITU admission frequency and 30-day hospital readmission rates (secondary to surgical or pulmonary complications). All patients were followed up during the study period for overall survival; defined as the time from date of surgery to the date of death or censored at the last date of confirmed living status. Cause of death was obtained from the death certificate and hospital records. Deaths were classified as a postoperative complication for those who

died within the initial hospital admission or within 30-days of surgery, cancer related for patients who died of disease progression or recurrence, non-cancer related from patients who died of other causes, or cause of death unknown where records were not available.

4.3.4. Statistical analysis

Results are expressed as mean (95%CI) or median (IQR) for continuous variables depending on normality of data and as a percentage for categorical variables. Following assessment of normality of data, univariate analysis was performed for categorical variables using Pearson's chi-square test or Fisher's exact test where appropriate, and for continuous variables using Kruskal-Wallis test for non-parametric testing or ANOVA for parametric testing. Bonferroni post-hoc multiple comparison test was used to assess differences between smoking cessation groups. Survival analysis using Cox proportionate hazard modelling was used to analyse the predictors of long-term survival, which is the standard approach to perform survival analysis using multivariate modelling. There was no selection bias in the variables included in the model. Covariates were assessed for validity of proportional hazard assumption prior to inclusion in the model. Standardized score process and cumulative martingale residuals plots were generated. The following covariates were well specified and met the proportional hazard assumption: stage, age, PPC, total LOS, ITU LOS, VATS surgery, hypertension, diabetes, ischaemic heart disease, COPD, percentage predicted FEV_1, sex, type of analgesia, preoperative activity level and smoking status. The statistical

significant covariates were identified following a backward elimination process. The level of significance was set at 0.05. Predictive survival curves adjusted for the different smoking status categories were generated based on the final Cox proportional hazards model at different levels of the predictor variables. Cox proportionate hazard modelling was chosen over other methods such as propensity matching between smoking groups, as the event of late death was not rare and attempting to perform propensity matching can increase hidden bias due to dormant unobserved confounders (236). All analyses were performed using the SAS statistical package version 9.3 (SAS Institute, Inc, Carry NC) and IBM SPSS version 20.0.

4.4. RESULTS

4.4.1. Study population

There were 462 patients with NSCLC who underwent pulmonary resection during the study period of which 277 were male (60%). 15 patients were not included from the group of NSCLC patients (n=477) within the same cohort previously analysed (192) as data on duration of smoking cessation (< or ≥6 weeks) was not available. Within the 462 patients, the mean ± SD age of the group was 68.8 ± 8.5 years. Mean predicted FEV_1 was 79.2 ± 20.4 %, mean BMI was 26.8 ± 8.9 kg/m^2, 237 (51.3%) had an ASA score ≥3 and 136 (29.4%) had COPD. There were 111 (24%) current smokers, 55 (12%) ex-smokers <6 weeks, 245 (53%) ex-smokers ≥6 weeks, and 51 (11%) never smokers.

The most frequent procedures was lobectomy (n=361, 78.1%), followed by wedge or segmentectomy (n=61, 13.2%), pneumonectomy (n=32, 6.9%), sleeve resections (n=6, 1.3%) and chest wall resection/reconstruction (n=2, 0.4%). VATS approach was used in 39 patients (8.4%). Patients were NSCLC staging of I (n=229, 50%), II (n=147, 32%), IIIA (76, 16%) and IIIB (n=6, 1%).

Age, FEV_1% predicted and BMI were all significantly lower ($p<0.05$) in current smokers than never smokers. There were significantly more males and patients with COPD diagnosis in the current smoking group ($p < 0.05$, **table 4.1**); though there were no significant differences in the severity of COPD between groups. Cancer staging, pre-operative physical activity level, ASA and diagnosis of cardiovascular disease were not significantly associated with smoking.

		Current smokers (n=111)	**Ex-smokers <6 weeks (n=55)**	**Ex-smokers ≥6 weeks (n=245)**	**Never smokers (n=51)**	***p*-value**
Mean (95%CI) age (years)		67 (65 - 68.3)[b]	68 (65.6 - 9.9)[a,b]	70 (68.8 - 70.8)[a]	72 (67.2 - 72.8)[a,b]	0.006 [1]
Median (IQR) BMI (kg/m^2)		24.1 (6.3)[b]	24 (7.5)[a]	26.9 (5.4)[a,b]	26 (6.4)[a,b]	<0.001 [2]
Mean (95%CI) predicted FEV_1%,		72 (68.6 – 75.7)[c]	78 (73.5 – 81.8)[b,c]	79 (76.6 – 81.8)[a,b]	94 (87.4 – 100.5)[a]	<0.001 [1]
COPD		50.4% (n=56)[c]	20.0% (n=11)[b,c]	26.9% (n=66)[a,b]	5.9% (n=3)[a]	<0.001 [3]
COPD Severity (FEV_1% pred)	Mild (≥80)	18.9% (n=21)	3.6% (n=2)	6.5% (n=16)	2% (n=1)	0.358 [4]
	Moderate (≥50, <80)	23.4% (n=26)	16.4% (n=9)	15.5% (n=38)	3.9% (n=2)	
	Severe (≥30, >50)	8.1% (n=9)	0%	4.5% (n=11)	0%	
Male sex		63.9% (n=71)[b]	60.0% (n=33)[a,b]	63.2% (n=155)[b]	35.2% (n=18)[a]	0.002 [3]
Ischaemic heart disease		14.4% (n=16)	18.1% (n=10)	15.1% (n=37)	7.8% (n=4)	0.476 [3]
Diabetes		12.6% (n=14)	5.5% (n=3)	13.1% (n=32)	11.7% (n=6)	0.503 [4]
Hypertension		42.3% (n=47)	41.8% (n=23)	45.3% (n=111)	52.9% (n=27)	0.604 [3]
ASA ≥3		58.5% (n=65)	52.7% (n=29)	49.8% (n=122)	41.2% (n=21)	0.195 [3]

Preoperative activity <400 m		29.4% (n=32/109)	25% (n=13/52)	29.7% (n=71/239)	21.6% (n=11/51)	0.634 [3]
Staging	I	54.1% (n=60)	47.3% (n=26)	48.2% (n=118)	49.0% (n=25)	0.369 [4]
	II	29.7% (n=33)	32.7% (n=18)	34.7% (n=85)	21.6% (n=11)	
	IIIA	14.4% (n=16)	18.2% (n=10)	15.9% (n=39	21.6% (n=11)	
	IIIB	0.90% (n=1)	0%	0.41% (n=1)	3.9% (n=2)	
VATS	No	91.9 % (n=102)	85.5% (n=47)	92.7% (n=227)	92.2 % (n=47)	0.396 [4]
	Yes	8.1% (n=9)	14.5% (n=8)	7.3% (n=18)	7.8% (n=4)	
Surgery	Lobectomy	81.1% (n=90)	78.2% (n=43)	77.6% (n=190)	74.5% (n=38)	0.592 [4]
	Subsegmectomy /wedge	10.8% (n=12)	9.1% (n=5)	14.3% (n=35)	17.6% (n=9)	
	Pneumonectomy	5.4% (n=6)	10.9% (n=6)	6.5% (n=16)	7.8% (n=4)	
	Sleeve	1.8% (n=2)	0%	1.6% (n=4)	0%	
	Chest wall	0.9% (n=1)	1.8% (n=1)	0%	0%	
Analgesia	Epidural	40.5% (n=45)	29.0% (n=16)	37.1% (n=91)	43.1% (n=22)	0.217 [4]
	Intrathecal morphine	23.4% (n=26)	36.4% (n=20)	21.6% (n=53)	21.6% (n=11)	
	Morphine infusion	11.7% (n=13)	10.9% (n=6)	8.6% (n=21)	5.9% (n=3)	
	PCA	5.4% (n=6)	7.3% (n=4)	5.3% (n=13)	5.9% (n=3)	
	Paravertebral	18.9% (n=21)	12.7% (n=7)	26.5% (n=65)	23.5% (n=12)	
	Other	0%	3.6% (n=2)	0.8% (n=2)	0%	

Table 4.1 Results of univariate analysis to determine factors associated with smoking status.

BMI, body mass index; FEV, forces expiratory volume in 1s; COPD, chronic obstructive pulmonary disease; ASA, American Society of Anaethesiologists; VATS, Video-assisted thoracoscopic surgery; PCA, patient controlled analgesia. [1] ANOVA with Bonferroni post-hoc analysis; [2] Kruskal-Wallis test with Dunn Bonferroni post-hoc multiple comparison test; [3] Chi-square test with Bonferroni post-hoc analysis; [4] Fisher's Exact test with Bonferroni post-hoc analysis. When overall *p*-value is less than 0.05; each subscript letter ($^{a-c}$) denotes a subset of smoking category whose column proportion does not differ significantly from each other (Bonferroni post-hoc multiple comparison test).

4.4.2. PPC incidence

PPC was present in 60 patients (13%); the most common positive factors to trigger a score of 4 were raised WBC (n=54, 11.7%), purulent sputum (n=48, 10.4%), CXR findings (n=48, 10.4%), and reduced oxygen saturations (n=48, 10.4%). Compared to never smokers, current smokers had a higher frequency of PPC (22% vs. 2%, $p = 0.004$) (**table 4.2**). Compared to current smokers, in the ex-smoking groups there was a trend for approximately half the frequency of PPCs (<6 weeks, 10.9% vs. ≥6 weeks, 11.8%) but there was no significant differences between ex-smoking groups, or when compared to current and never smokers.

		Current smokers (n=111)	**Ex-smokers <6 weeks (n=55)**	**Ex-smokers ≥6 weeks (n=245)**	**Never smokers (n=51)**	***p*-value**
PPC Frequency		21.6% (n=24)[b]	10.9% (n=6)[a,b]	11.8% (n=29)[a,b]	2.0% (n=1)[a]	0.004 [1]
ITU admission		14.4% (n=16)[b]	5.5% (n=3)[a,b]	4.5% (n=11)[a,b]	0%[a]	0.001 [2]
Median (IQR) LOS (days)		6 (5)[b]	5 (3)[a,b]	6 (4)[a,b]	5 (2)[a]	0.001 [3]
Median (IQR) ITU LOS (days)		4 (10) (n=16)[c]	2 (*) (n=3)[a,b,c]	3 (2) (n=11)[a,b]	0 (0) (n=0)[a]	0.002 [3]
30-day hospital readmissions		13.6% (n=15)	14.8% (n=8)	15.1% (n=36)	7.8% (n=4)	0.793 [1]
Mortality	Total	30.6% (n=34)	32.7% (n=18)	30.2% (n=74)	23.5% (n=12)	0.744 [1]
	30-day	1.8% (n=2)	0%	2.4% (n=6)	2.0% (n=1)	0.874 [2]
	90-day	5.4% (n=6)	1.8% (n=1)	3.2% (n=8)	5.9% (n=3)	0.575 [2]
Cause of death	Early Postoperative Complications	2.7% (n=3)	3.6% (n=2)	2.9% (n=7)	2.0% (n=1)	0.970 [2]
	Cancer related	18.9% (n=21)	20.0% (n=11)	20.4% (n=50)	17.6% (n=9)	0.968 [1]
	Non-cancer related	6.3% (n=7)	5.5% (n=3)	6.9% (n=17)	1.9% (n=1)	0.768 [2]

	Uncertain	2.7% (n=3)	3.6% (n=2)	0%	1.9% (n=1)	0.015 [2]
Mean (95%CI) overall survival (months) excluding early postoperative complications		42 (42-45)	42 (38-47)	43 (40-46)	46 (41-50)	0.778 [4]

Table 4.2 Morbidity and mortality in patients stratified by smoking status.

PPC, postoperative pulmonary complication; ITU, intensive care unit; LOS, length of stay. [1] Chi-square test with Bonferroni post-hoc analysis; [2] Fisher's Exact test with Bonferroni post-hoc analysis. When overall *p*-value is less than 0.05; each subscript letter (a-c) denotes a subset of smoking category whose column proportion does not differ significantly from each other (Bonferroni post-hoc multiple comparison test); [3] kruskal-Wallis test with Dunn-Bonferroni post-hoc multiple comparison test; [4] ANOVA with Bonferroni post-hoc analysis.;* interquartile range not applicable due to number of patients.

4.4.3. Short-term outcome

Compared to never smokers, current smokers had a higher frequency of ITU admission (14% vs. 0%; $p = 0.001$) as well as longer median (IQR) hospital LOS (days) (6 (5) vs. 5 (2); $p = 0.001$). Compared to current smokers, in the ex-smoking groups there was a trend for a lower frequency of ITU admission (<6 weeks, 5.5% vs. ≥6 weeks, 4.5%). but there was no significant differences between ex-smoking groups, or when compared to current and never smokers. Patients who stopped smoking ≥6 weeks prior to

surgery were found to have a significantly shorter ITU median (IQR) LOS (days) compared to current smokers (3 (2) vs. 4 (10); p = 0.002) There was no significant difference in 30-day hospital readmission rates between any of the groups. There were no significant differences in the 30-day mortality, 90-day mortality or causes of death (cancer or non-cancer related) between any of the groups.

4.4.4. Long-term survival

When assessing for survival, the mean follow up time for all patients was 29.8 months, (95%CI 28.4 – 31.1). After excluding early postoperative deaths, Cox regression analysis showed significant independent risk factors for late deaths following thoracic surgery to be development of PPC, age, cancer staging and hospital LOS (**table 4.3**).

Variables	**Parameter estimate**	**SE**	***p*-value**	**HR**	**95%CI**
Stage (II vs. I)	1.11	0.22	<0.0001	3.03	1.97 - 4.71
Stage (IIIA vs. I)	1.35	0.25	<0.0001	3.87	2.39 - 6.28
Stage (IIIB vs. I)	1.88	0.61	0.0019	6.54	1.57 - 18.33
Age	0.03	0.01	0.0115	1.03	1.01 - 1.05
PPC	0.53	0.27	0.0469	1.71	0.98 - 2.82
LOS	0.03	0.01	0.0324	1.03	1.01 - 1.05

Table 4.3 Independent risk factors for late deaths.

PPC, postoperative pulmonary complication; LOS, length of stay.

Smoking variable was not a significant predictor for late deaths ($p > 0.05$) but after adjusting for the significant covariates, the adjusted survival curves for the never smokers were clearly above the survival curves for the rest of smoking categories (figure **4.1**).

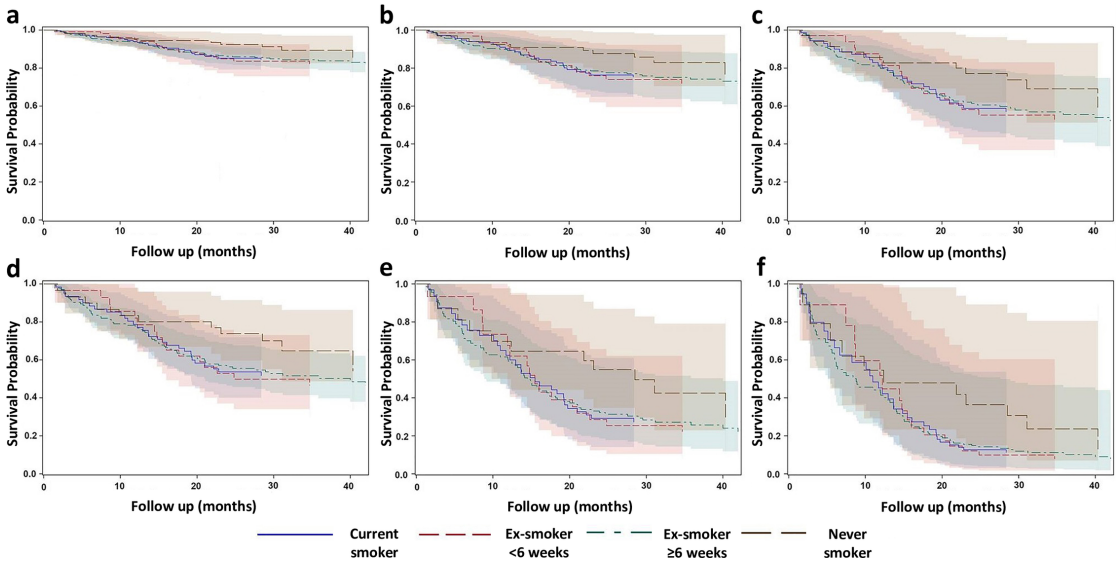


Figure 4.1 Predictive Survival curves for patients with different preoperative smoking status. (232)

Predictive Survival curves with 95%CI for patients with different preoperative smoking status, excluding early postoperative deaths, adjusted for the significant covariates from the Cox proportional hazards model (Table 3). Covariates set at: A – PPC 0, Stage I; B - PPC 1, Stage I; C – PPC 1, Stage II; D – PPC 0, Stage IIIA; E – PPC 0, Stage IIIB; F – PPC 1, Stage IIIB. Age and LOS were set at mean and median values respectively.

4.5. DISCUSSION

This single centre study demonstrated that patients who continue to smoke until the date of curative-intent lung cancer surgery had a higher postoperative morbidity, including higher frequency of PPC, longer hospital LOS, and a higher frequency of ITU admission. In ex-smokers there was trend for reduced frequency of PPC and ITU admission compared to current smokers, but there was no significant difference in observed outcome between patients who quit smoking less <6 weeks versus ≥6 weeks prior to surgery. We found no significant differences in early mortality or long-term survival between any of the groups within our follow up period.

Our study demonstrated that the incidence of PPC in current smokers is 22%, which is greater than 10 times that of never smokers and twice that of ex-smokers. Comparing this observed incidence with results from other studies is challenging because of the differences in the definition of PPC, which may extend to include potentially more severe yet less frequent complications such as pulmonary embolism, prolonged air leaks and bronchopleural fistulas (120). Despite this, the frequency of PPC in smokers using the MGS is within the wide range of pulmonary events reported in other studies (6.9 - 43.2%) (234, 237). Our finding of PPC to be higher in current versus never smokers is also supported by other studies (234, 238).

Despite the observed trend, our study showed no statistically significant difference in PPC frequency between ex-smokers and current/never smokers. Other studies have

found no significant difference in ex-smokers and current/never smokers which is most likely due to much smaller study sample sizes (9), and/or the exclusion of a never smoking control group (239, 240). In a general thoracic surgery cohort, we recently demonstrated that compared to never smokers, ex-smokers were more than twice as likely to develop a PPC (192). However, the timing of smoking cessation prior to surgery, and the subsequent effect of postoperative outcome was not investigated. This study did show that PPC rates in both groups of ex-smoker (10 and 11%) were halved compared to current smokers (22%); this was not statistically significant most likely due to smaller group sizes of the recent ex-smokers (n=55) and current smokers (n=111), as power calculations show that group sizes of n=145 with the above incidence are required to detect a significant difference ($p<0.05$) with 80% power between the two groups. Despite this, the halving of PPC rates in ex-smokers is still a clinically important finding due to the known poor short and long-term outcomes associated with PPC development (192).

In our prospective cohort, using 6 weeks as a cut off, we were unable to find an optimum duration of preoperative smoking cessation to reduce PPC incidence. Despite this, our study has demonstrated that stopping smoking 6 weeks prior to surgery seems to reduce, and not increase PPCs compared to current smokers. A study by Nakagawa et al (n=288) (121) also investigated the effects of smoking cessation on development of PPC incidence. This was also a single centre study, which was conducted in Osaka, Japan between 1997-1998. As their study is much older, all of

their cases were performed with thoracotomy approach and therefore no VATS procedures. The Japanese cohort had a similar percentage of pneumonectomy case to our cohort (4.1% vs 6.9%), but more wedge resection (43% vs 13%) and less lobectomy cases (50% vs 78%). There also was a much higher proportion of never smokers in the Japanese cohort compared to our cohort (40% vs 11%), and no record of COPD diagnosis. Their study found that the incidence of PPCs among ex-smokers (2-4 weeks prior to surgery) was 53.8%, whilst in current smokers was 43.6%, which were both significantly higher when compared to the never-smokers (23.9%; $p < 0.05$). In the same timeframe as the Russell Standard (89), the study found that at least 4 weeks of smoking abstinence was needed for a marked reduction in PPC (53.8% to 34.7%), whilst the incidence of PPC in patients who had a smoke-free period of 9–12 weeks or longer approached the same incidence as those in the never-smoked group. However, there was no significant differences in PPC incidence between the smoking and ex-smoker groups. In addition, the rate of PPC incidence was much higher than in our study (32.3% vs 13%), as they classified tube drainage > 7 days as a pulmonary complication, which comprised of 72/93 (77%) of all PPCs detected in their study, whereases only 15/93 (16%) of detected complications included pneumonia or significant atelectasis. The study is also limited by the retrospective nature of data collection. A large prospective review of the General Thoracic Surgery Database from the United States (n=7990) found that the risk of PPCs following primary resection for lung cancer steadily declined with longer intervals of smoking cessation (4 days - 1 month, 1-12 months, >12 months) (234). However, the differences were not

significant enough between the groups to recommend optimal interval of smoking cessation. A critique of this study is that the incidence of PPC was low (6.9% in smokers) as only major pulmonary events were reported, suggesting minor yet more frequent PPCs may have been missed. In additional, these studies looking at the effect of smoking and cessation on outcomes (121, 234) have use self-reported status and not biological confirmation to classify current/ex-smokers.

Smoking had no direct effect on postoperative mortality or long-term survival during in our study. The all-cause mortality was 30.6% in smokers compared to 23.5% of never smokers, which although was not statistically significant, is an approximate risk reduction by 25%, which could have clinical significance. Of interest there were no differences in 30 and 90-day mortality rates between the smoking and never smoking groups. The increase in mortality in smokers is most likely due to the increased non-cancer related deaths, found to be 6.3% in smokers compared to 1.9% in never smokers. This may in part be associated with the increased risk of PPC in the smoking groups, with PPC development having been shown to be associated with worse long-term outcome after thoracic surgery (192). The adjusted survival curves for never smokers were clearly above those of the other smoking categories especially after the first 12 months of follow up suggesting a likely more favourable outcome even though this did not reach statistical significance. The reason for this not reaching statistical significant could be explained by the short follow up period or the smoking habits of patients after surgery; some may stop whilst others may restart. Furthermore, other

studies have shown increased mortality in smokers compared to never smokers, but no significant difference amongst the current and ex-smoking groups (89, 234). The deleterious effect of continued smoking on long-term survival after being diagnosed with lung cancer is increasingly evident (241) but few papers have looked into the effect of pre-operative smoking cessation on long-term survival in lung cancer patients undergoing curative-intent surgery. A multicenter review of 169 patients undergoing NSCLC resection found that current smokers had a reduced 5-year survival in comparison to never smokers (72% vs. 91%, p = 0.02), and that stopping smoking a year prior to cancer diagnosis had no significant difference in long-term survival (237). Interestingly, though smoking was not an independent risk factor for late-deaths, PPC development and LOS both were, which were both significantly increased in the current smokers. Another longitudinal multicentre study investigated the impact of smoking cessation in patients (n=1124) with lung cancer diagnosis (surgical and non-surgical management) (119); they also found similar findings in that smoking was not an independent predictor of survival, as surgery itself was the biggest predictor. The study did however find that stopping smoking after a lung cancer diagnosis and continued abstinence was associated with 17% improvement in survival at 1-year; which was not statistically significant ($p>0.05$) however could be due to underpowering.

We found that lower predicted FEV_1%, BMI and COPD diagnosis were associated with current smoking on univariate analysis. It is important however to beware of co-

linearity between these variables; this study did not include a multivariate analysis of factors associated with smoking status or look at the interaction of these variables within such a model. Other investigators have found that the prevalence of low and normal BMI was greater among patients who are active smokers; those underweight patients had a greater risk-adjusted LOS compared to normal weight patients (226). We have previously investigated the risk factors for developing a PPC, and although lower pre-operative FEV_1% predicted and COPD physician diagnosis are associated with PPC on univariate analysis, only COPD diagnosis is regarded as an independent risk factor for PPC development or multivariate analysis (29, 192). Even in these models, current smoking remains the strongest independent risk factor for PPC development. Authors have found carbon monoxide diffusion capacity (DLCO) to be associated with PPC development (238); however, DLCO was not included in this study, as it was only performed in patients with limited exercise tolerance so the data was no collected.

The observed effects of smoking and increasing frequency of PPCs could be explained by the suppressive effect of cigarette smoking on the innate immune system. Smoking impairs the mucociliary escalator and the ability to remove foreign bodies/pathogens in the larger airways. This normalises after 15 days following smoking cessation implying that it is not the only etiological factor in PPC (109). Smoking seriously impairs antimicrobial (242, 243) and proinflammatory (244) functions of alveolar macrophages. Furthermore, there is a compounding effect on anaesthesia and

smoking on suppression of intraoperative antimicrobial/phagocytic activity in alveolar macrophages. Smoking cessation of 6 months was needed to normalise pro-inflammatory function whilst only 3 months was required to normalise phagocytic function (169). One possible explanation for the reduction in phagocytic capacity in AMS from smokers may be due to reduction in metabolic activity, which has shown to normalise after a 3-5 month smoke free period (245); another may be due to the changes in cell surfaces markers involved in regulation of phagocytosis, which are altered in current smokers (153, 155) and may resolve after a smoke free period at a level where phagocytosis is no longer impaired. The reason for finding of prolonged alteration in pro-inflammatory function at the transcriptional level (169), may be in part due to persistent genetic changes induced by tobacco smoke in exposed AM (246), and also may relate to the retained tobacco related autofluorescent bodies which can persist for over 6 months (147).

The question of whether a smoking cessation programme in surgery affects postoperative outcomes has been more recently reviewed (247). Implementation 6-8 weeks prior to elective orthopaedic surgery reduced postoperative complications (18% vs. 52%, p = 0.0003) (248), whilst implementation 2 weeks prior to colorectal surgery made no difference (249). Despite increasing numbers of small RCTs suggesting intensive smoking cessation treatments work in people with pulmonary diseases such as lung cancer, many patients continue to smoke (250). In thoracic surgery, pulmonary rehabilitation programmes have shown promise in increasing

smoking cessation, with a trend for fewer PPCs in the intervention group (224); however, RCTs are needed to confirm efficacy.

4.5.1. Limitations

In this study of prospectively gathered data, the main limitation is the reliance on self-reporting smoking status in preoperative patients to ascertain smoking cessation, which could result in recall bias and could underestimate the real smoking habits of patients. Use of biochemical confirmation by use of exhaled carbon monoxide testing and measuring cotinine levels from urine, saliva or blood could be used in future studies (251). Smoking cessation data was collected as a categorical variable, and therefore additional statistical analysis using ROC curve analysis could not be performed to further inform on a cut-off for the duration of smoking cessation needed to reduce PPC incidence. There are also limitations of using multiple regression analysis as discussed in Chapter 3. This study has included more variables in the analysis than the ratio of one predictive variable for every 10 events (230), however 5-9 events per predictive variable within multivariate analysis may be enough (231), suggesting this rule can be relaxed. Some of the variables including the surgery and analgesia variables have over 5 different categories, only the latter was included in the analysis, however with such small numbers in groups limits the ability to detect any signal. In our study, there were a low number of VATS cases in our cohort of prospectively gathered data (8.4%), and only 5.6% of patients with stage 1 NSCLC underwent VATS resection. The data of this study preceded the growth of VATS

lobectomies in NSCLC patients; the vast majority of lung resection over this time frame was performed via open thoracotomy. This low level of VATS resections could confound the results and make them less generalisable considering that many studies have demonstrated a higher postoperative pulmonary complication (PPC) rate with open thoracotomy vs. VATS resections (252, 253). Further observation studies could demonstrate the benefit of stopping smoking on PPC frequency in the setting of VATS and thoracotomy approach. Finally, this is a single centre study and therefore study findings may not be generalisable to other centres.

4.5.2. Conclusion

Our real-life study of patients undergoing thoracic surgery demonstrates that 1 in 4 patients continue to smoke prior to curative-intent surgery for NSCLC. Smoking cessation prior to surgery reduces PPC incidence as well as other important markers of postoperative morbidity, thus we recommend that all patients should undergo formal smoking cessation as part of the routine work up. The optimum timing for preoperative smoking cessation however is yet to be defined. Further research into effective short-term smoking abstinence programmes and their short term and long-term effects is urgently needed.

4.6. Introduction to Chapter 5

Following on from the cohort study work of Chapter 3 and 4, we sought to investigate further into the biological mechanisms by which smokers are at a higher risk of PPC development. The alveolar macrophage (AM) plays a key role in the innate immune system, and AM phenotype and function is known to be dysregulated following cigarette smoking. Understanding the role of the AM in the development of PPCs in those who smoke, and the effect of cessation may provide insight into alternative strategies to prevent PPC in those who have not had adequate time for risk modification prior to surgery.

CHAPTER 5: THE ROLE OF ALVEOLAR MACROPHAGES AND SMOKING IN THE DEVELOPMENT OF POSTOPERATIVE PULMONARY COMPLICATIONS AFTER LUNG CANCER SURGERY

My role in this chapter involved performing all the laboratory methods in obtaining translational data, as well as clinical data collection, data analysis, literature review, critical appraisal, and scientific writing including generation of the figures.

5.1. ABSTRACT

Introduction: Postoperative pulmonary complications (PPC) are the most common complication in patients following lung cancer surgery. Smoking is the biggest risk factor for PPC and has been shown to affect alveolar macrophages (AM), which have a key role in defending against infection and resolving inflammation. The role of the phenotype and function of AM in the development of PPC has yet to be determined.

Methods: Non-cancerous lung tissue was obtained from 50 patients undergoing lung surgery (REC number 17/WM/0272). Smoking history was determined using the patient's self-reported smoking status and confirmed by measuring exhaled carbon monoxide levels. AMs were isolated from the lung tissue and phenotype determined using flow cytometry. AM capacity to efferocytose was assessed using labelled apoptotic neutrophils from healthy donors. PPC was determined prospectively using the Melbourne Group Scale.

Results: AMs from smokers had increased surface expression of SIRPα and CD33, and reduced expression of CD206 and CD163 compared to long-term ex-smokers and never smokers ($p<0.05$). The efferocytosis index was lower in current/recent ex-smokers compared to long-term/never smokers ($p<0.05$). Patients who developed a PPC had significantly lower efferocytosis whilst SIRPα levels were significantly higher compared to patients who did not develop a PPC ($p<0.05$). Higher SIRPα levels and

lower efferocytosis index were associated with prolonged hospital length of stay (LOS).

Conclusions: AM from smokers have altered phenotype including increased SIRPα expression, which was lower in those who had stopped smoking for at least 1 month. SIRPα signalling inhibits efferocytosis. AM efferocytosis negatively correlates with PPC development and LOS. We conclude that to reduce smoking related PPC, smoking cessation strategies should be started early in the cancer diagnosis pathway. Further investigation is required to evaluate SIRPα as a potential target to rescue AM function and thus prevent PPC.

5.2. INTRODUCTION

Lung cancer is the third most common cause of cancer death in the UK (2). Surgery is the most effective strategy in treating patients with over 6800 patients in the UK undergoing lung resection annually (7). Postoperative pulmonary complications (PPC), including pneumonia and clinically significant atelectasis are the most frequent complications following lung resection. The development of a PPC has a dramatic effect on patient outcome, increasing hospital mortality (0.5% to 12%), intensive therapy unit (ITU) admission rate (1.5% to 26%) and hospital length of stay (5 to 14 days) (29). Development of a PPC is also associated with increased readmission rate (21% vs. 12%; p=0.023) and a poor long-term outcome (192).

Of the risk factors identified, smoking at the time of lung surgery is the strongest risk factor for developing a PPC (192). PPC risk following lung surgery reduces following smoking cessation, however 1 in 4 patients continue to smoke up until the date of lung cancer surgery and the optimum timing of cessation to reduce complications has not been defined (120, 232). It may be that a minimum of 4 weeks are needed to reduce the risk of PPC with increased benefit from longer periods of cessation according to a recent report by the WHO (254), who advocate postponing non-emergency surgery to achieve this. Due to cancer doubling time, lung surgery for suspected/proven cancer should be performed as early as possible to prevent upstaging of disease.

A major factor that likely contributes to the impact of cigarette smoke (CS) exposure on lung surgery outcomes is the effect on alveolar macrophages (AMs). AMs are sentinel cells of the innate immune system, representing over 90% of the alveolar immune cells, and have a role in initiating and resolving immune response, as well as surfactant/lipid processing. CS causes inflammation which increases cellular influx of AM and neutrophils into the alveolar space (255). Smokers have altered AM phenotype which is implicated in dysregulated inflammation (153). In patients undergoing surgery, anesthesia detrimentally effects AM phagocytic and antimicrobial function (167) (168), which is more pronounced in smokers (169).

Efferocytosis is the clearance of apoptotic bodies, mainly of apoptotic neutrophils in the setting of acute pulmonary inflammation. It requires the AM to adopt an anti-inflammatory state to prevent inflammatory responses (256). CS is known to reduce efferocytosis in AM however the mechanism of this suppression is unclear (176, 257). Cell surface markers of interest are highlighted in **Figure 5.1**. AM cell surface markers CD163 and CD206 are thought to help mediate efferocytosis (170), and have shown to be altered following cigarette smoke exposure (155).

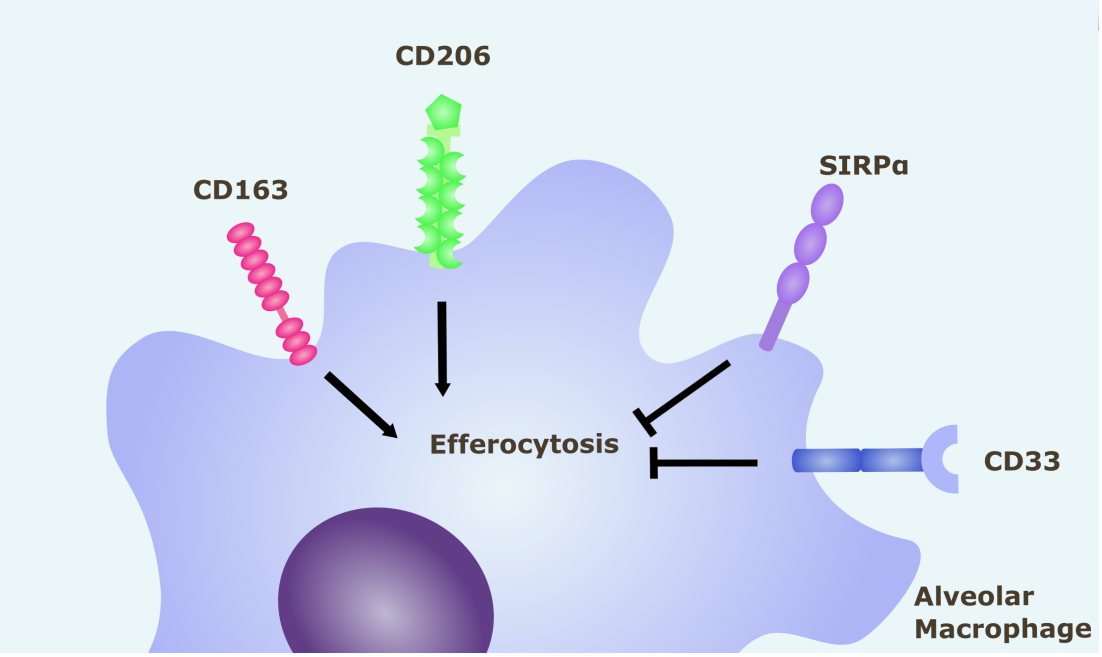


Figure 5.1 Cell surface markers of interest involved in AM efferocytosis

Signal regulatory protein alpha (SIRPα) is an inhibitory receptor which binds to CD47 on healthy cells as well as surfactant protein A and D (SP-A and SP-D). Activation of SIRPα suppresses efferocytosis, which has a key role in regulating immune homeostasis; blocking antibodies can interrupt SIRPα activation thus restoring efferocytosis function (258). CD33 is expressed in myeloid cells and AMs, and also has inhibitory role in macrophage efferocytosis, however the relationship with smoking status is unclear.

This work detailed in this chapter will investigate the role of the AMs in smoking and the development of PPCs in patients undergoing lung surgery, focusing on efferocytic capacity of AM and the expression of SIRPα in the same cells.

5.3. METHODS

5.3.1. Patient recruitment

The study was conducted at a single regional centre for thoracic surgery within the University Hospitals Birmingham NHS Foundation Trust between August 2018 and August 2021. Inclusion criteria were adult patients (≥ 18 years of age) undergoing surgery for lung resection of either suspected or proven lung cancer. Exclusion criteria included patients who could not understand English and provide written consent; those with COPD and severe or very severe airway obstruction (FEV_1 < 50% predicted (259)) which has been associated with altered AM phenotype (260, 261); those who had received pre-operative chemotherapy; those who were on or who had received immunosuppressant medication other than steroids in the 12 months preceding surgery.

Patients were recruited at the pre-operative thoracic surgical clinic typically 1 week before surgery for suspected lung cancer (REC number 17/WM/0272) and asked to self-report smoking status and perform an exhaled carbon monoxide (eCO) measurement (CareFusion). A smoker was defined as self-reported to continue to smoke some/most days or have an eCO ≥10 parts per million (ppm). A never smoker was defined as having smoked <100 cigarettes in a lifetime. An ex-smoker was defined as having quit and an eCO of <10ppm. Ex-smokers were further categorised into recent ex-smokers of cessation duration of <1 month before surgery, recent ex-smokers of cessation duration of 1-24 months before surgery; long-term ex-smokers with

cessation duration of >24 months before surgery. 1 month was chosen based on the Russel Standard of defining smoking cessation using eCO confirmation which is used in clinical practice (75, 89). In addition, a 6-week cut-off alone was unable to show a difference in PPC frequency in the ex-smoking groups in a cohort of patients undergoing thoracic surgery who had self-reported smoking status (232). Due to heterogeneity of the ex-smoking group, a further category of 1 month to 24 months and >24 months was chosen as smoking related changes in AM phenotype may persist by evidence of persistence of inclusion bodies withing this timeframe (150, 151).

On the day of surgery patients were admitted to hospital. Operations were performed with single lung ventilation under general anaesthesia. General anaesthesia included volatile gas sevoflurane or isoflurane. Patients also received intravenous paracetamol and morphine with the addition of an epidural or paravertebral catheter to assist with pain control. Patients were given intravenous dexamethasone for peri-operative anti-emesis. All patients were given one dose of prophylactic antibiotics at the time of surgery, with no further antibiotics prescribed unless complications developed.

Patients were subsequently scheduled for extubation in the operating room. Postoperatively, patients were managed in a dedicated thoracic high dependency unit HDU (level 2) and ward unless complications required their admission to the ITU. Postoperative pain control was achieved by continuous thoracic epidural analgesia, paravertebral infusion, intrathecal morphine and/or intercostal blocks or systemic

opioids (parenteral administration or intravenous patient-controlled administration). The choice of analgesic technique was made by the anaesthetist after discussion with the patient.

From the first postoperative day, all patients had a daily physiotherapy programme comprising deep breathing exercises, incentive spirometry, supported coughing and mobilisation. PPC were defined using the Melbourne Group Scale (MGS); a standardised and validated scoring system to define the presence of a PPC by meeting ≥ 4 of 8 factors including: chest x-ray (CXR) findings of atelectasis or consolidation; raised white cell count (WCC) (>11.2x10^9/l); temperature >38°C; signs of infection on sputum microbiology; purulent sputum differing from preoperative status; oxygen saturations <90% on room air; physician diagnosis of pneumonia; and prolonged high dependency unit (HDU) stay or readmission to HDU or intensive treatment unit (ITU) for respiratory complications (51).

5.3.2. Lung resection samples

Lung resections samples collected as part of the ethics (REC) were immediately examined in theatre by an experienced member of the surgical team or histopathologist. A section of the sample distant from the tumour, without any evidence of macroscopic malignancy and not required for diagnostic or clinical purposes was immediately placed in a sterile sealed container with 0.9% saline and

stored in a 4°C fridge. All samples were transported to the laboratory for processing and analysis within 24 hours.

5.3.3. Alveolar macrophage isolation

Upon arrival in the laboratory lung resection samples were immediately washed through with 500-1000mLs of sterile 0.9% sodium chloride using a 14-gauge needle. The needle was moved through the lung sample and small airways cannulated whilst continuous saline was allowed to run through freely using an intravenous giving set. The washed through fluid or lavage was collected and decanted into 50ml Falcon™ tubes and then centrifuged in a pre-chilled centrifuge (Eppendorf 5810R Centrifuge) with maximum acceleration and maximum brake at 560G for 10 mins at 4°C. The supernatants were discarded, and the cell pellets pooled and re-suspended in PBS (Sigma Aldrich) to make up a volume of 18mls in preparation for density gradient separation.

The 18ml volume of pooled cell pellets and PBS solution was carefully overlaid onto 12mls of Lymphoprep™ (STEMCELL Technologies) at a ratio of 3:2 sample to Lymphoprep™ in a 50ml sterile Falcon™ tube (Becton Dickinson Ltd) and centrifuged (Eppendorf 5810R Centrifuge) at 800G with minimal acceleration and zero brake for 30 minutes at 4°C. Lymphoprep™ is a density gradient medium for the isolation of mononuclear cells from peripheral blood as granulocytes and red blood cells have a higher density than mononuclear cells and sediment through the Lymphoprep™ layer

during centrifugation. The interphase created which contains mononuclear cells was then gently aspirated using a disposable 2.5 ml Pasteur pipette (Alphalabs, Eastleigh, UK) and transferred into a clean 50ml Falcon™ tube (Becton Dickinson Ltd) and washed by the addition of 40mls of sterile PBS and centrifuged at 500G for 10mins at 4^{o}C. This process enables removal of most of the platelet population. The supernatant was then discarded, and cells re-suspended with culture medium (RPMI 1640 media; 10% FBS; penicillin 100U/mL; streptomycin 100ug/mL (Sigma-Aldrich)).

Cell count was performed using a trypan blue exclusion method. Cells were then re-suspended in culture media dependent on macrophage cell percentage and total cell count to an initial volume such that the concentration was 1x10^{6}/ml for use in phenotyping.

Cells were then re-suspended in culture media at a concentration of 0.5 x 10^{6} macrophages/mL and plated at 1.0 x 10^{6} per well in a 6 well flat bottom cell culture plate, 0.25 x 10^{6} per well in a 24 well flat bottom cell culture plate (Costar®,Corning®, Wiesbaden, Germany) and 0.5 x 10^{5} in a 96 well black plate and incubated at 37 ^{o}C in a 5% CO_2 atmosphere. After 24 hours of culture, the macrophages adhere whilst remaining mononuclear cells are within the cell media suspension. The media is then changed in preparation for cellular experiments, thus removing non-adherent mononuclear cells.

5.3.4. Isolation of peripheral blood neutrophils

Peripheral blood was collected from healthy volunteers into lithium heparin vacutainers (Becton Dickinson). The individual lithium heparin vacutainers were emptied into a single 50ml Falcon and 1ml of 2% Dextran added per 6ml of blood before gently mixing by inversion. Both 56% and 80% Percoll solutions were removed from the fridge and left to stand at room temperature for approximately 30-40 minutes allowing the buffy coat to form. Once the buffy-coat had been sedimenting for 30 minutes, the Percoll gradient was prepared using two 15ml Falcons (1 tube per 12mls of blood): firstly 5mls of 56% Percoll was pipetted into each 15ml tube. Using a sterile Pasteur pipette, 2.5ml of 80% Percoll was added underneath the 56% Percoll. The buffy coat was overlaid on top of 56% Percoll using a sterile Pasteur pipette and centrifuged at 220G for 20 minutes at room temperature with minimal acceleration and no brake. The peripheral blood mononuclear cells (PBMCs) (boundary between buffy coat and 56% Percoll) were removed using a sterile Pasteur pipette and cells discarded into the waste pot. The neutrophils were removed (on the boundary between 56% and 80% Percoll) using a sterile Pasteur pipette and transferred into a fresh 15ml Falcon containing PBS before being centrifuged at 1,500G for 5 minutes at room temperature, maximum acceleration, and maximum brake. The supernatant was discarded, and the pellet resuspended in culture media (RPMI 1640 media; 10% FBS; penicillin 100U/mL; streptomycin 100ug/mL (Sigma-Aldrich)) for cell count using the haemocytometer and cytospin to determine purity (≥95% neutrophils).

5.3.5. Labelling of neutrophils

Neutrophils were labelled at a concentration of 4 x 10^6 cells per ml in a 15ml Falcon. A 15µg of Cell tracker deep Red (ThermoFisher) was resuspended in 20µl of dimethyl sulfoxide (DMSO), and the contents gently inverted to encourage mixing. Cell tracker was then mixed with neutrophils for labelling; incubated for 45 minutes at 37 ^oC in a 5% CO_2 atmosphere. Following incubation the cell tracker labelled neutrophils were centrifuged at 1,500G for 5 minutes at room temperature, maximum acceleration and maximum brake. The supernatant was removed, and cell tracker labelled neutrophils were re-suspended in serum-free RPMI media at a concentration of 2 x 10^6 neutrophils per ml in a 15ml Falcon and were incubated for 24 hours at 37 ^oC in a 5% CO_2 atmosphere to encourage apoptosis. Previous studies by our group have shown that 24 hours of incubation results in >90% neutrophil apoptosis for efferocytosis assays (262).

5.3.6. Phenotyping

After isolation of the alveolar macrophages (AMs) from the lung resection sample, 100,000 cells suspended in 100µl RPMI media were added to FACS tubes; 2mls of FACS buffer (PBS with 1% Ethylenediaminetetraacetic acid (EDTA, Sigma Aldrich) and 1% BSA)) was added to each tube prior to being centrifuged at 500G for 5 minutes (Eppendorf 5810R Centrifuge) with maximum acceleration and maximum brake at 5^oC. The supernatant was removed and 1µl of Fc block (BD Biosciences) added and incubated for 15 minutes at room temperature to prevent non-specific binding of

antibodies to the AMs. AMs were incubated for 30 minutes in the dark at room temperature (RT) with target cell surface antibodies and isotype controls (1:20 dilution). Unlabelled control cells were treated equally (30min incubation at RT). All antibodies and isotype controls used in experiments are shown in **Table 5.1**. The cells were then washed twice with 2mls of FACS buffer and centrifuged at 500G for 5 mins with maximum acceleration and maximum brake at 4^{o}C. After the second wash step, the supernatants were removed and cell pellets resuspended in 300µl of FACS buffer in preparation for flow cytometry. A representative gating strategy is presented in Figure 5.1. Using the Accuri C6 Plus flow cytometer the unlabelled samples were used to set the gating for FSC against FSC-height to eliminate doublets. AMs were gated based on high forward scatter (FSC) and side scatter (SSC) and auto-fluorescence particularly in the yellow-green spectrum corresponding to the FITC fluorophore. 2µl of propidium iodide (PI) were added to each subsequent sample as a live/dead stain; live cells were PerCP-A florescent low. 5,000 events of live AMs were assessed for APC fluorescence using median fluorescence intensity (MFI) (**Figure 5.1**). The overall MFI of each antibody was calculated by subtracting the MFI of the isotype control from the MFI of the specific antibody-stain.

Antibody	Clone	Fluorophore	Manufacturer	Isotype Control	LOT
CD14	61D3	APC	Invitrogen	Mouse IgG2ak	4344410
CD80	2D10	APC	BioLegend	Mouse IgG1k	B257710
HLA-DR	LN3	APC	Invitrogen	Mouse IgG2bk	B297875
CD33	WM-53	APC	Invitrogen	Mouse IgG1k	1995341
CD163	eBioGH/6I	APC	Invitrogen	Mouse IgG1k	4322930
SIRPα	15-414	APC	eBioscience	Mouse IgG2ak	4322930
CD206	19.2	APC	BD Biosciences	Mouse IgG1k	9183662
IgG1k	P3.6.2.8.1	APC	eBioscience	-	4323294
IgG2ak	eBM2a	APC	Invitrogen	-	1931044
IgG2bk	eBM2b	APC	eBioscience	-	4319923

Table 5.1 Antibodies used in experiments.

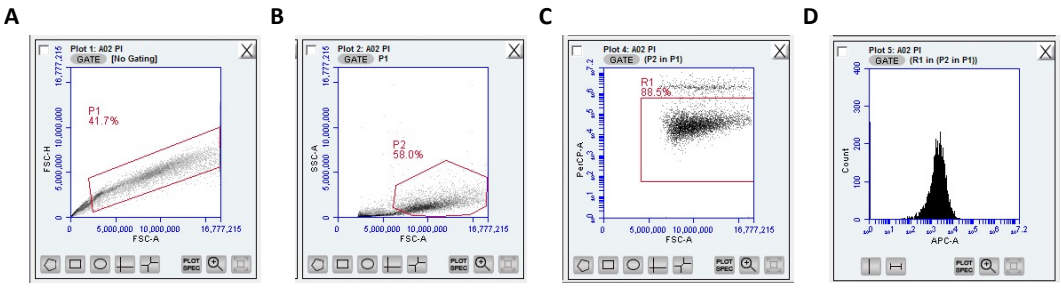


Figure 5.2 Representative flow plots of phenotyping.

Plot A: Includes mononuclear cells from lung tissue lavage, doublets were excluded using FSC-H against FSC-A (P1). **Plot B:** Gating of macrophages based on high FSC-A and SSC-A (P2). **Plot C:** Gating of live cells based on low florescence in PerCP-A after exposure to PI (R1). **Plot D:** The histogram of APC-A of R1 in (P2 in P1) to calculate the median fluorescence of isotype control or specific antibody-stained macrophages.

5.3.7. Efferocytosis

An efferocytosis assay was performed to assess the ability of the AMs to phagocytose apoptotic neutrophils. This assay has been previously validated by our group (35, 262). AM were plated at 0.25 x 10^6, 0.5ml media in a 24 well plate for these experiments. Cytochalasin D was added to the control well(s) at a concentration of 5µg/ml and incubated for 30 minutes at 37 ^oC in a 5% CO_2 atmosphere to inhibit cytoskeletal rearrangement, meaning these cells will be incapable of efferocytosis. Any fluorescent signal generated from these cells will therefore denote surface bound labelled apoptotic neutrophils or background. After 30 minutes the supernatant was then removed from each well and stored for future analysis. Apart from the macrophage only control, 0.5ml of the cell tracker labelled neutrophils (1 x 10^6 in total) were added to each well, thus flooding the macrophages with 4-fold excess of apoptotic neutrophils. The plate was then incubated for 2 hours at 37 ^oC in a 5% CO2 atmosphere. During this time a desired volume of PBS was kept on ice to be used in the wash step. Following incubation, the serum-free media containing neutrophils were removed from each well, followed by 2 washes of 1ml of ice-cold PBS. Following the wash, 0.5ml of trypsin was added to each well to encourage macrophage removal from the plate and were incubated for 8 minutes at 37 ^oC in a 5% CO_2 atmosphere. The trypsin was then neutralised by adding 0.5ml of fresh RPMI 1640 media (containing 10% FBS; penicillin 100U/mL; streptomycin 100ug/mL (Sigma-Aldrich). Cells were then agitated and pipetted into FACS tubes, with the addition of 2mls of FACS buffer and centrifuged at 500G for 5 minutes with maximum acceleration and maximum brake at

4°C. The supernatant was removed and 300µl of FACS buffer was added to each tube in preparation for flow cytometry.

Using the Accuri C6 Plus flow cytometer, gating FSC against FSC-height to eliminate any doublets. The AM population was gated against FSC and SCC. AMs pre-treated with cytochalasin D and exposed to neutrophils were used as the negative control. The positive population percentage in experimental groups was compared with cytochalasin D control treated cells to calculate the efferocytosis index. Representative plots are shown in **Figure 5.2**. Experiments were run in duplicate.

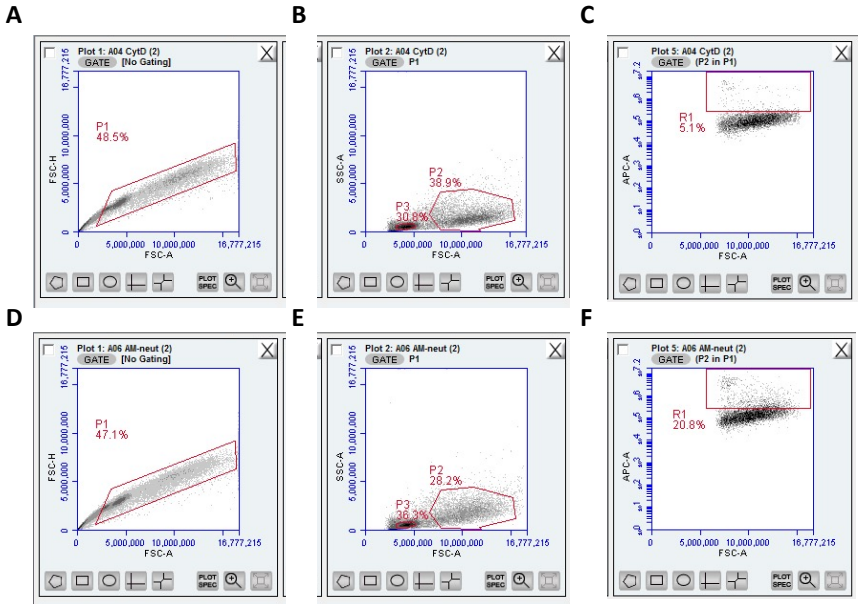


Figure 5.3 Representative flow plots of efferocytosis.

Plot A: Cytochalasin D treated macrophages exposed to fluorescent neutrophils (negative control); doublets were excluded using FSC-H against FSC-A (P1). **Plot B:** Demonstrates gated macrophages (P2) and gated neutrophils (P3). **Plot C:** Represents the florescent cells in APC

above that of the macrophage population (R1). **Plot D, E and F:** Use of the same gating strategy with macrophages exposed to fluorescent neutrophils without cytochalasin D control (R1). The efferocytosis index was calculated using R1 without cytochalasin D control minus cytochalasin D control ((R1 in F) – (R1 in C) = X).

5.3.8. Statistical methods

Data were analysed using GraphPad PRISM v8 software (GraphPad). Continuous data were analysed for normality using the D'Agostino & Pearson omnibus normality test. Non-parametric tests included Mann-Whitney U testing between groups and ANOVA Kruskal-Wallis test used to test difference between all groups. Spearman's correlation was used for correlation of non-parametric data. Categorical data were assessed using the Chi squared test.

5.4. RESULTS

5.4.1. Patient demographics

A total of 50 patients included n=11 current smokers, n=6 recent ex-smokers (<1 month quit), n=8 recent ex-smokers (1-24 months quit), n=20 long-term smokers (>24 months quit) and n=5 never smokers **(Table 5.2)**. The median age was 67 (IQR; 62-75). There were 45 patients (90%) who had a lobectomy, with 2 pneumonectomy (4%) and 3 sleeve lobectomy cases (6%; 2 long-term ex-smokers and 1 never smoker) ($p<0.05$). The eCO (ppm) and the FEV1% predicted differed between the groups ($p<0.05$). There were more patients with COPD diagnosis in current and recent ex-smoking groups

compared to the long-term ex-smokers and never smokers ($p<0.05$). There was no significant difference in age, sex, approach between the groups.

	Current Smoker **n=11**	**Recent ex-smoker (<1 month)** **n=6**	**Recent Ex-smoker (1-24 months)** **n=8**	**Long-term Ex-smoker (>24 months)** **n=20**	**Never Smoker** **n=5**	**P Value**
Age (years) (IQR)	63 (53-66)	66.5 (63-74.8)	64.5 (59.5-76.8)	73.5 (64-76.8)	72 (64.5-79)	0.107
Sex (%)						
Male	6 (55)	4 (67)	4 (50)	10 (50)	1 (20)	0.634
Female	5 (45)	2 (33)	4 (50)	10 (50)	4 (80)	
FEV1 (%pred) (IQR)	103 (79.5-110.5)	70.5 (60.8-76)	70.5 (62.5-87)	92.5 (79.3-97)	92 (90-128)	**0.001**
FEV1/FVC % (IQR)	68.5 (59.8-74)	58 (51-73.8)	58 (54.3-69)	71.5 (65-77.8)	72 (61-76)	0.099
Pack Years (IQR)	42 (35-60)	53.9 (30.1-90)	43.2 (24.4-60.3)	23.5 (16-37.4)	0	**0.0002**
eCO (ppm) (IQR)	25 (9-29)	3.5 (0.8-9)	1 (1-5.5)	2 (0.3-2)	3 (2-3.5)	**0.0001**
COPD (%)						
Yes	5 (45)	5 (83)	4 (50)	4 (20)	0 (0)	**0.017**
No	6 (55)	1 (17)	4 (50)	16 (80)	5 (100)	
E-cigarette (%)						
Yes	5 (45)	2 (33)	1 (13)	2 (10)	0 (0)	0.095
No	6 (55)	4 (67)	7 (87)	18 (90)	5 (100)	
VATS (%)						

Yes	7 (64)	4 (67)	6 (75)	9 (45)	3 (60)	0.710
No	4 (36)	2 (33)	2 (25)	11 (55)	2 (40)	
Resection (%)						
Lobectomy	10 (91)	5 (83)	8 (100)	18 (89)	4 (80)	**0.046**
Sleeve lobectomy	0	0	0	2 (10)	1 (20)	
Pneumonectomy	1 (9)	1 (17)	0	0	0	

Table 5.2 Baseline patient demographics and surgery approach/type

VATS, video-assisted thoracoscopic surgery; COPD, chronic obstructive pulmonary disease; eCO, exhaled carbon monoxide; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; IQR, interquartile range.

5.4.2. Phenotype

Current smokers had reduced expression of CD206 and CD163 and increased expression of SIRPα, compared to recent ex-smokers, long-term ex-smokers and never smokers (**Figure 5.4 A-C**). Over 1 month of smoking cessation was associated with reduced SIRPα and increased CD163 levels; SIRPα and CD163 levels inversely correlated across all patient groups ($p<0.05$; **Figure 5.4D**). CD33 levels were elevated in smokers, with levels lower after <1 month smoking cessation (**Figure 5.5**). There were no differences in expression of CD14, HLA-DR between patient groups. CD80 expression differed between groups with lower expression seen in current/recent ex-smoker groups. SIRPα expression was associated with increase eCO, pack/year history and FEV1/FVC ratio (**Figure 5.6**). Efferocytosis Index was not significantly different

across all smoking groups. The current smoking and recent <1 month ex-smoker groups were combined to clinically reflect those who have not had 1 month of smoking cessation, in addition there were no differences in SIRPα levels between these groups. Efferocytosis was significantly lower in current/recent <1 month smokers versus long-term/never smokers (p=0.040; **Figure 5.7**). Efferocytosis Index was inversely related to SIRPα levels (p=0.007) with no significant association to CD163 expression.

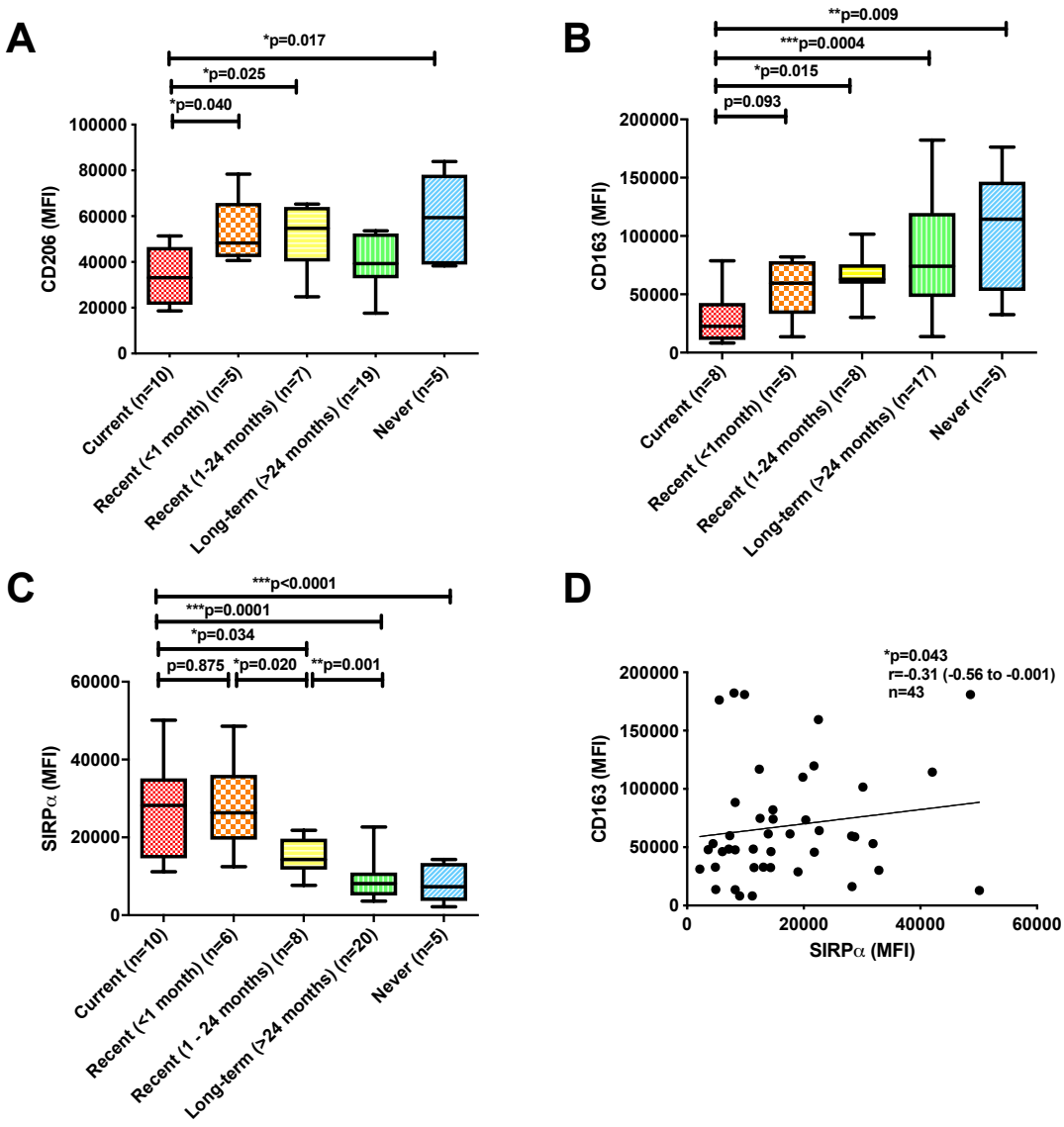


Figure 5.4. AM phenotype between groups including CD206, CD163 and SIRPα.

AM cell surface marker expression is shown as absolute median fluorescent intensity (MFI) of the specific antibody-stained cells minus the isotype control. Box and whisker plots showing median, IQR and range. Individual groups were compared using the Mann-Whitney U test, with ANOVA Kruskal-Wallis test used to test difference between all groups. Spearman's correlation was used for correlation of non-parametric data. **A:** CD206 is expressed differently across groups (p=0.017). **B:** CD163 was expressed differently across groups (p=0.009) with

lower levels in current smokers compared to recent (>1 month) smokers (p=0.015). **C:** SIRPα was expressed differently across groups (p<0.0001) with higher expression in current smokers compared to recent (1-24 month) smokers (p=0.034). **D:** Expression of CD163 was inversely related to SIRPα expression (p=0.043).

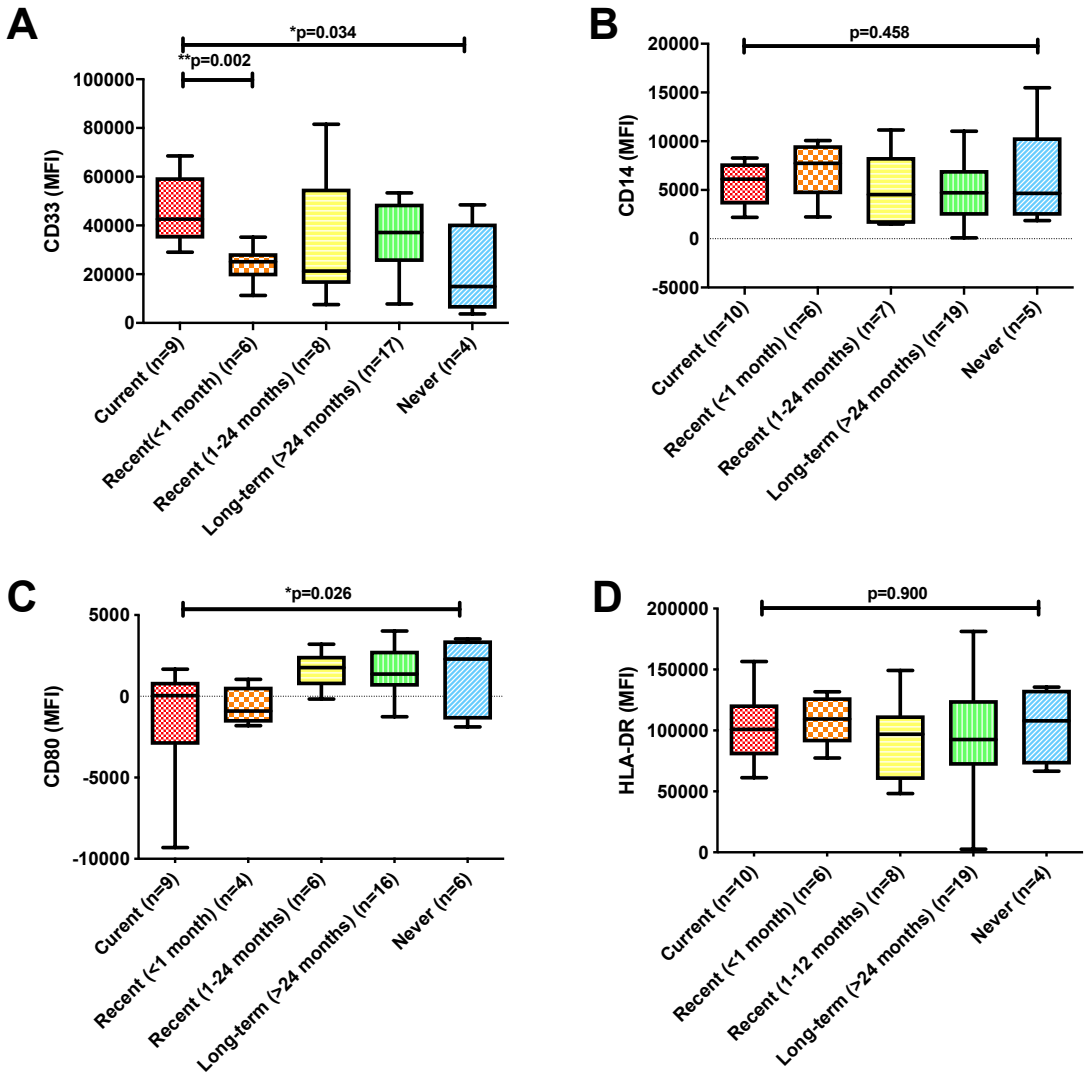


Figure 5.5 AM phenotype between groups including CD33, CD14, CD80 and HLA-DR.

AM cell surface marker expression is shown as absolute median fluorescent intensity (MFI) of the specific antibody-stained cells minus the isotype control. Box and whisker plots showing median, IQR and range. Individual groups were compared using the Mann-Whitney U test, with ANOVA Kruskal-Wallis test used to test difference between all groups. **A:** There were significant differences in CD33 expression between groups (p=0.034) **B:** No differences in CD14 expression between groups. **C:** There were significant differences in CD80 expression between groups (p=0.026). **D:** There were differences in HLA-DR expression between groups.

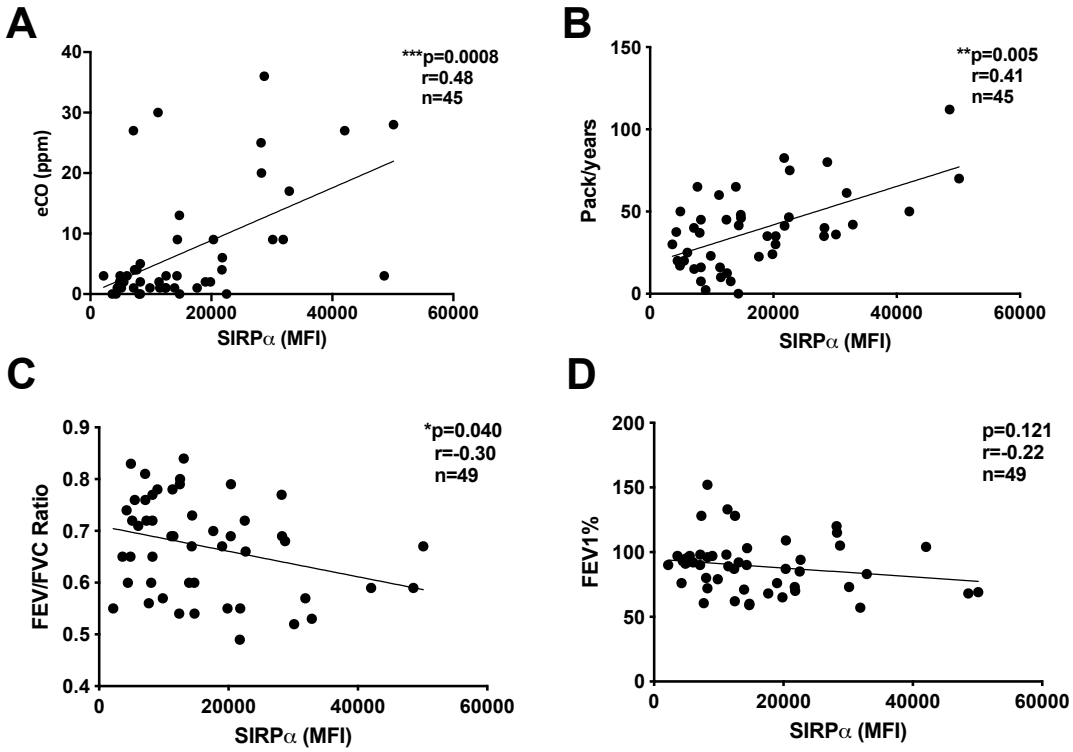


Figure 5.6 Association of SIRPα and smoking and lung function.

SIRPα expression is shown as absolute median fluorescent intensity (MFI) of the specific antibody-stained cells minus the isotype control. Spearman's correlation was used for correlation of non-parametric data. **A:** There was significant correlation between exhaled carbon monoxide (eCO) and SIRPα expression (p=0.0008). **B:** There was significant correlation between pack/years and SIRPα expression (p=0.005). **C:** There was significant correlation between FEV1/FVC (FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity) ratio (p=0.040). **D:** There was no significant correlation between FEV1% predicted and SIRPα expression.

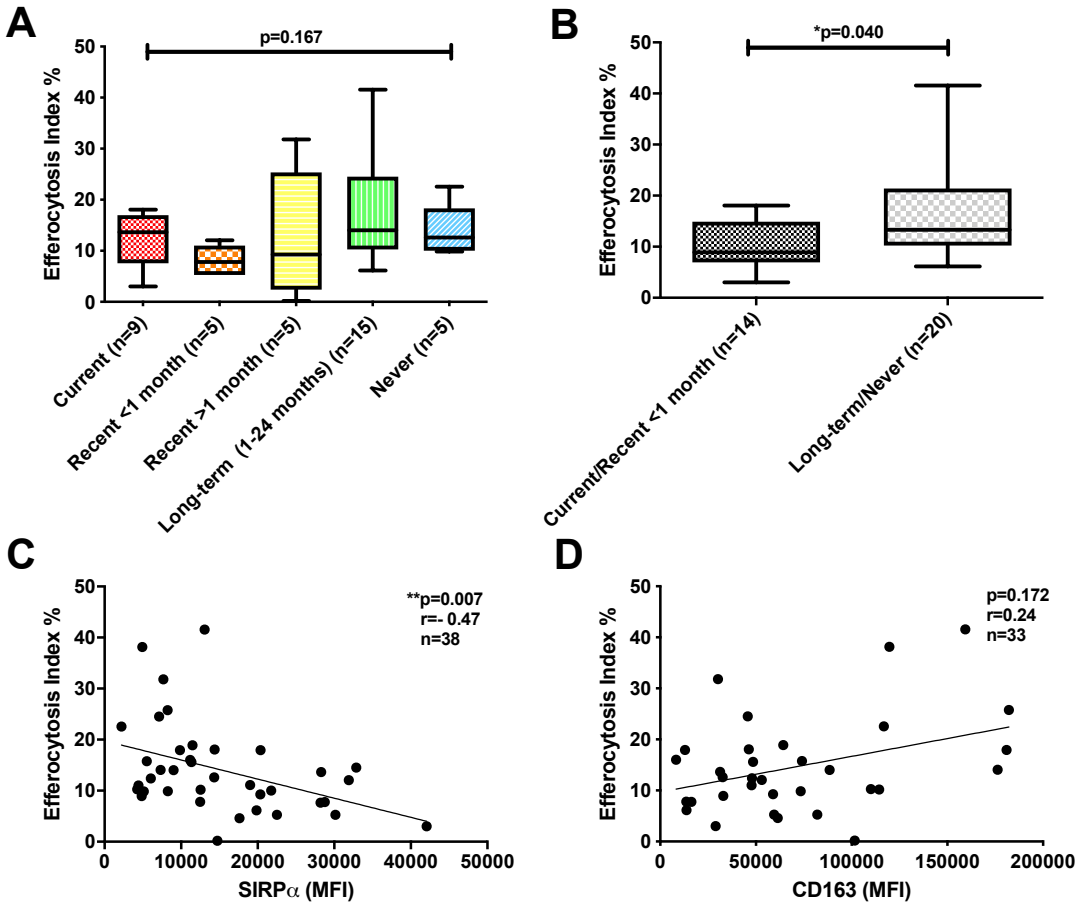


Figure 5.7 Efferocytosis of patient groups and association with SIRPα and CD163.

SIRPα and CD163 expression is shown as absolute median fluorescent intensity (MFI) of the specific antibody-stained cells minus the isotype control. Box and whisker plots showing median, IQR and range. Individual groups were compared using the Mann-Whitney U test, with ANOVA Kruskal-Wallis test used to test difference between all groups. Spearman's correlation was used for correlation of non-parametric data. **A:** No significant differences in efferocytosis index (%) was found between all groups. **B:** Efferocytosis was lower in current/recent <1 month compared to long-term/never smokers (median % index (IQR) 8.9 (6.9 to 14.9) vs 13.3 (10.2 to 21.4); p=0.040). **C:** Efferocytosis index was inversely related to

SIRPα expression (p=0.007). **D:** There was no significant correlation between efferocytosis index and CD163 expression.

5.4.3. Postoperative pulmonary complications

Of the 50 patients, n=14 (28%) developed a PPC as defined by the MGS. There were significant differences in PPC incidence between groups **(Table 5.3)**. There were no PPCs in the never smoker group, whereas 27% (3 in 11) of those in the current smoker group and 83% (5 in 6) of those in the recent ex-smoker group developed a PPC. There were significant differences in LOS between the groups ($p<0.05$). None of the patients died within the hospital stay. Two of the 3 patients with sleeve lobectomy developed a PPC (67%) compared to 12/44 (27%) in the lobectomy group. Due to the higher incidence of PPC in those who had sleeve lobectomy, translational data linked to postoperative outcomes have been presented as including and excluding sleeve lobectomy cases. Outcomes excluding the sleeve lobectomy cases are represented in **Table 5.4.** This is important as sleeve lobectomy was not equally distributed amongst smoking groups and was only performed in the never/long term ex-smokers and could confound results.

	Current Smoker n=11	Recent ex-smoker (<1 month) n=6	Recent Ex-smoker (1-24 months) n=8	Long-term Ex-smoker (>24 months) n=20	Never Smoker n=5	P Value
PPC (%)						
Yes	3 (27)	5 (83)	1 (13)	5 (25)	0 (0)	**0.016**
No	8 (73)	1 (17)	7 (87)	15 (75)	5 (100)	
LOS (days) (IQR)	5 (4-25)	6.5 (3.8-26)	3.5 (2-5.8)	5.5 (3-8)	3 (2-4)	**0.039**

Table 5.3 Postoperative pulmonary complications in different smoking groups

PPC, postoperative pulmonary complication; LOS, length of stay

	Current Smoker n=11	Recent ex-smoker (<1 month) n=6	Recent Ex-smoker (1-24 months) n=8	Long-term Ex-smoker (>24 months) n=18	Never Smoker n=4	P Value
PPC (%)						
Yes	3 (27)	5 (83)	1 (13)	3 (16)	0 (0)	**0.009**
No	8 (73)	1 (17)	7 (87)	15 (84)	4 (100)	
LOS (days) (IQR)	5 (4-25)	6.5 (3.8-26)	3.5 (2-5.8)	5.0 (2.7-7.3)	2.5 (2-3.8)	**0.040**

Table 5.4 Postoperative pulmonary complications minus sleeve lobectomy cases

PPC, postoperative pulmonary complication; LOS, length of stay

SIRPα levels were higher in patients who developed a PPC compared to those who did not; excluding those with sleeve lobectomy (median MFI (IQR) 25,431 (13,055 to 32,630) vs 11,944 (7,587 to 19,232; p=0.011) (**Figure 5.8**). Higher SIRPα levels were associated with increased hospital LOS, in patients with all resection types (p=0.017), and in those without sleeve lobectomy (p=0.005).

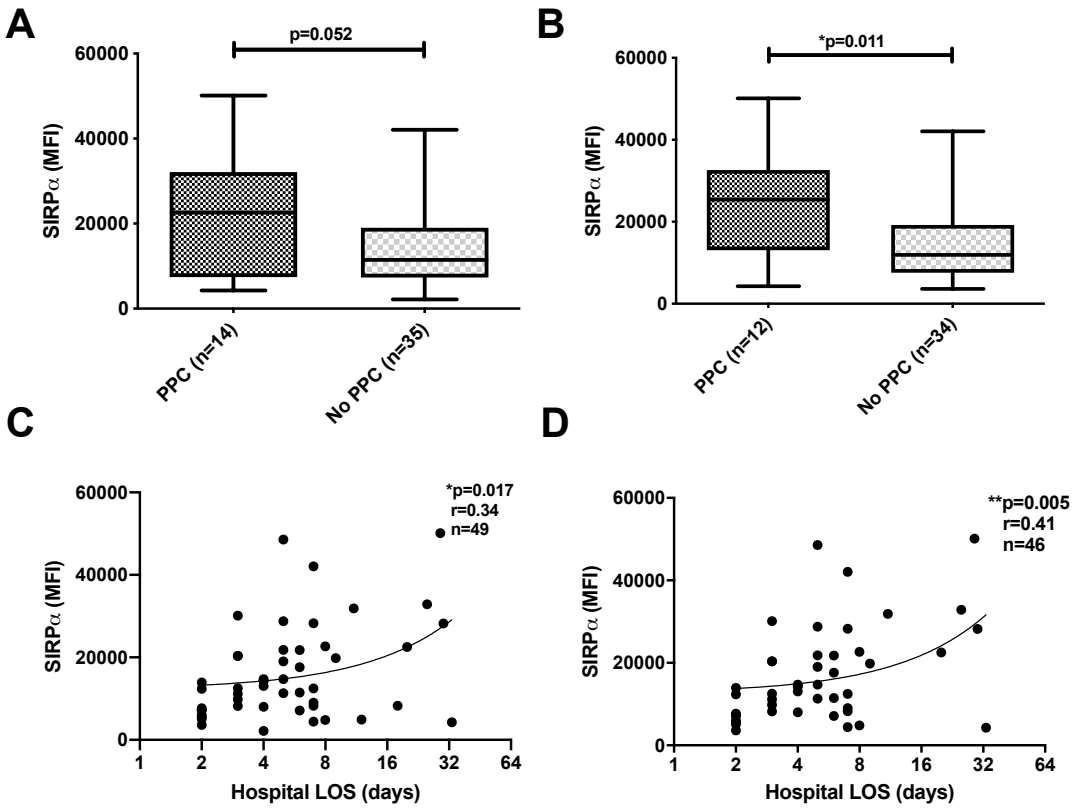


Figure 5.8 Association between AM SIRPα levels and PPC development and LOS

SIRPα expression is shown as absolute median fluorescent intensity (MFI) of the specific antibody-stained cells minus the isotype control. Box and whisker plots showing median, IQR and range. Individual groups were compared using the Mann-Whitney U test. Spearman's correlation was used for correlation of non-parametric data. Hospital length of stay (LOS) is

expressed in log2 scale. **A:** Including all surgery types, there was a strong trend for an increase in SIRPα expression in PPC compared to non-PPC patients but this was not statistically significant (median MFI (IQR) 22,586 (7,431 to 32,143) vs 11,496 (7,325 to 19,028); p=0.052). **B:** Excluding sleeve lobectomy cases, PPC patients had significantly higher SIRPα expression compared to non-PPC patients (median MFI (IQR) 25,431 (13,055 to 32,630) vs 11,944 (7,587 to 19,232; p=0.011). **C:** Including all surgical cases, SIRPα expression was positively correlated to hospital LOS (length of stay) days (p=0.017). **D:** Excluding sleeve lobectomy cases, SIRPα expression was positively correlated to hospital LOS (length of stay) days (p=0.005).

Efferocytosis Index was reduced in those patients who developed a PPC, particularly in those without sleeve lobectomy (p=0.006) (**Figure 5.9**). Higher efferocytosis index was associated with reduced hospital LOS in all patients and excluding the sleeve lobectomy cases (p=0.032).

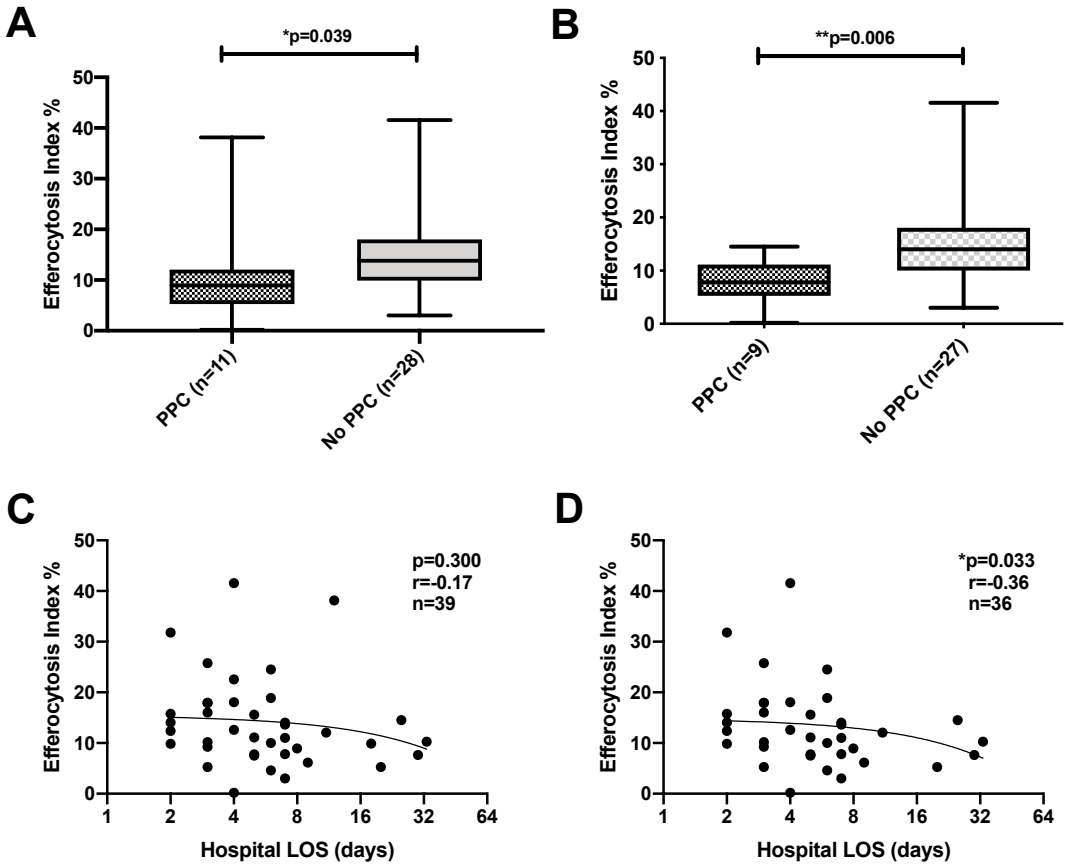


Figure 5.9 Association between AM efferocytosis index and PPC and LOS

Individual groups were compared using the Mann-Whitney test to test difference between all groups. Box and whisker plots showing median, IQR and range. Correlation data is expressed as nonparametric correlation using Spearman. Hospital length of stay (LOS) days data is presented in log2 scale. **A:** Including all surgical cases, efferocytosis index (%) was lower in PPC cases compared to non-PPC cases (median % index (IQR) 9 (5.3 to 12.1) vs 13.8 (9.9 to 18); p=0.039). **B:** Excluding sleeve lobectomy cases, efferocytosis index (%) was lower in PPC cases compared to non-PPC cases (median % index (IQR) 7.8 (5.2 to 11.1) vs 14 (10 to 18.1); p=0.006). **C:** Including all surgical cases, there was no significant correlation between

efferocytosis index (%) and hospital LOS (days). **D:** Excluding sleeve lobectomy cases, lower efferocytosis index (%) was significantly associated with higher hospital LOS (days) (p=0.033).

5.5. DISCUSSION

AMs from current and recent ex-smokers have altered phenotype and reduced efferocytosis compared to long-term/never smokers. The phenotype of smokers showed reduced expression of receptors involved in phagocytosis and efferocytosis (CD206 and CD163) and increased expression of inhibitory receptors (SIRPα and CD33). Higher SIRPα levels were related to smoking status and correlated inversely to efferocytosis. SIRPα expression was related to postoperative outcome; increased expression of SIRPα was seen in AMs from patients who had developed a PPC and longer hospital LOS. Efferocytosis was also lower in those who had developed PPC and in those with longer hospital LOS.

CS has been previously shown to influence the phenotype of pulmonary macrophages, resulting in more immature monocyte-like macrophage phenotype with reduction in surface markers which have a role in play a role in phagocytosis and efferocytosis (161). CD206 has a key role in macrophage phagocytosis of micro-organisms and endocytic clearance of host derived glycoproteins in efferocytosis. In COPD, AMs have a smoking related reduction in CD206 expression which is associated with reduced AM phagocytosis (155). CD163 is a high affinity scavenger receptor which also has role in phagocytosis. Expression of CD163 is reduced in smokers compared to ex-smokers in

COPD (155), expression of CD163 have been shown to normalise after >1 month of smoking cessation.

CD14 levels of expression on AM were unchanged between smoking groups. CD14 expression has previously been found to be increased in AMs from smokers (153). CD14 is expressed in higher levels in monocytes compared to AMs, and AMs were selected through flow cytometry gating based on the size, granularity and auto-fluorescence. Therefore, this gating strategy would not capture newly infiltrated monocytes which are CD14 high; these cells are likely to be in higher abundance in smokers and may explain the difference between this study and previously reported results.

CD33 levels of expression on AM were found to be higher in the current smokers and reduced in those who had recently quit. CD33 is a transmembrane receptor which binds sialic acids from glycoproteins or glycolipids, upon binding the phosphorylation of the immunoreceptor tyrosine-based motif (ITIM) acts as a docking site for Src homology 2 (SH2) which inhibits phagocytosis. Increased expression of CD33 may play a role in downregulating inflammation, as previously studies have shown it to be inversely related to systemic inflammation in patients with COPD (263).

CD80 expression on AM was lower in current smokers and those who have recently quit smoking. CD80 is expressed by antigen presenting cells such as AM and has a key

role in regulating the innate and adaptive immune system. Previous studies have shown that in AMs from former smokers with COPD, CD80 expression is reduced (157, 264). Lower CD80 expression was related to higher amounts of black carbon content and increased and more severe COPD exacerbations (264).

This is the first study to show the association between smoking and SIRPα expression in AMs. SIRPα is an inhibitory receptor which interacts with transmembrane CD47 on a live self-cell (also called the 'don't eat me signal'), resulting in inhibition of effector function including efferocytosis. This study findings showed the association of increased expression of SIRPα in current and recent smokers (<1 month) compared to those who had stopped ≥1 month prior to surgery. SIRPα expression was lower still in those who were long-term ex-smokers and never smokers. SIRPα expression was associated with current smoking but also pack year history and degree of airway obstruction. This study found that SIRPα expression was inversely associated with efferocytosis and was higher in those patients who went on to develop a PPC. Efferocytosis of apoptotic neutrophils is impaired in AM from patients who have recently smoked compared to long-term ex-smokers/never smokers. This is particularly important given the increased pulmonary influx of circulating neutrophils found in smokers (144, 265). Efferocytosis is a pro-resolving process, therefore reduced efferocytosis could prolong the time needed for tissue repair and resolution of inflammation. This is key in the setting of thoracic surgery, where resident AM need

to recover from the initial inflammatory insult of surgery and prevent a large second inflammatory hit in the form of a postoperative pulmonary complication (PPC).

The potential mechanisms for upregulation of SIRPα and inhibition of efferocytosis are displayed in **Figure 5.10**. This may be through CS-induced damage to pulmonary surfactant (266, 267); as surfactant proteins A and D also bind to SIRPα to cause downstream inhibitory signalling. AM are a key player in the catabolism/recycling of damaged surfactant (268). CD47 is expressed on neutrophils and during apoptosis expression is reduced/altered; higher expression of SIRPα may inhibit efferocytosis through remaining CD47 'don't eat me signal' to further prevent efferocytosis. Furthermore, a recent study looking at the role of SIRPα in pneumonia (258), found that SIRPα levels had a dual role in inducing paralysis in the AM resident population; increasing expression in the early stages directly impacted phagocytosis and then triggered immunosuppressive environment on AMs that had not directly encountered the pathogen. *In vitro* addition of SIRPα antagonist restored phagocytic function of monocytes from patients with sepsis-induced systemic inflammation. Thus, SIRPα would be an attractive target to block to restore AM function.

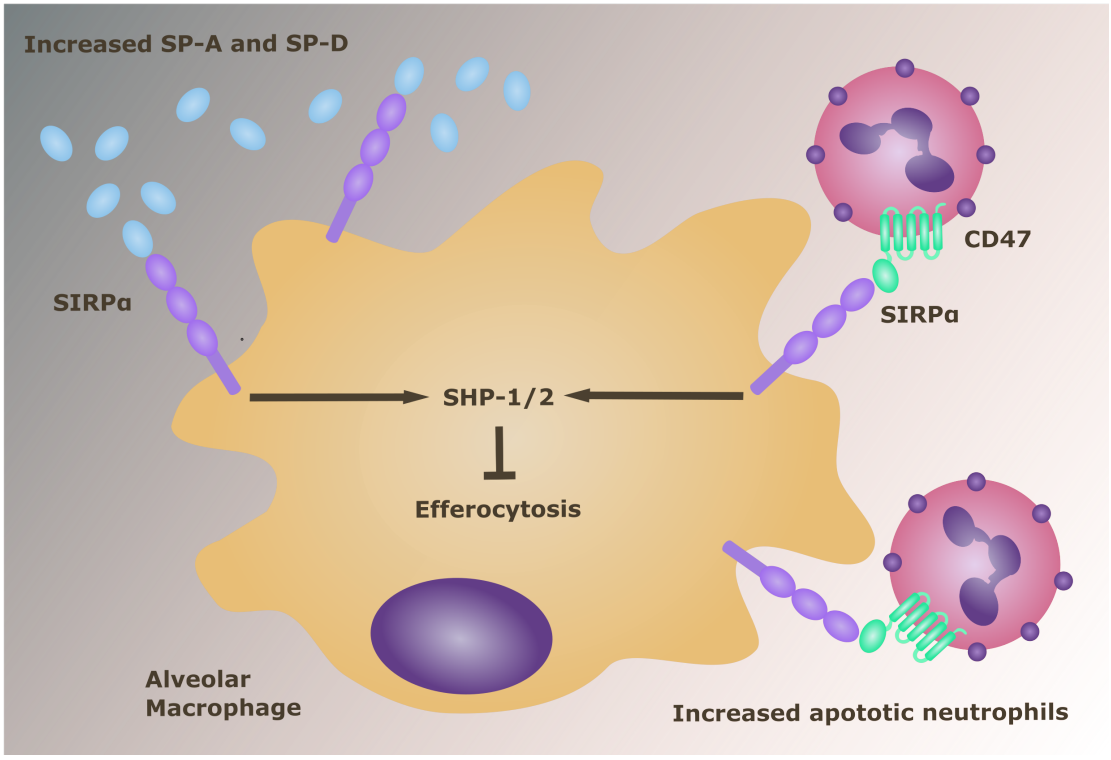


Figure 5.10 Possible mechanisms for effect of smoking on AM SIRPα levels and efferocytosis.

SP-A, surfactant protein A; SP-D, surfactant protein D; SIRPα, signal regulatory protein alpha; SHP-1/2, Src homology region 2 domain-containing phosphatases 1/2.

There were 14/50 (28%) cases of PPC in this study, which is typically higher than the 13-15% of cases seen in previous cohorts (192, 232). The reason for this may be the increase in patients recruited to the current and recent-ex smoking groups which represent half of the translational cohort. Also, in order to obtain sufficient lung tissue for AM isolation, there is selection bias as all these patients had a substantial lung resection with minimum resection of lobectomy, and none had explorative/biopsy or segmentectomy and wedge resection. Also, this cohort had 3/50 (6%) sleeve

lobectomy cases, which typically would be seen in 1% of all major surgery and is associated with increased risk of postoperative complications. Two of the 3 patients (67%) with sleeve lobectomy developed PPC. We therefore presented the postoperative outcome data as including and excluding excluded sleeve lobectomy; previous studies have excluded sleeve lobectomy based on the increased risk of complications in this group (269). Sleeve resection has been previously been found to carry 12 times increased incidence of postoperative pneumonia compared to standard lobectomy (270). Another study found sleeve lobectomy to have greater risk of postoperative pulmonary complications than pneumectomy in those under 70 years of age; in the older age group there were no significant differences in incidence in pulmonary complications (271). The mechanisms for increased risk of PPC in sleeve lobectomy cases include bronchial anastomotic complications and atelectasis.

These results are supported by other studies showing that smoking impairs AM efferocytosis (176, 257). In our study, combining current/recent ex-smoker < 1 month and long-term ex-smoker/never smokers found difference in the efferocytosis index, which suggests a biological signal probably 'diluted' by other intracellular pathways affecting efferocytosis. Even in studies in patients with COPD, there was greater AM efferocytosis capacity in subjects with COPD who had ceased smoking, compared with those who were still smoking, suggesting a smoking-related effect on AM in COPD that may be partially resolved upon smoking cessation (153). COPD as well as smoking related changes on apoptosis and efferocytosis of AMs, would contribute to the

inflammatory response and lead to greater tissue disruption. Of interest, there were a higher proportion of patients who developed a PPC in the recent-ex smoker (<1 month) group compared to the current smoker group. There was a higher proportion of patients with COPD in that group which is a known independent risk factor for PPC development (192). The presence of patients with COPD in the recent ex-smoker <1 month group may also account for higher SIRPα expression in this group; this group also had an increased pack year history, which correlated with higher levels of SIRPα expression. CD33 levels were lower in the ex-smoker <1 month group and are inversely related to systemic inflammation in the setting of COPD (263). Thus, the balance of SIRPα expression and CD33 expression is worst in recent ex-smokers < 1 month compared to current smokers and may in part explain the differences in PPC outcome between the groups. In addition, the group of recent ex-smokers (<1 month) were also older and had a higher proportion of males. Little is known on macrophage expression of SIRPα and the effect of ageing and differences in sex. However, ageing has been observed to phagocytosis/efferocytosis of monocytes-macrophages (272).

Earlier studies suggested an increased risk following smoking cessation before surgery, however more recent cohort studies have shown risk reduction in patients who have quit, though many of these studies will not have used eCO verification to group those into current and recent ex-smokers. Our previous study did not show a difference in PPC incidence between those who had stopped smoking before and after 6 weeks (232), however eCO verification was not used and the >6 weeks group is a very

heterogenous group of patients, including patients who have stopped smoking within the last 2 years, and those who have smoked many years prior.

E-cigarettes are increasingly being used in patients undergoing surgery; of the 10 patients who were vaping, half were dual using with cigarettes. There were no differences in PPC between those who were vaping and were not vaping. However, the numbers are small, and the study was not designed to investigate the effect of e-cigarettes. A larger cohort study is needed to see if switching from cigarette smoking to e-cigarettes demonstrates harm reduction and impacts on reducing incidence of PPC.

5.5.1. Limitations

The limitations of the study include AMs may be influenced by other factors than smoking habits such as underlying disease and medical therapy. We cannot rule out that some of the AMs could be tumour associated and therefore have a more pro-resolving phenotype than resident AMs. In addition, there was a significant difference in FEV_1/FVC between sub-groups. COPD is known to effect AM function, and many of the patients undergoing lung surgery in this setting have been diagnosed or have an obstructive spirometry in-keeping with the diagnosis, particularly in those who are current and recent ex-smokers. In addition, glucocorticoids can enhance efferocytosis and inhaled corticosteroid usage was not recorded and investigated within this study. Dexamethasone was given intravenously as part of the anaesthetic protocol; however,

this was the case for all patients recruited in the study. Excluding these patients would not make the results generalisable to smokers undergoing lung surgery, therefore this study includes patients with or without diagnosed COPD with a ratio (FEV_1/FVC <0.7) and mild/moderate obstruction (FEV1 >50%). Data on these variables have been prospectively collected however a small translational study would not allow correction for analysis. The strength of this study is the use of human AMs from patients undergoing lung surgery, rather than the use of circulating monocyte-derived macrophages or immortalised monocytic cell lines (e.g. THP-1).

5.5.2. Conclusion

AMs isolated from lung resection of patients undergoing curative intent lung cancer surgery showed increased SIRPα expression in smokers and recent ex-smokers, with reduced expression in patients who had quit for more than 4 weeks. SIRPα levels were inversely related to efferocytosis function, and directly correlated with PPC development and hospital LOS. Thus, in higher risk patients whom smoking cessation strategies are not effective, SIRPα may be a potential target to restore macrophage function and reduce incidence of PPC.

5.6. Introduction to Chapter 6

Following on from the results of Chapter 3 to 5, pre-operative smoking cessation is a strategy to modify PPC risk after major lung surgery. Smoking cessation is part of the national and speciality specific guidance for patients undergoing surgery. However, in practice, a large proportion of patients continue to smoke prior to surgery. Therefore, there is need for delivery of timely and effective smoking cessation in this setting. Therefore, we sought to develop a web-based smoking cessation application 'Quit4Surgery' for use in the surgical pathway prior to major lung surgery. This is detailed in Chapter 6, with a section of the way forward for the set-up of a feasibility study using 'Quit4Surgery' as part of integrated smoking cessation intervention in this setting.

CHAPTER 6: DESIGN OF AN INTEGRATED, WEB-BASED SMOKING CESSATION INTERVENTION IN THE THORACIC SURGICAL PATHYWAY ('QUIT4SURGERY')

My role in this chapter involved chairing patient and healthcare practitioner workshops and stakeholder meetings, thematic analysis, literature review, critical appraisal, and scientific writing including generation of the figures. This chapter includes the manuscript which has been submitted for peer review.

Lugg ST, Kadiri S, Budacan A, Kerr A, Golby C, Skirth B, Farley A, Perski O, West R, Brown J, Thickett DR, Naidu B. User-centered design of an integrated, web-based smoking cessation intervention in the thoracic surgical pathway ('Quit4Surgery'): A case study of the Double Diamond method.

6.1. ABSTRACT

Objective: 1 in 4 patients continue to smoke before major thoracic surgery, with higher risk of developing postoperative complications. This is a case study of the UK Design Council's Double Diamond user-centered design process to develop a web-based smoking cessation intervention in major thoracic surgery.

Methods: The Double Diamond process includes four phases 'discovering' insights to the problem, 'defining' the areas to focus on, 'developing' potential solutions and selecting 'deliverable' solutions. These were achieved through workshops involving the core research team of academic researchers and software designers (n=9), patient groups (n=6) and healthcare practitioners (HCPs) (n=8).

Results: Barriers for implementing smoking cessation in the thoracic surgical pathway (discovered) included patient factors of education, behavioural change, motivation and continuing support; HCP factors included training and feedback on patient outcomes and medication support. The resultant design brief included a digital passport/coach for patients, outcome tracking tool for clinicians, and a HCP training tool (defined). A smoking cessation web app (Quit4Surgery) was designed for patients and HCPs, with a focus on promoting user engagement (i.e. biofeedback, motivational support, medication support and access to information). The prototype app was tested (developed) with patients and HCPs, who judged the intervention acceptable.

The final minimal viable product was developed, through collaboration between HCPs and the core research team (delivered).

Conclusion: The 'Double Diamond' process enabled the development of an acceptable integrated web app to complement the delivery of face-to-face smoking cessation support in the thoracic surgery pathway. Quit4Surgery is currently being piloted in a feasibility study.

6.2. INTRODUCTION

Smoking before major lung surgery is associated with increased risk of developing postoperative complications, with associated worse short and long-term outcomes (192). In lung cancer surgery, patients who continue to smoke have reduced survival and increased risk of cancer recurrence (118). Smoking cessation at any stage prior to lung cancer surgery is beneficial (273). Despite this, 1 in 4 patients continue to smoke before surgery (232).

Pre-operative smoking cessation is recognised by the European Association of Cardiothoracic Surgery (EACTS) as a priority (274) and the UK National Institute of Clinical Excellence (NICE) guidance states support should be offered to all patients undergoing elective surgery as early as possible (275). The most effective support combines stop-smoking medications with behavioural support, offered as weekly sessions, preferably face-to-face, for a minimum of 4 weeks after the quit date. Available UK smoking cessation services depends on local provisions and are predominantly community-based, which would require patients to attend several appointments and may not be available in time for the date of surgery, especially in patients with lung cancer where avoiding delays in receiving counselling and pharmacological intervention are of paramount importance. There is however a paucity of evidence of smoking cessation for people with lung cancer; with recent systematic review finding no RCTs that determined the effectiveness of any type of smoking cessation programme in lung cancer (276).

Research shows that patients prefer to receive bespoke smoking cessation support, integrated into the existing hospital visits during the surgical pathway (277). This study included 22 patients with a smoking history who had undergone lung cancer surgery and involved semi-structured qualitative interviews and framework analysis to explore views on smoking and patient's preferences for smoking cessation support. The reasoning of continuing smoking was because patients found it enjoyable, they found it helped with phycological coping or had health benefits. With regards to the harms of smoking, some patients had a fatalistic attitude on continuing to smoke, which would limit cessation attempts. Despite clinicians believing smoking cessation would be difficult and unwanted in lung cancer patients, all those in the study wanted cessation support, and for this to be raised by healthcare professionals (HCPs) as early as possible and within the pathway for their treatment. Furthermore, a recent published report of a survey of a hospital-based smoking cessation service in the North-West England has been shown to be acceptable to patients, prompting consideration of further quit attempts in 96% patients of the 106 patients surveyed. It also highlighted the opportunity to improve smoking cessation outcomes by providing access to the most evidence based NICE recommended treatments (varenicline, e-cigarettes and dual NRT) as part of individualized treatment programmes (278).

There are however many potential barriers to the delivery of smoking cessation support, including lack of access to smoking cessation resources (279, 280), lack of

clinical knowledge or skills (280, 281), and lack of time to deliver the intervention (279). A potential solution would be to provide the patient and HCPs with an electronic tool, such as a mobile phone/device web application for smoking cessation. However, none are currently available which have been designed specifically for lung cancer surgery. 'Quit2Heal' is a smartphone app designed to support patients with cancer diagnosis (282), though not specific to lung cancer with 21/59 (39%) of adults tested having lung cancer diagnosis, showed promising acceptability and effectiveness though has not been tested in a full scale RCT. 'QuitIT' is another application (283), designed for use in the peri-operative setting for patients undergoing cancer surgery, though not specific to lung cancer surgery, with only 12/38 (32%) having lung cancer diagnosis. The mobile application was specific to using a game to help with coping strategy, though found although satisfaction was high, the majority (60%) did not play the game. Thus, the importance of integration of the application with healthcare practitioner smoking cessation support to improve engagement.

A hybrid approach with an electronic tool in addition to structured behavioural support delivered face-to-face may aid successful quit attempts and support the HCPs in delivering the intervention throughout the clinical pathway. A review of over 100 smoking cessation apps showed only 6 were deemed technically high quality in terms of the 'Mobile Application Rating Scale' (284). One high quality application, SF 28, has published smoking cessation efficacy data (285). However, the majority of available

apps have not been designed for use alongside support from trained smoking cessation practitioners.

Due to the complex nature of smoking cessation in high-risk patients about to undergo major thoracic surgery, it is important to involve patients and HCPs in the design process, both to identify barriers to smoking cessation and to create the solution. The Double Diamond is a user-centered co-design process (286) that has previously been used in healthcare settings (287-289). It distinguishes itself from others by systematically splitting the design process into two divergent-convergent processes, with two-stage engagement in end users. It is a creative process where a number of ideas are created (i.e. 'divergent thinking'). These ideas are then narrowed down to the best concept or solution (i.e., 'convergent thinking'). Therefore, in the Double Diamond, this happens twice, once to define the problem and once to create a feasible solution.

We report a case study to demonstrate how the Double Diamond method can be used to develop a novel smoking cessation tool for use within the pathway of elective major thoracic surgery.

6.3. METHODS

6.3.1. Study design

We used the Double Diamond approach to intervention development, which is split into four phases: the first diamond consists of two phases, Discover and Define, whilst the second diamond consists of further two phases, Develop and Deliver. The Double Diamond method including workshops involved are displayed in **Figure 6.1.**

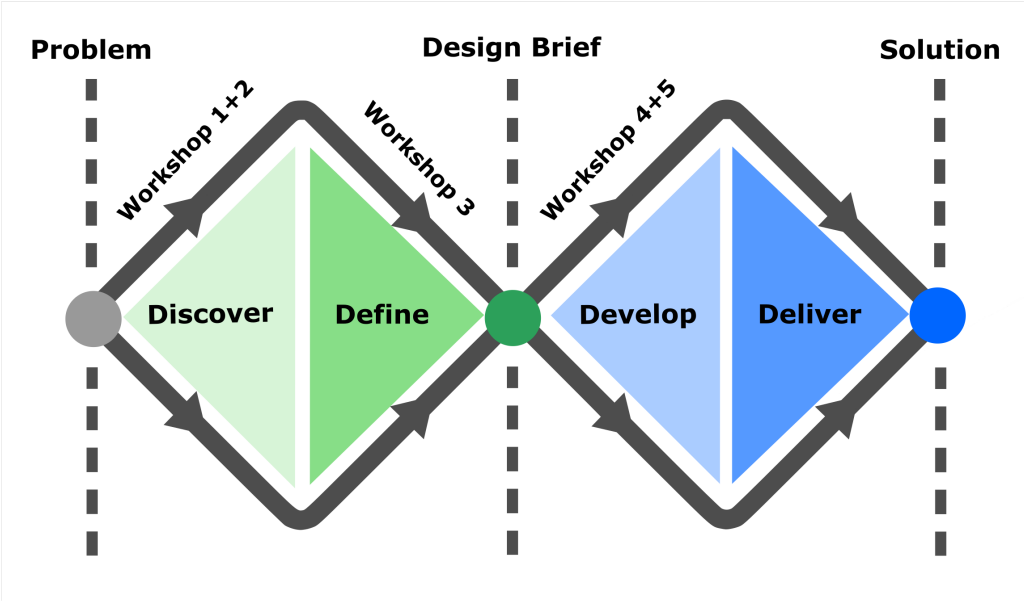


Figure 6.1 Overview of the Double Diamond design approach and workshops in methods.

Discover*:* The aim of this phase was to develop a shared understanding of behavior change, medication usage and technology use from both the perspective of service users and HCPs. This phase is *divergent* in its approach as it explores the subject matter

from a variety of viewpoints. **Define:** The aim was to refine and reframe the breadth of insights emerging for the Discover phase, and therefore was *convergent* in its approach. **Develop:** The aim was to resume a *divergent* process by focusing on creating a wide range of ideas for addressing the design brief. **Deliver:** The aim was to refine the breadth of concepts generated in the Develop phase and was *convergent*.

6.3.2. Participants

A series of workshops were held as part of service development. Local approval was granted from the audit and quality improvement department under code CARMS-16784 within a tertiary thoracic surgery hospital, University Hospitals Birmingham NHS Foundation Trust (UHBFT). Ethical approval was not required based on Health Research Authority guidance (https://www.hra-decisiontools.org.uk/research).

Workshops were complemented with insights arising from HCPs who were part of the thoracic surgery clinical pathway. The Discover Phase included Workshop 1 and 2; the Define phase Workshop 3, and Deliver phase included Workshop 4 and 5.

In Workshop 1, members of the core research team were recruited from a range of expertise, including n=9 in total. From the University of Birmingham and UHBFT this included (n=6): a thoracic surgical consultant, a respiratory medicine doctor, a lung cancer specialist nurse, and two research associates with background in psychology and a lead research nurse in thoracic surgery. The role of this part of the team was to

provide insight into the clinical pathway and HCP and patient needs. From University College London, this included a team of individuals (n=3) with an extensive background in smoking cessation research and behavioral psychology, who have also had previous experience in health app development. The role of this part of the team was to provide insight into the evidence based behavioral interventions and integration into the pathway and app. The core research team continued to provide input throughout the development of the app. Following these tasks, Workshop 1 included user profiles of prototypical service users and HCPs were created, together with mapping of the contexts in which therapy is delivered.

In Workshop 2, n=6 service users were invited to join by telephone and email who were part of the thoracic surgery patient representative group (RESOLVE). The RESOLVE group included previous patients who had undergone major thoracic surgery. HCPs working within UHBFT as part of the lung cancer and thoracic surgical pathway were invited by email. This included n=8 in total: a thoracic surgeon (n=1), pre-operative nurses (n=2), a lung CNS (n=1), respiratory medicine consultants (n=2) and thoracic advanced clinical practitioner (ACP) (n=2).

In Workshop 3, this included the core research team members from Workshop 1 (n=9). In Workshop 4, service users (n=4) who were members of the RESOLVE group were invited by telephone and email to test a prototype of the app to consider its

acceptability from a patient perspective. Participants were asked to complete a few tasks including onboarding, and goal setting.

In Workshop 5, regional training sessions for the HCPs were for delivering smoking cessation intervention in the thoracic surgical pathway, where (n=12) were asked to comment on the acceptability of the app from an HCP perspective.

6.3.3. Data collection and analysis

Data were collected from the workshops including field notes, thematic analysis was used to identify major themes and sub-themes which were validated against the insights arising from therapy observations, service shadowing, and context mapping.

6.3.4. User personas and user stories

Form the output of previous Workshops with the patient groups, HCPs and core research team, it was possible to create what is known in software as a User Persona. A User Persona is used to identify who the potential users are, creating an example person and then detailing who they are, and why they will be using the system.

6.3.5. Agile project management

In developing the Quit4Surgery, 'agile' and 'scrum' project management (290) was used in the process to develop an initial project outline, and a 'Product Backlog' where suggested changes were broken down into small 'app features'. Work was then

conducted in 2-week cycles, termed, ‘sprints’ where a selection of items from the product backlog are dealt with. At the end of each sprint, a working product was delivered. Following user testing, a final version of the web-based app, suitable for release and ready for the feasibility study, was developed (291).

6.4. RESULTS

6.4.1. Discover

The outputs from the Discover phase and Workshop 1 included user mapping of the thoracic surgery pathway showing the service users’ journey, including interactions and touchpoints from when the user first hears about the service, as well as including people encountered on the journey and places visited (**Figure 6.2**).

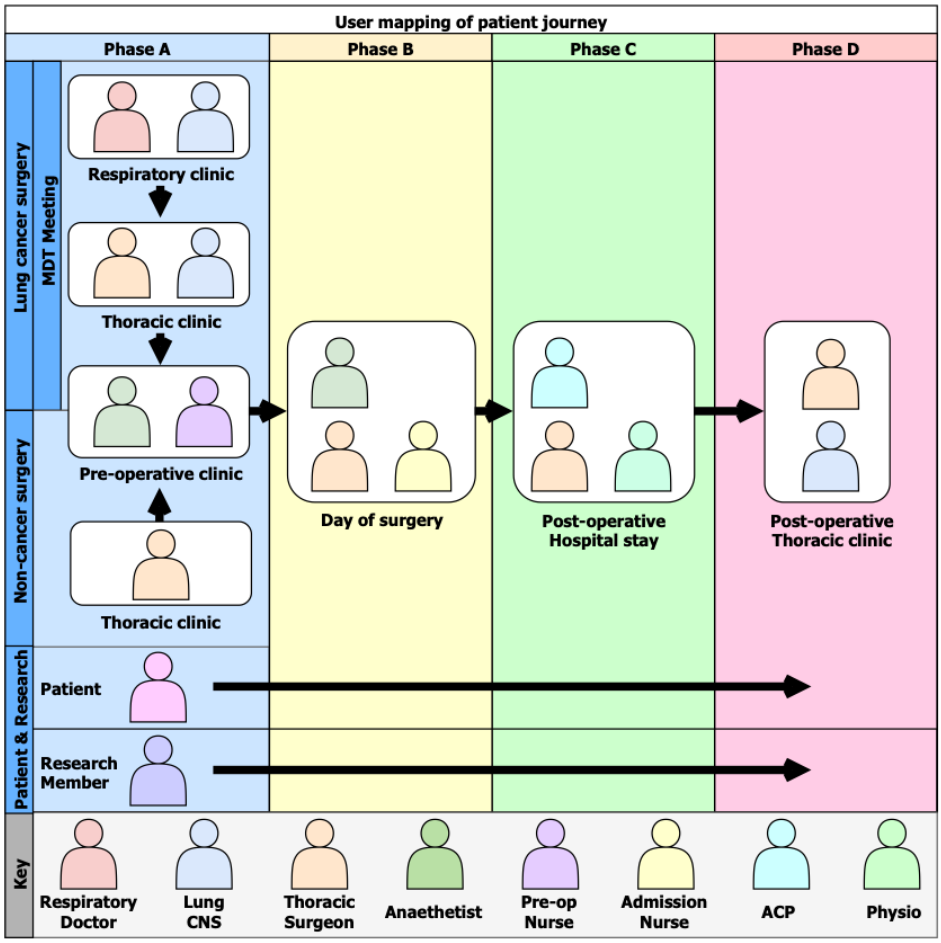


Figure 6.2 Results from Workshop 1 - user mapping of patient journey in the major thoracic surgery pathway.

MDT, multidisciplinary team; CNS, clinical nurse specialist.; ACP, advanced clinical practitioner.

The user mapping of the patient journey included 4 Phases, Phase A (pre-operative outpatients), Phase B (day of surgery), Phase C (post-operative hospital stay) and Phase D (post-operative outpatients). Phase A was initially split, depending on

whether patients will undergo lung cancer surgery or non-cancer surgery, whereas the remaining Phases (B-D) are common for all patient pathways. In those with lung cancer diagnosis, the patient cases are discussed in a multidisciplinary team meeting (MDT), which the patient is not present, but determines further progression in the pathway. The lung cancer clinical nurse specialist (CNS) initially would see the patient in the respiratory clinic, contacts the patient of the MDT outcome and attends the thoracic surgery clinic. The patient and research team member are present at all parts of this pathway. The core research team agreed that the important timepoints to assess smoking cessation would be at Phase B (point of surgery) and Phase D (quit rate 30 days after surgery). Note, in some UK thoracic surgical centers the thoracic clinic and pre-operative clinic in Phase A may be combined to form a one-stop clinic.

In Workshop 2, through focus group discussions and analysis of data generated during these discussions the following sub-themes were identified, which group together to form major themes (**Figure 6.3**).

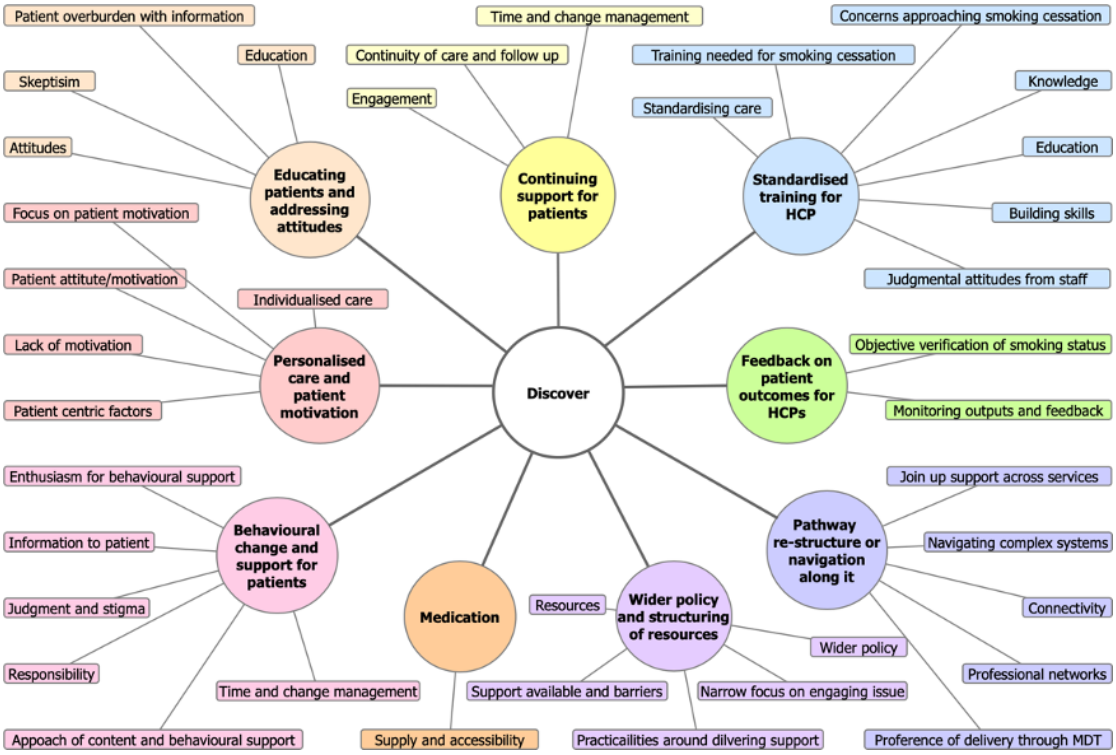


Figure 6.3 Sub-themes and major themes generated through discover phase.

HCP, healthcare practitioner; MDT, multidisciplinary team.

From the discovery phase, in total 38 sub-themes were generated by the group and were narrowed down to 9 major themes. The major themes included: behavioural change and support for patients; personalised care and patient motivation; educating patients and addressing attitudes; continuing support for patients; standardising training for HCP; feedback on patient outcomes for HCPs; pathway re-structuring or navigation along it; wider policy and structuring of resources; medication.

6.4.2. Define

The define phase involved defining the design brief based on the insights from the discover work. This consists of workshops to synthesise the research insights into themes and identify the most salient areas for improving smoking cessation in thoracic surgery. A list of service users' and clinicians' needs in relation to the therapy was developed, with each need rated according to potential impact and ease of implementation. Based on these insights, a design brief was developed which specified the desired area of impact and aims for the redesign. During ideation from the workshop held with the stakeholders, out of the major themes, the following solutions were judged deliverable and prioritised into three major categories to support the design brief (**Table 6.1**).

Digital Passport/Coach for patients	**Outcome tracking tool for HCPs**	**Training tool for HCPs**
Goal setting support	Hub to discuss outcomes	E-Learning
Delivery of information	Review current roles and process maps	Set expectations with patients
Tailored and bespoke tech	Opt out smoking cessation support	Digital training
Behavioural support	Follow up phone calls	Face to Face training
Incentivise engagement	Text messaging	Management support for education
Gather patient commitment	Link up with CQUIN	Tailor patient information
Counselling	National driver	Behavioural support training

AI feedback to patients	Funding support for smoking cessation	Bespoke training
On demand support: telehealth	Tech infrastructure to enable connectivity across disciplines and systems	Continual professional development
IT Feedback/monitoring	Digital analytics	Regular HCP events
Integrate digital into pathway	Standardise follow up	Training sessions for HCPs
Digital passport	Digital tools to show smoking cessation status	Increased networking between HCPs
Face to face support	Joint primary and secondary care networking	Smoking cessation expert & GP to attend MDT
Informing patients of risks	Change to policies around medication use	Champions in trust
Single point of contact	Patient satisfaction surveys	
Chat bot		
NRT education		
Biochemical feedback		
Tailor patient information		
Patient friendly information		

Table 6.1 The following solutions were derived to define the design brief.

IT, information technology; HCP, healthcare practitioner; CQUIN, Commissioning of Quality and Innovation; GP, general practitioner; MDT, multi-disciplinary team.

6.4.3. Design Brief

Following ideation, the team identified priority areas for further investigation, which constituted the design brief (set out below). The design brief specified the aim to develop a web-app to support the smoking cessation therapy process. This project aimed to build a digital hub to include the three following major components:

1. A digital Passport/Coach for patients
2. An outcome tracking tool for clinicians
3. A training tool for HCPs (primarily for lung CNS)

As part of the design brief, each of the above components were broken down, to further specify the intentions of the web-app.

6.4.4. Digital Passport/Coach for patients

- This involved the need for development of a patient inclusion tool, which will help patients to feel 'involved' in the pathway, and their own smoking cessation decisions. The tool will be designed for the major thoracic surgery pathway. The aim of the tool was to increase the level of engagement of the patient with the healthcare system.
- The tool will be an intensive support package including behavioral change support tools from National Center for Smoking Cessation Training (NCSCT) (75). The tool will focus around motivation of the individual and coping with craving. It will also

aim to boost patient confidence overall, and can be tailored and personalised to individual needs.

- The tool will aim to store where patients are along the thoracic surgical pathway and track smoking status. There is aim for an area, such as within a content management system, where the latest evidence-based advice can be inputted. Patients often self-refer, so there needs to be option of direct booking not just signposting for entry into the smoking cessation programme. In addition to this, a social interaction section with the HCP will need to be researched. Another factor to include, is a social commitment (such as e-signing) by patients and HCPs, to commit to giving up at different stages of the pathway.
- Nicotine replacement therapy (NRT), varenicline (Champix) or e-cigarette usage will be an important part of the system. The tool will need to inform the patient about the benefits and risks of the smoking cessation medications/aids with education around it.
- Within the web-app there could be an area where patients are able to get electronic support from HCPs. This aspect, will require further investigation, including the type of support required, how this is communicated (e.g., messaging), and how it is received by the HCP and then fed back to the patient.
- There needs to be an investigation into technologies used in this field, with decisions on how the tool will look and how it is navigated. It will also need to be decided, whether the patient is given a physical device with the web-app included

or virtual software to access. This includes consideration of costs of additional physical devices and possible benefits of compliance of the tool.

6.4.5. Outcome tracking tool for HCPs

- An outcome tool is needed to monitor patient progress during the thoracic surgical pathway. The system requires areas for feedback to both HCPs and stakeholders. Extensibility and Interoperability with clinical systems must be considered.
- The outcome tracking tool may be in the form of a web dashboard and will contain patient reported outcome measures (PROMs) or clinician reported outcome measures (CROMs) (which can be inputted on the system).

6.4.6. Training tool for HCPs (primarily lung CNS)

- The tool needs to incorporate HCP training, primarily for the lung CNS to use as part of an integrated smoking cessation support. This includes making it easier for HCPs to access behavioural support tools, in order to help patients with smoking cessation and delivery of a positive patient experience.
- The stakeholders agreed that the training will be most effective by breaking current NCST online training down into bitesize chunks. This could be delivered in a different form to face-to-face training.
- The timing of training will coordinate with the digital tool given to patients and include specific training on using the web-app. A reward mechanism for

completing training needs to be investigated, though there will be a designated area for HCPs to upload certificates for evidence of training.

6.4.7. Develop

At the beginning of the develop phase, concepts to address the design brief were generated. These concepts were then developed, evaluated, and refined by the project designers, technologists, and stakeholders. The selected concepts were made and validated with service users (patients and staff).

6.4.8. Prototype testing of service users

The selected concepts were prototyped digitally and validated by presenting them to participants on a tablet. The participants were first informed of the reason behind the development of the application and were given a brief overview of the functions. The validation process focused on both participants' verbal reports and observation of their physical and cognitive ability to use the prototypes. They were tested on their ability to navigate the application on the device without aid and were asked for their feedback in real time. The initial prototype was tested by a small group of service users (n=4) who were members of the RESOLVE group.

6.4.9. Logging in and navigation

No patient had any problems logging into the application. The group felt the initial medication selection page was clear including the advantages and disadvantages of

each smoking cessation medication. However, the group identified issues with not being able to change the smoking cessation medication during the quit attempt and discussed the need to maintain flexibility to change the medication, due to side effects for example, which would be guided initially by face-to-face consultation. The home screen on the prototype listed information entered on each day such as medication usage, which the group found would be better categorised initially into weeks to prevent a long list of data to scroll through for each day the application is used.

6.4.10. Information content

With regards to the information given to educate patients, the focus group felt the e-cigarette information was well set out with no specific comments related to this content.

6.4.11. Input of medication to aid compliance

With regards to the daily in input of medication, members of the group found it easier to see what they had used the previous day when inputting data. All members of the group discussed potential use of graphical representation of nicotine replacement therapy (NRT) usage.

6.4.12. Feedback for service user

The group also discussed use of graphical representation of exhaled carbon monoxide (eCO). The group liked the concept of inputting smoking craving scores and receiving

craving tips when the user inputs high craving scores. The group also felt that the patient should be able to input data on cigarette usage, which would be a prompt for advice within the application and/or at a future face-to-face session.

6.4.13. Style of content

Regarding the style of the prototype, the participants commented on the font size being too small, and would need to increase size, particularly in sub-headings and information page. It would also be more easily read in a tablet over a smart phone. They recognised this as priority. One of the members commented on the blue text but did feel it was easy to read and would be better than just using black and white.

6.4.14. Prototype testing for HCPs

HCP feedback on the web-based app prototype was acquired during half day face-to-face training of the novel smoking cessation intervention. The training addressed the feasibility study and introduction to the web-based app, overview on smoking cessation, making a quit attempt, cravings, NRT and smoking cessation medications and managing cravings and changing behaviour. The feedback from the HCPs of a range of users from lung CNS, to research nurses and thoracic surgeons was grouped into sub-themes and then major themes (**Table 6.2**).

Aesthetics	Intervention content	Functionalities
Simplicity	Usefulness	Regular updated
Visual appeal	Personalisation	Feedback capacity
	Motivational qualities	

Table 6.2 The following themes and sub-themes derived from the healthcare practitioner feedback.

With regards to aesthetics, the HCPs found the design of the web-based app easy to use. Many praised the clarity of the pages when navigating through them. However, when asked about improvements, it was suggested that some aspects of the visuals were not desirable particularly use of just jet blue and white. Introduction of more pictures were recommended. Generally, the HCPs found the content useful both for themselves and from the patients' perspective. They particularly liked the personalisation aspect, and they could easily see that it would help patients comply with smoking cessation. Some suggested the personalisation can hold the patient accountable for their own health behaviour. HCPs were keen on having extra feedback capability of the web-based app so they could support their patients. The regular updates of the patient progress through the clinician dashboard was appreciated and they acknowledge the positive outcomes of having the consistent updates on the 'how to cope with cravings' tips for the patients.

6.4.15. Deliver

In the “deliver phase”, wireframe storyboards of the session and out-of-session content were developed based on the selected concepts and then iteratively coded. The selected concepts for therapy redesign were finalised and storyboards developed. The new version of the design was iteratively produced through rapid prototyping in software code.

User Personas were done to cover both HCPs and Patients. In that same session, it was possible to then use these User Personas to identify what the users are looking for the system, known as Epics. These are the high-level areas of the software mapped out against how long they take to develop, and how important they are to the users (292).

This can then be used to identify the most important areas to begin development work on. Once the high-level areas are decided, it was then possible to ‘drill down’ into what makes up each area. This is done by creating ‘User Stories’. The User Stories follow a step a user might take, and what benefit it would have. For example, one of the Epics was a ‘Patient Tracker’. This was the top priority. One of the linked User Stories for this Patient Tracker was being able to track E-Cigarette usage. This was a priority based on what was extracted from the User Personas and their needs from the what the tool would do. Once each Epic was fleshed out and had User Stories, it is possible to create a ‘Look and Feel’ design document. The purpose of this document is to detail what elements of the tool will look like and how they act as a base when designing the

application. For example, it would contain the design elements in the software such as colours, fonts and example button styles. Once this is approved alongside the original prioritised Epics and User Stories it was possible to begin development work.

The application name, '*Quit4Surgery'*, was the product of a brainstorming with the stakeholders including clinicians, research phycologists and software developers. Popular digital brand names were reviewed for inspiration, and name concepts were generated based on the themes of stopping smoking, motivation and benefits around surgery. Quit4Surgery was selected.

6.5. DISCUSSION

6.5.1. Principal findings

We have developed a novel bespoke smoking cessation web-based mobile application for patients and HCPs for use in the lung surgery pathway. To our knowledge, this is the first study to have used a Double Diamond design approach for development of a smoking cessation application. Understanding the thoracic surgery pathway and key users enabled a map for which the smoking cessation would run alongside. Discussion in the discover phase with multidisciplinary HCP members and service users allowed the stakeholders to understand the main barriers to developing an integrated smoking cessation service. The define phase allowed the stakeholders to utilise the information from the previous step to produce solutions, which subsequently developed into the design brief to facilitate primarily a digital passport for smoking cessation in major lung

surgery, an outcome tracking tool for HCPs, and an opportunity to integrate training and professional development. The develop phase allowed feedback from both patients of prototype testing followed by the deliver phase to produce a final viable product.

Mobile/device based 'applications' have become an integral part of day to day life for many people, and there are increasing number of applications available to improve health (293). Mobile apps may be considered as a feasible and acceptable means of administering health interventions. A systematic review was conducted and included 24 studies including primarily feasibility and pilot studies with small sample sizes, with all studies informed by behavioural theories or strategies, and self-monitoring the most common way of implementing the health intervention (294). The review found that acceptability of mobile phone apps was high in mobile phone users, and there was early evidence that health intervention apps were well received. However, there is a lack of studies and those with large sample sizes, and more research is therefore needed to determine efficacy. Although smoking cessation apps exist, there is no clear evidence that their use alone improves smoking cessation compared with low intensive smoking cessation support. This has been demonstrated within a Cochrane review including 5 studies (3079 participants) with no difference in relative risk of smoking cessation using smartphone apps compared to lower-intensity smoking cessation (RR 1.00 95%CI 0.66 to 1.52) (295). However, the same review showed that automated text messaging interventions were more effective than minimal smoking

cessation support (RR 1.54, 95%CI 1.19 to 2.00; 13 studies; 14,133 participants). The reasoning for the lack of effectiveness in web-apps may be related to imprecision of the smoking cessation support provided or due to inconsistencies between effectiveness of the apps developed and the findings of the studies of which they are tested. However, there is need for innovation in healthcare services for delivery of smoking cessation including use of smoking cessation applications.(94).

Quit4Surgery has been designed to be part of hybrid smoking cessation intervention alongside support by trained HCPs in the thoracic surgical pathway. Quit4Surgery incorporates evidence-based smoking cessation behavioral therapy and pharmacotherapy, an effective smoking cessation application in the setting of major surgery will be able to bridge the gap between patient visits by providing continuation of care, irrespective of the location used. Depending on the clinical environment, contact would either be face-to-face, or by telephone which has shown to be non-inferior to face-to-face support (296).

There is lack of clear and accepted standards for the development of digital health apps which is a major problem in this field. This is the subject of a planned systematic review which aims to identify criteria from applicable standards, guidelines, frameworks, and best practice for health app development (297). Recently NICE have published UK guidelines on the use of digital and mobile health interventions for behaviour change, including recommendation on development, advising to involve a

wide range of stakeholders including potential users as early as possible throughout the development process (298). NICE has also produced an evidence standard framework for digital health technologies (299) including guidance on regulation of medical devices in the UK (300) and regulation of the Care Quality Commission.

We have used the Double Diamond co-design process (301), which has previously been implemented in digital health tools for mental health and wellbeing (287, 302). The Double Diamond process focuses on the needs of the users. The design of new features is based on findings and from previous research carried out by the group and informed by behavioural frameworks/motivation theory. The result was a list of content/features to be developed.

6.5.2. Strengths and Limitations

The basis of the behavioral techniques and pharmacotherapy recommendations for Quit4Surgery are based from best evidence practice as implemented in the NCSCT guidance (75). Therefore, outside specific information relating to the benefits of stopping in major thoracic surgery and lung health, much of the information content has been modified from these tried and tested resources used by smoking cessation practitioners throughout the UK. However, this has been delivered to patients in a bitesize format and complements their individual journey in major lung surgery, with for HCP rather than be just for service users as of other smoking cessation applications. The options for smoking cessation medication in the design brief and developed web

app include varenicline, which although showed superiority of effectiveness to dual NRT (82), has unfortunately since been withdrawn in the UK due to the concern regarding nitrosamine impurities (84). Further iterations of the web app may include alternate smoking pharmacotherapy such as bupropion as an option, which is NICE approved and relatively safe and effective (80). Furthermore, the information on e-cigarettes pre-dated the case series epidemic in the US (101), which was associated with the use of vitamin E acetate (103); Current UK NICE guidelines recommend nicotine-containing e-cigarettes are accessible as an option for a stop smoking intervention, which when combined with behavioral support are more likely to result in a successful quit attempt (303) .Another limitation of this study are the group sizes, for example for prototype testing were small which may limit detection of useability issues (304), however such numbers were not unusual for a workshop setting. The alpha testing was performed on older individuals; however, we do not have the data to describe the service users in detail. Thus, the alpha testing may be limited by the service users, for example younger service users are likely to be different in attitude and probably more experienced in using digital technology. A strength of the study is that both expert clinicians, academics and patient and public involvement (PPI) meant there was a breath of perspectives in the production. Finally, this is not a finished product as it has only undergone alpha testing within the developer's and stakeholders' sites. The feasibility study of the web-app would involve beta testing within the users' environment, including further testing of usability, functionality, and reliability. The results of the feasibility study, including reports from qualitative

interviews of both service users and HCPs, would help guide future iterations for use in a full scale study of integrated smoking cessation in the thoracic surgical pathway.

6.5.3. Implications

In applying the Double Diamond methodology within the healthcare setting to create an app, allowed users to be involved in the process from the very beginning of the design process. Rather than being led by the stakeholders, the design process allowed both patients and HCP to brainstorm barriers within the discover stage of the process. It also allowed further involvement of users in the development phase. Thus, the Double Diamond was user-centric with user input at either end of the design process. Both user processes were ran within single workshops thus to minimise time spent for patients and HCP, whilst maximizing output to make the final product as relevant to the end user as possible.

6.5.4. Conclusion

This is the first app created for stopping smoking in major thoracic surgery, with use of the Double Diamond Design process to achieve this objective**.** Thoracic surgery is a high priority area, as effective smoking strategies have not been tested in this cohort of patients. Quit4Surgery is an innovative blended digital therapy for people to stop smoking in thoracic surgery. The design process highlights the importance of focus groups of patients, HCP and stakeholders**,** with product testing on devices satisfied

usability for patients and HCP**.** Quit4Surgery will be assessed for use in a multi-center feasibility study of integrated and bespoke smoking cessation versus usual care (291).

6.6. The Way Forward

The observational and translational data though Chapters 3 to 5 demonstrate that smoking prior to major thoracic surgery is the biggest risk factor for development of PPC, with approximately 1 in 5 patients continuing to smoke before surgery. NICE guidelines state that smokers undergoing 'elective' surgery should receive behavioural support and stop-smoking pharmacotherapy as early as possible in their outpatient or pre-operative assessments, which should be offered weekly, preferably face-to-face, for a minimum of 4 weeks after the quit date (305). The Cochrane review supports this type of 'intensive' intervention (306). Despite this, few patients undergoing thoracic surgery are offered smoking cessation support (307), and many patients would prefer bespoke support during hospital visits (277).

To improve smoking cessation in thoracic surgery, an integrated smoking cessation (INT) package has been developed using best practice from NICE/NCSCT (75) involving pharmacotherapy and a hybrid of behavioural support delivered by trained HCPs in the thoracic surgical pathway and the complementary web app Quit4Surgery. Support may be particularly effective because of site of the surgery, the lungs, makes this a clear 'teachable moment' for patients (308, 309).

The project MURRAY feasibility study aims to determine the feasibility of integrated (INT) smoking cessation support in the surgical pathway of major thoracic surgery for use in a future a multi-centre RCT. The feasibility study will assess if INT improves smoking cessation rates compared with usual care (UC) of standard community/hospital-based NHS smoking cessation support (291). Feasibility studies are a recommended pre-requisite to assess feasibility of a large and expensive full-scale trial (310). The full scale study will likely take the form of a stepped wedge cluster randomised controlled trial (311), and therefore the feasibility study does not include individual patient randomisation.

The protocol for the Project MURRAY feasibility study details the intervention, study process including the trial schema and schedule of assessments (291). The feasibility study will be conducted in 5 adult thoracic centres including the University Hospitals Birmingham NHS Foundation Trust. The primary objective Is to establish the number of patients who agree to participate in the intervention as a proportion of those eligible to enter the study. Secondary objectives include evaluation of study processes. The feasibility and acceptability are predefined such as the ACCEPT model for pilot evaluations and pilot RCTs (312).

Inclusion criteria are adults (>18 years) who are current tobacco smokers (including those smoked within the last 28 days), those undergoing major thoracic surgery

(including both open and minimally invasive approach), ability to provide written informed consent and having at least 1 weeks' time to surgery. Exclusion criteria are those undergoing emergency thoracic surgery and unable to perform exhaled carbon monoxide (CO) measurements.

Data collected will include demographic information and co-morbidities. Postoperative complications will be defined by the European Society of Thoracic Surgery (ESTS) (313), Thoracic Morbidity and Mortality (TM&M) system (314) and the StEP-COMPAC Group (55) with hospital re-admission rate determined within 30 days of discharge. The feasibility of the following questionnaires will be tested including self-reported quit rate and exhaled CO measurement, Fagerstrom Test for Nicotine Dependency (315), Mood and Physical Symptoms Scale (MPSS) (316), generic health related quality of life (EQ-5D-5L) (317), NRT and support usage questionnaire and health resource usage questionnaire.

Patient telephone interviews will be conducted until saturation is achieved, which is likely to be around 25-30 patients across all sites (318). Key HCPs in the clinical pathway will be invited to attend a focus group or individual interviews, which will also explore both the acceptability and recommendations for optimisation of the intervention. Digitally recorded interviews and focus groups sessions will be transcribed verbatim and anonymised, and analysed for patients and staff separately following Braun and Clarke's method for thematic analysis (319).

In this feasibility study, selecting 5 units whose overall elective thoracic patient throughput amounts to 4000 patients a year, of whom 1 in 5 are smokers and 20% agree to take part using our own criteria, however >40% recruitment should be achievable as per the ACCEPT model (312), and from previous recruitment rates of patients into smoking cessation trials (320). As this is a feasibility study no formal sample size calculation has been performed. Billingham et al in 2013 conducted a survey of 79 trials from the UK Clinical Research Network Database to assess target sample sizes in feasibility and pilot studies (321). Pilot trials were found to have a lower sample size per arm (median = 30, range 8 to 114) than feasibility studies (median = 36, range = 10 to 300). However, the study also concluded that whilst sample size calculation is important, a formal sample size calculation may not be appropriate.

It is expected that 60 eligible patients will be recruited to the INT group and 60 patients to the UC group of the study, with recruitment of 120 patients in total. The aim therefore is to recruit 5 patients/month to each group over the 12 months recruitment period across all sites. Recruitment of 60 participants per group will allow us to measure recruitment and compliance rates with 95% CI width between 10 and 20%. Analysis of feasibility and patient-reported outcomes will take the form of simple descriptive statistics and where appropriate, point estimates of effects sizes and associated 95% CIs.

The study has obtained ethical approval from NHS Research Ethics Committee (REC number 19/WM/0097). Dissemination plan includes informing patients and HCPs, engaging multidisciplinary professionals to support a proposal of a definitive trial and submission for a full application dependent on the success of the study. The trial registration number is NCT04190966.

CHAPTER 7: THESIS SUMMARY

7.1. OVERVIEW

The development of PPCs in lung surgery remains a complication which is associated with dramatically worse outcomes. Risk factors have been identified, such as smoking, but risk modification and preventative strategies in clinical practice are limited. It has previously been shown that those who developed PPC had a worse in-hospital outcome however the long-term effects had not been assessed. Previous studies have shown smoking to be a risk factor of PPC development, however, the optimum timing required to reduce the risk of smoking is unknown. Furthermore, the role of alveolar macrophages in the development of PPC has not been assessed and could point towards potential future therapeutic targets in those at risk. Any smoking cessation intervention in thoracic surgery would need to be integrated into the pathway to be effective. This thesis investigated the following questions: 1) What is the long-term impact of developing a PPC after lung surgery? 2) What is the optimum time to stop smoking before surgery? 3) What is the role of the alveolar macrophage in PPC development? 4) Can an application be developed to help patients stop smoking before major lung surgery?

7.1.1. Long-term impact of developing a PPC after lung surgery

Chapter 3 shows that the incidence of PPC in those undergoing thoracic surgery was 13%. PPC was associated with increased in-hospital mortality, ITU admission, HDU and hospital length of stay. There was also an increase in hospital readmission after discharge, including an increase in readmission for respiratory tract infections in those

who had developed a PPC. This study demonstrated PPC was an independent risk factor for poor long-term outcome after lung surgery. Of the risk factors for PPC identified, cigarette smoking was the biggest risk factor for PPC development. This study highlighted the significance of developing a PPC both short and long-term; recognising cigarette smoking as a major modifiable factor in their development.

7.1.2. Smoking and timing of cessation on PPCs after lung cancer surgery

Chapter 4 assessed the smoking history in patients who were undergoing curative intent lung cancer surgery. One in four patients were smoking at the time of lung cancer surgery. Current smokers had the highest risk of PPC and ITU admission and longer LOS compared to never smokers. Those who had stopped smoking had reduced incidence of PPC, however no difference could be found between those who had stopped before or after 6 weeks of surgery. This article demonstrated that smoking remained common before lung cancer surgery and with associated risk of PPC. The optimum timing to improve outcomes could not be defined in those who had self-reported, which would require further objective biochemical validation in future prospective studies.

7.1.3. The role of AMs and smoking on PPCs after lung cancer surgery

Chapter 5 involved a translation study of alveolar macrophage phenotype and function in patients undergoing lung cancer resection, to investigate the role of smoking on alveolar macrophages and PPC development. The study found that those

who smoke prior to surgery had lower levels of expression of cell surface markers involved in phagocytosis/efferocytosis (CD206 and CD163), and higher levels of SIRPα, which has an inhibitory role in efferocytosis. The levels of SIRPα was lower in patients who had stopped smoking for >4 weeks duration. Efferocytosis was found to be lower in those current/recent ex-smokers compared to long-term/never smokers. SIRPα levels and efferocytosis related to clinical outcomes of PPC and LOS; those with higher SIRPα levels and lower efferocytosis having worse outcomes. This is the first study to have shown an association between SIRPα and smoking, and the importance of SIRPα in AMs as a biomarker and potential future target to improve clinical outcomes including PPC.

7.1.4. Design of an integrated, web-based smoking cessation intervention in the thoracic surgical pathway

Chapter 6 described the development of the web-based application to be used within the smoking cessation feasibility study. This was developed using the UK Design Council Double Diamond methodology involving four stages of process where problems and solutions regarding smoking cessation in thoracic surgery are generated and defined. The use of patients and health-care engagement at multiple points in the design process was key to develop a web-based application for use as feasibility and potentially could be used in clinical practice depending on the results of the smoking cessation study.

7.2. LIMITATIONS

1) In assessing the impact of PPC in patients undergoing lung surgery, there were relatively few patients undergoing VATS which has subsequently shown to reduce the risk of PPC. The data on smoking was reliant on self-reported status. With regards to classification of PPC, the use of the MGS, although demonstrating those with worse outcomes, does not specify the type off PPC or severity of complication.
2) The assessment of smoking on PPC and timing of cessation was limited by the use of self-reported status and did not include the use of exhaled carbon monoxide. Furthermore, patients had been prospectively categorised into either stopping within or after 6 weeks before surgery. This cut-off prevented any further analysis on timing of smoking cessation.
3) The translational study of alveolar macrophages cannot fully exclude effect of tumor associated macrophages. Recruitment was severely affected by the COVID-19 pandemic, with the structural changes in thoracic surgery, being moved between hospital sites and cancellation of surgeries even after the 2^{nd}/3^{rd} wave due to hospital pressures. The numbers were small in the never smokers and recent ex-smokers which represent the minority of patients being referred to surgery.
4) The intervention application 'Quit4Surgery' is to be tested as part of the feasibility. The group sizes for initial prototype testing were small, with larger groups of the health-care practitioners. More detailed information on acceptability and usage of

the application will be assessed throughout the feasibility from quantitative usage and qualitative patient interview.

7.3. FUTURE RESEARCH

The future research from the generation of work from this thesis is the following:

1) Further *in vitro* studies are required to determine the whether the observed increase in SIRPα expression in AMs from smokers can be replicated in AMs long-term ex-smokers/never smokers following exposure to CS extract. Additionally, if a SIRPα inhibitor would be able in part or fully restore function in CS exposed AMs. Further investigation is required to understand the mechanism of smoking related changes in SIRPα expression in AMs, and the potential therapeutic strategy of SIRPα inhibition to restore AM function in those at risk of PPC.
2) The optimum timing of cigarette smoking prior to lung cancer surgery is yet to be established. However, the earliest this can be attempted during the patient pathway the greater the benefit of cessation. The results from the feasibility study of Project MURRAY will answer the question on the recruitment and acceptance of an integrated smoking cessation programme in the thoracic surgical pathway in both patients and health care practitioners. This will include the integration of the web-based app Quit4Surgery. The results from the feasibility will inform a future large scale multi-center study on smoking cessation in lung cancer surgery, which if successful in showing clinical effectiveness could alter standard care for patients who smoke and are referred for lung surgery.

3) Finally, there is much interest in the harm reduction of e-cigarettes, with the uptake increasing in those undergoing major surgery. However, the effect of e-cigarette vaping on PPC is yet to be investigated and could inform smoking cessation pharmacotherapy and nicotine replacement strategies in those who are undergoing a quit attempt prior to surgery. Following feasibility, this could be investigated in a large-scale observation study and potentially span across major surgical disciplines to understand this in more detail.

7.4. CONCLUSIONS

This thesis has shown that the development of PPC following major thoracic surgery is associated with worse short-term and long-term outcomes. Those who smoke prior to surgery had the highest risk of developing a PPC. The risk of developing PPC was lower in those who had stopped smoking but the optimum timing of cessation prior to surgery could not be determined in those who had self-reported to have stopped either before or after 6 weeks of surgery. In AMs from lung tissue in those undergoing surgery, smoking was associated with changes in phenotype including SIRPα expression, which was higher in current smokers, and lower in patients who had stopped smoking for over 4 weeks. Efferocytosis was lower in those current/recent-ex smokers compared to long-term/never smokers. Higher SIRPα levels and reduced efferocytosis was associated with PPC development and increased LOS. Further *in vitro* studies area required to investigate the mechanism of smoking related changes on SIRPα and if a SIRPα-inhibitor would be able to restore AM function, as a potential strategy to prevent/ameliorate PPC. A newly designed web-application has been developed for use in the feasibility study of integrated smoking cessation in the thoracic surgical pathway, to assess the acceptance of the intervention and trial ahead of a full scale multi-centered study.

CHAPTER 8: ABBREVIATIONS

AE	Adverse Event
AM	Alveolar Macrophage
ANOVA	Analysis of Variance
APC	Allophycocyanin
ARDS	Acute Respiratory Distress Syndrome
ASA	American College of Anaesthetists
BMI	Body Mass index
BSA	Bovine Serum Albumin
BTS	British Thoracic Society
CI	Confidence Interval
CO	Carbon Monoxide
CNS	Clinical Nurse Specialist
CRF	Case Report Form
CS	Cigarette Smoke
CXR	Chest X-ray

COPD	Chronic Obstructive Pulmonary Disease
DLCO	Carbon Monoxide Diffusion Capacity
DMSO	Dimethyl Sulfoxide
ERS	European Respiratory Society
ESTS	European Society of Thoracic Surgery
EVALI	E-cigarette or Vaping Associated Lung Injury
FACS	Fluorescence-Activated Cell Sorting
FBS	Fetal Bovine Serum
FEV_1	Forced Expiratory Volume in 1 second
FSC	Forward Scatter
HCP	Health Care Practitioner
HDU	High Dependency Unit
HR	Hazard Ratio
HTN	Hypertension
IHD	Ischaemic Heart Disease
INT	Intervention

ITU	Intensive Therapy Unit
IQR	Interquartile Range
LOS	Length Of Stay
LRTI	Lower Respiratory Tract Infection
MDT	Multi-Disciplinary Meeting
MFI	Median Fluorescence Index
MGS	Melbourne Group Scale
MHRA	Medicines and Healthcare products Regulatory Agency
NLCA	National Lung Cancer Audit
NCSCT	National Center for Smoking Cessation Training
NICE	National Institute for Health and Care Excellence
NRT	Nicotine Replacement Therapy
NSCLC	Non-Small Cell Lung Cancer
OR	Odds Ratio
PBS	Phosphate Buffered Saline
PCA	Patient Controlled Analgesia

PIS	Participant Information Sheet
PPC	Postoperative Pulmonary Complications
PPI	Patient and Public Involvement
RCP	Royal College of Physicians
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
RESOLVE	Thoracic Patient's Representative Group
RPMI	Roswell Park Memorial institute of cell culture media
SD	Standard Deviation
SIRPα	Signal Regulatory Protein Alpha
SSC	Side Scatter
UC	Usual Care
UoB	University of Birmingham
VATS	Video-assisted Thoracoscopic Surgery
VBA	Very Brief Advice
WCC	White Cell Count

CHAPTER 9: REFERENCES

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