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Circulating biomarkers of malignant pleural effusion deriving from extrapulmonary tumours.

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TABLE OF CONTENTS

Page

1 INTRODUCTION

1.1 Physiology and pathophysiology

A malignant pleural effusion (MPE) is defined by the National Cancer Institute as "a condition in which cancer causes an abnormal amount of fluid to collect between the thin layers of tissue (pleura) lining the outside of the lung and the wall of the chest cavity". In these cases, cancer cells can be found in the pleural fluid or in the pleural tissue. A paramalignant effusion, also called paraneoplastic or secondary effusion, is a pleural effusion in the setting of a known malignancy but in the absence of tumour cells in either the effusion or the pleura. Examples of this are proximal bronchus obstruction with postobstructive atelectasis and/or pneumonia, chylothorax, pulmonary embolism, liver metastases associated with hepatic hydrothorax and tumour-related malnutrition (Aydin et al., 2009; Egan et al., 2014; Karkhanis & Joshi, 2012; Thomas & Musani, 2013). It is important to differentiate between malignant and paramalignant effusions because the prognosis is very different and different management strategies are used. Both the parietal and visceral pleura are responsible for both the production of pleural fluid as the absorption of it. In normal circumstances, pleural fluid production is about 0.01 mg/kg body weight per hour (BW/h) with a pleural fluid absorption capacity of 0.2 ml/kg BW/h, which is about 250-450 ml/day. Hydrostatic and oncotic differences in the systemic pressure of the pulmonary circulation and the pleural space are responsible for the production rate. Pleural effusion results from an imbalance between pleural fluid production and absorption. Congestive heart failure and pneumonia are the commonest cause of pleural effusion, followed by cancer. Pleural effusion can be divided in transudate and exudate. A transudate is low on protein, lactate dehydrogenase (LDH), cholesterol, has a low specific gravity and has a clear appearance. It is usually caused by increased hydrostatic pressure (e.g. congestive heart failure) or decreased colloid pressure (e.g. hypoalbuminemia, peritoneal dialysis). An exudate on the contrary is high on protein, LDH,

cholesterol, has a high specific gravity and has a cloudy appearance. It is usually caused by an increased vascular permeability (e.g. pneumonia, malignancy, pulmonary embolism). The Light criteria are used to differentiate transudate from exudate. In almost all cases malignant pleural effusions are exudates with high protein and LDH levels, although very occasionally, e.g. in cases of carcinomatous lymphangitis, they temporarily may have characteristics of a transudate (Fernandez et al., 2000; Thomas & Musani, 2013).

Malignancy can cause an imbalance between pleural fluid production and absorption directly or indirectly. Direct causes are an increased capillary permeability due to tumour implants or inflammatory response and/or a decrease in lymphatic drainage due to lymphatic obstruction caused by a tumour. Indirect causes are the afore mentioned increased hydrostatic pressure and/or decreased oncotic pressure or an increase in negative intrapleural pressure caused by atelectasis or trapped lung. A vascular obstruction (e.g. superior vena cava syndrome) or chylothorax can be cause of the imbalance as well (Arber, Clackson & Dargan, 2013; Mueller & Marcher, 2014; Thomas & Musani, 2013).

1.2 Molecular background of malignant pleural effusion production

Since the rise of molecular biology during recent years, several mechanisms have been outlined that explain the different tumour-host interactions. Although occurring often at the same time as part of the signalling network, these can roughly be divided in 3 mechanisms. Firstly, molecules like interleukin-2 (IL-2), tumour necrosis factor (TNF) and interferon (INF) stimulate pleural inflammation. Secondly, tumour angiogenesis is stimulated by molecules like angiopoietin 1 & 2 (ANG-1 & ANG-2). A third group of molecules influence vascular hyperpermeability (e.g. vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMP), osteopontin (OPN), etc.) (Skok et al., 2019). These factors or a combination of them can all lead to the development of excess pleural fluid.

1.3 Epidemiology

About 15% of all cancer patients develop a malignant pleural effusion (Skok et al., 2019). The majority (ca. 95%) of all malignant pleural effusions are caused by metastases in the pleural space, which are unfortunately very common (ca. 56 000 cases per year in Germany and ca. 375 000 – 400 000 in Europe) (Ried & Hofmann, 2013). Pleural metastatic disease can occur either by direct local tumour extension or by distant tumours with spreading via a haematogenous route. The types of cancer that metastasise to the pleura most often are lung cancer in men and breast cancer in women. These two cancers combined account for 50 - 65% of all MPE (Arber, Clackson & Dargan, 2013; Asciak & Rahman, 2018; Psallidas et al., 2016; Thomas & Musani, 2013). Together with lymphoma, genitourinary and gastric cancer, these malignancies account for 80% of all MPE. Histologically, adenocarcinoma is most frequently found (70-77%) (Egan et al., 2014; Kastelik, 2013; Ried & Hofmann, 2013; Skok et al., 2019). Even after thorough investigation, in about 10% of cases with MPE the primary tumour cannot be identified (Egan et al., 2014).

1.4 Symptoms

Dyspnea is the most common symptom, which may be disproportional to the volume of fluid present. Patients may also present with cough, sometimes a dry cough, an uncomfortable feeling in the chest, chest pain (especially in mesothelioma), and constitutional symptoms such as weight loss, generalized fatigue, malaise, and anorexia. Patients may feel shortness of breath during activity or at rest depending on the size of the effusion. Some patients with a small amount of pleural effusion may be asymptomatic, with the effusion only noticed on routine chest X-ray. Symptoms may arise gradually or with sudden onset. Symptoms of the underlying malignancy may also be present, such as haemoptysis, change in bowel habit or gastrointestinal bleeding. Occasionally paraneoplastic syndromes occur, such as the syndrome of inappropriate antidiuretic hormone

secretion, haematological, neurological, or renal abnormalities. On physical examination there will be decreased air entry, dullness to percussion, decreased vocal resonance, and decreased vocal fremitus over the pleural effusion. Signs of underlying primary malignancy such as breast lumps or palpable lymph nodes may be present (Arber, Clackson & Dargan, 2013; Asciak & Rahman, 2018; Kastelik, 2013).

1.5 Imaging

Often the posterior-anterior (PA) chest X-ray is the first imaging tool to establish the diagnosis and identify its extent. A pleural effusion of greater than 200 ml is routinely detected, and blunting of the posterior costophrenic angle has been reported to correlate with as little as 26 ml of pleural fluid. Effusions can be bilateral or unilateral. The chest X-ray is considered the first radiologic investigation of choice for patients with a presumed MPE, however nowadays further imaging is generally indicated to assess the characteristics of the effusion in more detail (Asciak & Rahman, 2018; Egan et al., 2014).

Over the course of the years, more and more focus has been put on the use of thoracic ultrasound (TUS). It is a fast and inexpensive diagnostic tool and it also does not expose the patient to radiation. Ultrasound is particularly helpful for critically ill or ventilated patients in the supine position, a situation in which chest X-ray is less sensitive. It can add valuable information about the size and depth of the pleural effusion, any contralateral pleural effusion, echogenicity, septations, loculated fluid, pleural thickening and nodularity, diaphragm position, and movement. Therefore, it is recommended that TUS is used as a guide for pleural procedures including thoracocentesis and chest drain insertion (Asciak & Rahman, 2018; Corcoran et al., 2018; Egan et al., 2014; Jany & Welte, 2019; Maskell, 2010).

Computed tomography (CT) scanning can be used to evaluate complex situations in which the anatomy cannot be fully assessed by plain chest X-ray or TUS. It can distinguish pleural fluid from pleural tissue proliferation, and it provides clues to

the potential causes of the effusion (pneumonia, cancer, pulmonary embolism). It contributes greatly to clinical tumour staging. Imaging criteria for distinguishing benign from malignant pleural changes have been prospectively validated, but chest CT cannot be used to distinguish pleural carcinosis from mesothelioma (Asciak & Rahman, 2018; Egan et al., 2014; Karkhanis & Joshi, 2012).

F-18 fluorodeoxy-glucose (FDG) positron-emission (PET) CT can be used for staging as well. Its use to differentiate between benign and malignant pleural diseases is limited by the false-positive results obtained from infection, inflammation, or after talc pleurodesis (Asciak & Rahman, 2018; Egan et al., 2014; Karkhanis & Joshi, 2012).

Magnetic resonance imaging (MRI) has a limited role but is superior in determining invasion of the tumour into the thoracic margins: chest wall, diaphragm and apex around the subclavian vessels (Egan et al., 2014; Scherpereel et al., 2020). It can be combined with PET to help with tumour staging.

1.6 Cytology

As stated before, to define a malignant pleural effusion, cytological or histological proof of malignancy is needed. Thoracocentesis is in almost any case the easiest way to obtain a fluid sample for further examination. This can be performed blind or under TUS or CT guidance. MPE can be diagnosed after the first cytological examination in about 60% of cases. If negative, sending a second pleural fluid sample can be sufficient for diagnosis of an additional 30%. The diagnostic yield is among others dependent on the primary tumour and tumour load, the quality of the fluid sample and its preparation and the experience of the cytologist. There is no consensus over the optimal quantity of fluid, but for a first analysis 20 to 40 ml should suffice. It is recommended that a second sample – if necessary – is larger than the first one, about 75 ml. Besides the direct smear examination, a cellblock preparation can be conducted as well. If both examinations are being carried out, a sample of 150 ml is recommended. If a lymphoma is suspected, flow cytometry

can give valuable information. Besides the diagnosis, cytology may be used to determine the therapeutic options for the patient (Asciak & Rahman, 2018; Bibby et al., 2018; Dixit et al., 2017; Psallidas et al., 2016).

1.7 Pleural biopsy

Malignant cells may not be discovered by cytology alone, thus in persisting suspicion a pleural biopsy is needed. The most frequently used method is video assisted thoracoscopic surgery (VATS), which has a sensitivity up to 95%. Besides getting a biopsy of the pleura, the VATS can at the same time be used for therapeutic interventions (discussed below). If this procedure is not possible, an ultrasound or CT guided percutaneous biopsy can be performed. Percutaneous biopsy, however, has a much lower accuracy (Arber, Clackson & Dargan, 2013; Aydin et al., 2009; Bhattacharya et al., 2012; Egan et al., 2014).

1.8 Management

Due to the poor prognosis of patients with MPE, treatment is focused on palliation of symptoms rather than cure. Many different management techniques are available.

1.8.1 Thoracocentesis

By first occurrence of the pleural effusion, a needle thoracocentesis is often conducted for both symptom treatment and for diagnostic purposes. In most cases though, the pleural effusion is recurrent and further treatment is necessary. Repeated thoracocentesis are restricted to patients in poor general conditions, who are not candidates for more invasive treatment modalities and have a slowly increasing amount of pleural effusion.

1.8.2 Pleurodesis

Pleurodesis creates a local inflammatory response, which causes the visceral and parietal pleura to fuse together and obliterates the pleural space by doing so.

Consequently, there is no room for a pleural effusion to accumulate. There are several agents and several techniques that can be used to generate the local inflammatory response. Independent of the chosen technique, success mainly depends of the capability of the lung to fully expand after pleurodesis in order to allow complete adaption of parietal and visceral pleura.

The most commonly used and preferred agent is talc or talcum. It was first used as a pleurodesis agent in 1935, and since then a lot of clinical research data has been accumulated. Talc is a trilayered magnesium silicate sheet. The talc used for intrapleural administration is free of asbestos and is sterilized effectively by dry heat exposure, ethylene oxide, and gamma radiation. After exposure to talc, there is an accumulation of fibroblasts in both the parietal and visceral pleura which causes the inflammatory response. In the early years, severe complications or even death occurred after talc pleurodesis. Complications included severe pain, fever, chock, acute respiratory distress syndrome (ARDS) or cardiac arrest. Most adverse effects were caused by microembolisation by the talc particles. Talc particles could be found in bronchoalveolar lavage (BAL) and an autopsy report even found talc particles in almost every organ in a patient who died shortly after talc pleurodesis (Campos et al., 1997; Wcrcbe et al., 1999). It became clear that both the amount of talc used as well as the particle size where responsible for the majority of complications. Talc particles with size of <10 µm are easily absorbed through the pleura and can so be transported over the bloodstream in nearly every organ. Since then, the consensus is reached to use talc with a large particle size and to not exceed 10g per pleurodesis attempt. There are two different ways to apply the talc in the pleural space: during a thoracoscopy as a poudrage, or as installation through a chest tube after mixing the talc powder with a saline solution and creating a so called slurry. Recent studies show no significant difference in success rates between poudrage and slurry (Aydin et al., 2009; Feller-kopman et al., 2018). A post-hoc analysis showed a survival benefit in patients with MPE who underwent

a successful pleurodesis compared to those who were refractory to the pleurodesis (Hassan et al., 2019).

Besides talc, a chemical pleurodesis can be performed using a wide variety of agents. Tetracycline is an antibiotic and is the most commonly used. It is mostly applied in the pleural space using a tube drainage, which is then clamped for 2 hours and released afterwards. Success rates found in literature vary greatly between 50% and 95%. Side effects include chest pain and fever. Because of the limited side effects, reasonable efficacy and low costs, it is still a widely used technique. Similar to tetracycline, the antibiotic doxycyclin can be used for chemical pleurodesis. However, success rates are much lower and the procedure often has to be repeated several times (Aydin et al., 2009).

Pleurodesis using a wide variety of antineoplastic agents has been investigated, but, with the exception of bleomycin, the sclerosing effect was to low and the side effects to high. Bleomycin, which is used in lymphomas or cancers of the head and neck, is installed in the pleural space the same way as tetracycline. Success rates are lower than tetracycline, ranging from 35% to 85%. Side effects are pain, fever or gastrointestinal complaints. Toxicity is rare because of the limited absorption. It is not recommended because of the high cost and relatively low effectiveness (Aydin et al., 2009).

1.8.3 Indwelling pleural catheter

An indwelling pleural catheter (IPC) is a soft silicone, multichannelled, 15.5 French tube which is inserted percutaneously into the pleural cavity. The catheter is tunnelled subcutaneous and is equipped with a polyester cuff which promotes tissue ingrowth to prevent ascending bacterial infections and holds the catheter in place. A one-way valve allows the intrapleural air and liquid to be removed when the catheter is connected with a vacuum bottle. The placement of the indwelling catheter is minimally invasive and can be performed as an inpatient or outpatient procedure. After placement, drainage is performed intermittently at the patient's

home by the patient, caretaker, or a district nurse, initially 2 to 3 times a week. Over the weeks, the fluid tends to decrease in volume and drainage frequency can be revised accordingly. In some cases, spontaneous pleurodesis occurs over time, which allows the catheter to be removed. The use of an IPC instead of pleurodesis is recommended in patients with non-expandable lung or after failed pleurodesis. Recent studies show no difference in effectiveness between IPC and pleurodesis to relieve dyspnea. However, IPC patients had shorter hospital stay, fewer admissions and fewer re-interventions. The choice of either technique depends on the case and patients wish (Asciak & Rahman, 2018; Boshuizen et al., 2017; Feller-kopman et al., 2018; Kastelik, 2013).

1.8.4 Pleuroperitoneal shunt

Another possible technique is a pleuroperitoneal shunt, by which the pleural fluid can transfer from the pleural space into the peritoneal cavity by means of either a manual pump (Denver shunt) or passive drainage (LaVeen shunt). It may be considered for patients with trapped lung who are not candidates for decortication, after failed pleurodesis, or for patients with refractory chylothorax, which allows for recirculation of chyle. Contraindications are pleural or systemic infection or if the patient had major abdominal surgery. Unfortunately, complications are common: shunt failure by clotting of the catheter or shunt infections, which both require re-surgery (Mueller & Marcher, 2014; Thomas & Musani, 2013).

1.8.5 Pleuro-venous shunt

A pleuro-venous shunt is a rare technique that is mainly used in benign pleural effusion. Due to potential seeding of tumour cells its value is very limited in patients with MPE (Artemiou et al., 2003; Bayram et al., 2008; Fremont et al., 2007).

1.8.6 Pleurectomy

A surgical parietal pleurectomy can be performed as VATS or via a small thoracotomy. Compared with the above mentioned techniques, it has an elevated morbidity and mortality risk. It is usually not indicated in patients with MPE, even though some authors report about its use after failed pleurodesis in a selected patient group with good performance status and expected survival of >6 months (Mueller & Marcher, 2014; Thomas & Musani, 2013).

1.8.7 Fibrinolytics

Patients with pleural effusion can form fibrinous adhesions within the pleural space, building septations with non-communicating chambers filled with pleural fluid. If there are multiple fluid collections, or the pleural fluid is divided into separate pockets, this is called a loculated pleural effusion. If, on the other hand, there is a pleural effusion within which fibrinous strands have built, this is called a septated pleural effusion. This makes initial drainage difficult and often incomplete. These fibrinous chambers can be destroyed either mechanically (e.g. videothoracoscopically) or by the use of a fibrinolitic agent such as urokinase. Although several studies have shown partly contradictory outcomes (Bibby et al., 2018) it is safe to say that in patients with MPE, the use of fibrinolitics may have an effect on the volume of fluid drainage and the radiological appearance in case of loculated effusions. However, there is no difference in the rate of success of pleurodesis or on the patient's dyspnoea when compared to placebo. Therefore, the use of fibrinolitics is not recommended, even though alternatives are limited for patients who are not suitable for surgery.

1.8.8 Management algorithm

In a collaborative effort from the American Thoracic Society (ATS), Society of Thoracic Surgeons (STS), and Society of Thoracic Radiology (STR), a management algorithm has been created for patients with known or suspected MPE. The

flowchart is seen in Figure 1. In asymptomatic patients, an intervention is only indicated for diagnostic purposes. In symptomatic patients, a TUS guided thoracocentesis is recommended for both diagnostic assessments and symptom relief. If there is no improvement of dyspnea, other causes should be investigated. If the effusion is refractory after initial thoracocentesis and the lung does not fully expand, an IPC is recommended. If the lung does fully expand, it is up to the patient's choice if an IPC is installed or a talc pleurodesis (either as poudrage or as a slurry) is performed. In patients who are not fit for Intervention or with a low expected survival (<1 month), palliation of symptoms (repeated thoracocentesis, oxygen, morphine) should be considered (Feller-kopman et al., 2018).

1.9 Primary pleural tumours

Although pleural metastases occur far more often, there are many types of primary tumours of the pleura, and all of them are rather uncommon. Based on the point of origin, these can be divided into 2 groups: mesothelial tumours and mesenchymal tumours. According to the 2015 classification of the World Health Organisation (WHO) of tumours of the pleura, the lymphoproliferative disorders should also be taken into account, but these will not be discussed in this dissertation (Galateau-Salle et al., 2016).

1.9.1 Mesothelial tumours

Diffuse malignant pleural mesothelioma (MPM) is by far the most common type of primary pleural tumour. There is also a localised form of MPM, which has a better prognosis then the diffuse counterpart, but it is extremely rare. The MPM will be discussed in more detail below (1.10 Malignant pleural mesothelioma). The well-differentiated papillary mesothelioma, which is seen more often in the peritoneum of young women, can rarely occur in the pleura. It is extremely rare and has low malignant potential. It has a high potential to spread, but local invasion is minimal or completely absent. Lastly, the adenomatoid tumour is a benign tumour that most commonly occurs in the genital tract, but is rarely seen in the pleura

(Galateau-Salle et al., 2016; Karpathiou, Stefanou & Froudarakis, 2015; Murali, Park & Leslie, 2010).

1.9.2 Mesenchymal tumours

First to mention is the solitary fibrous tumour of the pleura (SFTP), which is the commonest benign tumour of the pleura, even though it only occurs in about 5% of all pleural tumours. It is treated by en-bloc complete resection. In about 10% to 15% of all SFTP however, a malignant form occurs which shows local recurrence or distant metastasis. Before there was consensus over the origin of the malignant SFTP, there was a diversity of names being given to it (e.g. localized mesothelioma, solitary fibrous mesothelioma, pleural fibroma, etc.) which lasted for several decades. Next, the calcifying fibrous tumour is a benign disease which is treated by radical resection, although it may recur in about 10% to 15% of cases. The desmoid-type fibromatosis is a tumour that does not metastasize, but is rather aggressive locally as it tends to infiltrate in the soft tissue of the chest wall. Other mesenchymal tumours consist of the very rare but highly aggressive epithelioid hemangioendothelioma, angiosarcoma, synovial sarcoma and desmoplastic round cell tumour (Attanoos & Pugh, 2018; Galateau-Salle et al., 2016; Karpathiou, Stefanou & Froudarakis, 2015; Murali, Park & Leslie, 2010).

1.10 Malignant pleural mesothelioma

Diffuse MPM is related to exposure to mineral fibres in almost all cases, of which asbestos is the commonest. The aetiology is complex and not 100% clear, but the different types of asbestos fibres are all biopersistent in both humans and animals and all have carcinogenic potential. The malignancy can occur as soon as 15 years or as late as 67 years after exposure to asbestos, with a mean latency of 40 years. The higher the exposure to asbestos, the greater the risk of developing the malignant disease. At this point however, no threshold of cumulative exposure could be established below which there is no increased risk. In recent years, a mutation of the breast cancer-1 (BRCA-1) associated protein-1 (BAP-1) gene has

been identified, meaning a genetic predisposition is possible. This may be the case in patients with peritoneal mesothelioma, minimal asbestos exposure, young age or a second cancer diagnosis. Another risk factor for developing MPM is ionising radiation. MPM is associated with MPE in up to 95% of cases (Arber, Clackson & Dargan, 2013; Psallidas et al., 2016; Scherpereel et al., 2020).

1.10.1 Histopathological types

Histopathologically, MPM is divided in different types: epithelioid, sarcomatoid and biphasic (also called mixed form). Clinically, MPM is often split in 2 classes: epithelioid and non-epithelioid. The epithelioid and sarcomatoid types are divided in subtypes, based on the architectural patterns and their cytologic and stromal features. Some of these subtypes are important for their prognostic value, while others are just mentioned to help the pathologist with the diagnosis. The biphasic type has any combination of epithelioid and sarcomatoid patterns (at least 10% of each). Epithelioid MPM is the most common (50-80%) and least aggressive form with the best prognosis (median survival of 13 months). The sarcomatoid type is the most aggressive and has the worst prognosis of all (median survival of 4 to 6 months) (Nicholson et al., 2020; Skok et al., 2019).

1.10.2 Diagnosis

The diagnosis is rather difficult. Occasionally, cytological examination is sufficient to diagnose the rare malignancy. In most cases however, a biopsy with sufficient material of the parietal pleura is necessary. It is recommended to use immunohistochemical examination using both mesothelial markers to indicate MPM and related markers of other cancer types to exclude a non-MPM malignancy. The diagnosis of sarcomatoid MPM is particularly difficult, since tests for mesothelial markers are often negative. On top of that, there is a lack of specific antibodies that can differentiate between sarcomatoid MPM and other sarcoma-type tumours (Bibby et al., 2018; Fels Elliott & Jones, 2020).

1.10.3 Therapy

The evidence on options of MPM is limited, however recent guidelines provide guidance for appropriate treatment selection (Scherpereel et al., 2020). Due to its rarity mesothelioma patients should be treated in dedicated centres of excellence.

1.10.3.1 Surgery

The two main surgical techniques offered for patients with MPM are pleurectomy/decortications (P/D) and extrapleural pneumonectomy (EPP). In the former, the parietal and visceral pleura are stripped from the surface of the lung and fissures. If furthermore the diaphragm and pericardium of the ipsilateral hemithorax is removed and reconstructed, the technique is called extended P/D (eP/D). An EPP is essentially an extended P/D with additional removal of the ipsilateral lung. Complications include cardiac arrhythmias, myocardial infarction, hypotension, mediastinal shift, pneumonia, ARDS, bleeding and empyema (Kim, Sterman & Haas, 2019; Scherpereel et al., 2020; Woolhouse et al., 2018).

1.10.3.2 Radiotherapy

Radiation treatment is technically difficult, because it is hard to deliver an adequate dose of radiation to the pleura without damaging the radiosensitive lung or mediastinal structures. Radiotherapy alone has shown a lack of efficacy and has a high toxicity, and consequently has been abandoned. Therefore, radiation is mostly used as combination therapy after surgical EPP. On the other hand, radiation therapy can be used as palliation in patients who have tumour induced pain, dyspnoea, oesophageal symptoms or superior vena cava syndrome (Kim, Sterman & Haas, 2019; Scherpereel et al., 2020; Woolhouse et al., 2018). The Toronto group reported on good outcomes with neoadjuvant radiation according to the SMART protocol prior to EPP and is currently investigating its use prior to ePD (Cho et al., 2021).

1.10.3.3 Chemotherapy

Different types of chemotherapy have been used in treating MPM, but only the antifoliates pemetrexed or raltitrexed in combination with cisplatin have shown a significantly better overall survival (OS) (median OS of 13.3 months) compared to best supportive care. It also has a role in the palliative setting.

A relatively new therapeutic option is the local administration of a chemotherapeutic agent in the pleural space. The most performed technique is the *hyperthermic intrathoracic chemotherapy* (HITOC or HITHOC), in which the chemotherapeutic agent (mostly cisplatin) is heated to 42°C and perfused in the pleural space for 60 to 90 minutes. The reason why the chemotherapeutic agent is heated to 42°C, is because the membrane of tumour cells are destroyed at this temperature, whereas the same effect on healthy tissue is only reached in higher temperatures. The HITOC can be performed as monotherapy or in combination with the above-mentioned surgical techniques. Because the monotherapy has a primarily sclerosing effect with limited tumour response, it is mainly used in the palliative setting. When performed after cytoreductive surgery, effects are much higher. Although it can be performed after both (e)P/D as EPP, morbidity and mortality is significantly higher in the latter. Besides HITOC, the hypotonic intrapleural chemotherapy is an alternative technique, although it is performed far less frequently due to the lack of clinical data (McNamee et al., 2014; Ried et al., 2019; Zhou et al., 2017).

1.10.3.4 Immunotherapy

Several clinical studies have been conducted or are still in progress to look at the efficacy of immunotherapy in patients with MPM. Immune checkpoint inhibitors have been used as monotherapy in combination with chemotherapy and/or targeted therapies as well as in combination of two immune checkpoint inhibitors. Both their use as first-line therapy or as salvage therapy have been examined (Scherpereel et al., 2020). The MAPS-Trail (Mesothelioma Avastin Cisplatin

Pemetrexed Study) from 2016 showed that adding bevacizumab to a cisplatin/pemetrexed treatment resulted in a significant longer survival with only a mild increase in toxicity (Zalcman et al., 2016). In the NIBIT-MESO-1-Trail patients received a combination of tremelimumab and durvalumab as first-line therapy or second line after tumour progression was seen under platinum-based chemotherapy, showing encouraging results (Calabrò et al., 2018). The prospective, single-centre phase 2 trial INITIATE showed promising results of the combination of ipilimumab and nivolumab in patients which showed disease progress after at least one line of platinum-containing chemotherapy (Disselhorst et al., 2019). In the CheckMate 743 trial, a combination therapy of nivolumab plus ipilimumab in patients with non-resectable MPM showed a significant better survival compared to patients receiving standard-of-care chemotherapy (Baas et al., 2021). These are preliminary results and many validating studies are needed before these drugs are available for routine treatment.

1.10.3.5 Multimodality treatment

It has been shown that a monotherapy of any of the above-mentioned treatments has little or no effect on the OS of patients with MPM, which led to the introduction of a multimodality treatment for patients who are fit enough. At this point, there is no consensus yet about which combination of treatments is best and it is the focus of many current studies (Kim, Sterman & Haas, 2019; Scherpereel et al., 2020; Woolhouse et al., 2018). Studies in patients with sarcomatoid MPM demonstrated no benefit in survival after aggressive treatment, so palliation or best supportive care is recommended in these patients (Balduyck et al., 2010).

1.11 Biomarkers

A biomarker is defined as “a biological molecule found in blood, other body fluids or tissues that is a sign of a normal or abnormal process, or of a condition or disease” (National Cancer Institute, n.d.). It can be a protein (e.g. enzyme or

receptor), nucleic acid (e.g. micro RNA), antibody or peptide. It can almost always be relatively easily obtained noninvasively. The potential use of biomarkers is mainly threefold: it can be used for 1) diagnosis, 2) prognosis and/or 3) treatment outcome. Additional possible uses are screening, follow-up or individualising therapeutic interventions (Aronson & Ferner, 2017). A wide range of biomarkers in serum, plasma and/or pleural fluid has been studied. Many biomarkers have poor specificity and sensitivity, or the investigation of these markers is not widely available outside the research setting. Additionally, many studies have contradictory results, most likely due to variations in study measurement techniques or sample preparation, in study populations or other reasons due to a lack of standardisation in biomarker sampling. Because of this, the clinical use of these markers is limited at this time (Blyth, 2015). That being said, the following biomarkers have the potential to being useful in the future, but further research with a greater number of studies is required.

1.11.1 Inflammatory markers

Systemic inflammation is associated with worse morbidity and mortality in patients with malignant disease (Clive et al., 2014). The emergence of cancer is a complicated process, and in many cases there is an involvement of the inflammatory response in the cancer development. At the start of the inflammatory response, cytokines and other proinflammatory agents are released which activate the inflammatory cascade. When the cancer cells are not eradicated, a chronic inflammation may occur, which may help the cancer cells to survive and spread due to tissue damage and higher levels of reactive oxygen and nitrogen. Tumour cells themselves can also emit proinflammatory substances, which in their turn may help with angiogenesis, tumour growth, invasion, and metastasis (Allin & Nordestgaard, 2011; Lu, Ouyang & Huang, 2006). Inflammatory markers like C-reactive protein (CRP), white blood cells (WBC), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) are easily measured in the blood and are potential useful biomarkers. Since albumin is a negative acute-

phase protein, it is more of a marker of inflammatory status than of nutritional status, and therefore another possibly valuable biomarker.

1.11.2 Platelets

In recent years, mounting evidence suggests platelets contribute in all steps of tumorigenesis, including tumour growth, tumour cell extravasation and metastasis. Furthermore, platelets have been found to partake in protecting tumour cells against chemotherapy induced cell death and in maintaining the integrity of tumour vasculature. Since many inflammatory mediators are produced by and stored in platelets, they contribute greatly to the inflammatory response. Thrombocytosis also adds to the higher risk of deep vein thrombosis (DVT) and venous thromboembolism (VTE), especially in patients receiving chemotherapy (Haemmerle et al., 2019). The paper of Lin *et al.* states that thrombocytosis is to be seen as a paraneoplastic occurrence where malignant tumour cells produce cytokines that stimulate thrombocytosis, and the platelets are activated directly or indirectly by those tumour cells to promote tumour growth, creating a positive feedback loop (Lin, Afshar-Kharghan & Schafer, 2014). This makes platelet count a potential useful biomarker.

1.11.3 Haemoglobin

Low levels of haemoglobin (Hb) are not rarely seen in cancer patients. The mechanism is complex and often multifactorial. Besides treatment-related anaemia like chemo- or radiotherapy induced bone marrow suppression or blood loss in case of surgery, cancer itself can cause bone marrow destruction, blood loss, haemolysis or cytokine induced suppression of erythropoietin (EPO) production. Moreover, poor appetite and nutritional deficiencies lower blood levels of Hb even further (Abdel-Razeq & Hashem, 2020). It should not come as a surprise that Hb levels in the blood have been extensively researched as a possible valuable biomarker.

1.11.4 Lactate dehydrogenase

The more energy cancer cells have at their disposal, the faster they can grow. This is why cancer cells change their metabolism of glucose, which is the most efficient method of generating energy within a cell. Although the aerobic metabolism of glucose is far more efficient, it is much slower than the anaerobic metabolism. This is why normal cells, in particular skeletal muscles, use this pathway when the energy need is greater than the availability of oxygen. It has been found that cancer cells, even when oxygen is available, reprogram their metabolism to use the anaerobic pathway. This change is called the Warburg effect. Even though a lot of potential energy of glucose is wasted due to this change, the amount of energy produced per second is far greater. In addition, since there is no production of CO_2 and H_2O, there are more carbon molecules available to be used in the cell proliferation. Another positive side effect for cancer cells is the accumulation of lactic acid, which acidify the microenvironment, which in turn degrade the extracellular matrix and facilitate angiogenesis, tumour invasion and protect the cancer cell against attacks of the immune system. Since the last step of the anaerobic cycle is to convert pyruvate into lactate by the enzyme LDH, the production of LDH is upregulated in cancer cells. LDH is present in all human cells and is only released in the bloodstream due to cell damage. High levels of LDH in the blood mean a high level of cell injury. This can be caused by tumour cell destruction, but also non-malignancy related causes like haemolysis, infection, infarction, acute liver or kidney disease, shock and hypoxia can lead to elevated LDH levels in the blood. Levels of LDH can be measured in blood or pleural effusion quite easily and its use as a useful biomarker has been studied extensively (Gallo et al., 2015).

1.11.5 Malignant pleural mesothelioma specific molecular markers

A plethora of biomarkers was identified in serum, plasma, pleural fluid and exhaled breath that play a role in MPM. Many of the recently found biomarkers (e.g.

transforming growth factor-beta (TGF-β)) (Stockhammer et al., 2020) lack validation, but other markers like mesothelin, fibulin-3 (Fb-3), OPN and megakaryocyte potentiating factor (MPF) have been analysed in many studies (Arnold & Maskell, 2018; Panou et al., 2015). They are mostly used to potentially diagnose the disease, but data about survival prognosis and treatment outcome is being investigated as well. The problem with these markers and following studies – as stated before – is the large heterogeneity in study populations, different cut-offs being used and different measuring techniques. Due to this fact, although results show greater diagnostic accuracy than other tumour markers, they cannot be used as a sole diagnostic biomarker in clinical practice just yet. There are however studies being conducted in combining these markers with other biomarkers or variables. A more detailed discussion of the discovered biomarkers goes beyond the goal of this thesis, but are summarised in the *ERS/ESTS/EACTS/ESTRO guidelines for the management of malignant pleural mesothelioma* (Scherpereel et al., 2020).

1.12 Prognostic factors for malignant pleural effusion

Not all patients with pleural metastases develop a MPE. Those who do (about 55-60%) have a worse prognosis than those who do not. From the aspects of cancer staging, the presence of malignant pleural effusion usually defines advanced malignancy and therefore once it is diagnosed the median survival depending on the site of the primary neoplasm (shortest for lung cancer, longest for ovarian cancer) has been reported as 3 to 12 months (Asciak & Rahman, 2018; Egan et al., 2014; Kastelik, 2013; Mueller & Marcher, 2014; Skok et al., 2019). Beside the location of the primary tumour, the histological type also plays a role in the patient's prognosis. This is especially true in MPM, in which the epithelioid type has a better prognosis than non-epithelioid types. Another example is the poor prognosis of small cell lung cancer compared to lung adenocarcinoma or squamous cell carcinoma.

The TNM (Tumour – Node – Metastasis) classification of the Union for International Cancer Control (UICC) puts close to all patients with MPE in stage IV, which is the last and worst stage of the malignant disease. Exception to this is MPM, in which the TNM stage is independent of the presence of MPE. Studies have shown that in patients with MPM, pre-therapeutic CT measurement of tumour volume can predict pT, pN and OS (Rusch et al., 2016; Scherpereel et al., 2020).

Since the discovery that a chronic inflammatory process plays an important role in the formation and spread of cancer, researchers have looked for ways to measure its effects in the blood. This resulted in many studies being conducted about the use of serum CRP, NLR, PLR, platelets and so on, to predict survival in patients with malignancy. For patients with MPM, there have been several studies to look at the prognostic value of NLR. Results show great variation, and in a study of Meniawy *et al.* they compared their own data with that of 4 other large studies (Meniawy et al., 2013). They conclude that the cut-off value for NLR in those papers is variable and the independent predictive value shows too little consistency to be used in daily clinical practise.

Other known prognostic factors are the patients' performance status (PS), age, gender, smoking habits or parameters that reflect the tumour burden in the pleural space (pleural fluid pH, glucose, LDH, adhesions) (Dixit et al., 2017). Again, results vary strongly among studies, showing statistical significant prognostic values of the independent factors in some cases but not in others.

In an attempt to better predict the OS of patients with malignant disease, many research has been conducted in combining several biomarkers with or without clinical markers. This resulted in several prognostic scoring systems that can help in the decision making of several therapeutic options.

1.12.1 European Organisation for the Research and Treatment of Cancer prognostic score

Published in 1998, the European Organisation for Research and Treatment of Cancer (EORTC) devised a prognostic scoring system from pre-treatment patient data with MPM which were included in clinical trials of chemotherapy, and found 5 factors which had a negative predictive value on survival: poor PS, high WBC count (≥8,3 x 10^9/l), male gender, uncertain diagnosis of MPM and sarcomatoid histology (Table 1) (Curran et al., 1998). Even though low Hb (<1 g/dl lower than normal) showed a statistical significant effect on survival, it was not included in the prognostic index. Although the use of the EORTC prognostic score system has been validated by several research groups (Edwards et al., 2000; Fennell et al., 2005), its use hasn't been integrated in routine practice (Scherpereel et al., 2020).

1.12.2 Cancer and Leukemia Group B prognostic score

Published in the same year, the Cancer and Leukemia Group B (CALGB) devised a prognostic score for patients with MPM, which were in phase II clinical trials of chemotherapy (Herndon et al., 1998). Showing the bad prognostic effect of pleural involvement, serum LDH >500 IU/l, Eastern Cooperative Oncology Group (ECOG) PS >0, chest pain, dyspnoea, weight loss, platelet count >400 x 10^9/l, low Hb, high WBC count, non-epithelioid histology & age >75 years, they made a regression tree using some but not all variables. The regression tree has been adapted and is shown in Table 2. The CALGB prognostic score has also been verified in several studies (Edwards et al., 2000; Fennell et al., 2005), but isn't used in daily practice due its complexity (Scherpereel et al., 2020).

1.12.3 Glasgow prognostic score

For decades, it was known that involuntary weight loss and the resulting cachexia was linked to increased morbidity and mortality in cancer patients. After the discovery that, besides the loss of appetite and the resulting decrease in food

intake, a chronic inflammatory process was partially responsible for this phenomenon, Professor Donald C. McMillan published a paper in 2008 in which he looked at the serum levels of albumin and CRP to create the Glasgow prognostic score (GPS) (McMillan, 2008). Both serum levels of albumin <35g/dl and CRP >1.0 mg/dl were allocated points, and the higher the resulting score, the worse the patient's prognosis (Table 3). This simple scoring system was not just easy to use but it also required just a minimum of laboratory results. In the following years after its discovery, many studies have validated its prognostic use in almost any kind of cancer. After observing that isolated hypoalbuminemia was not associated with poor survival and that it was rarely seen without elevated CRP, the modified GPS (mGPS) was created (Table 3). Many studies since then have confirmed the better prognostic value of the mGPS compared to the GPS in most types of cancer, including MPM (Woolhouse et al., 2018). Later, the high-sensitivity mGPS (HS-mGPS) was created in an attempt to optimise the prognostic ability even further (Table 3). However, the superiority of the HS-mGPS compared to the mGPS was only confirmed in a handful of cancer types (Chen et al., 2017; Hanai et al., 2018; Nakamura et al., 2015; Osugi et al., 2016; Takeno et al., 2014). Currently it is also examined if the mGPS can be used as a prognostic tool in non-malignant diseases (McMillan, 2013).

1.12.4 LENT score

In the year 2014 Clive *et al.* published the results of a prognostic score they created for patients with MPE (Clive et al., 2014). They looked at 14 variables that were measured at the time of diagnosis of the MPE. They found that a combination of pleural fluid **L**DH, **E**COG PS, **N**LR and **t**umour type formed a good prognostic score in this patient population. As seen in Table 4, the scoring system puts the patient in a low, moderate or high risk group with a median survival of 319 (228 – 549), 130 (47 – 467) or 44 (22 – 77) days respectively. Not only did the research group come up with a scoring system, they also validated it on a different cohort of 76 patients with MPE, showing similar results. This relatively simple

scoring system looks promising, but more validating studies are required before it can be put to everyday practice.

1.12.5 Zurich Multimodality Prognostic Score (MMPS)

The Zurich research group of Opitz *et al.* looked at their patient population with MPM which received a multimodal therapy consisting of induction chemotherapy followed by EPP (Opitz et al., 2015). They made a prognostic score, allocating 1 point to any of the following variables: pre-treatment (before chemotherapy) tumour volume >500ml, pre-treatment serum CRP >30 mg/l, non-epithelioid histology in pre-treatment biopsy and progressive disease according to modified Response Evaluation Criteria in Solid Tumours (RECIST) criteria. A higher score means a lower predicted survival. Validating studies have yet to be conducted.

1.12.6 Brims decision tree analysis score

The research group of Brims *et al.* published a classification and regression tree (CART) analysis of the interaction of multiple variables with a given outcome for patients with MPM (Brims et al., 2016). Retrospectively using the data of 482 patients with MPM, they looked at 29 variables and ultimately designed a model that included weight loss (the most significant parameter), serum Hb and albumin, ECOG PS and histological type. This resulted in patient separation into 4 groups, ranging from predicted median OS of 34 months for group 1 to 7.4 months for group 4 (Table 5). They validated the scoring system prospectively on a cohort of 174 patients. The model had a sensitivity of 94.5% and positive predictive value of 76% for predicting death at 18 months. According to the European respiratory society (ERS) and the European Association for Cardio-Thoracic Surgery (EACTS), the Brims decision tree is the most useful tool to predict survival in patients with MPM at this point (Bibby et al., 2018). That being said, in the recent literature there has only been 1 paper that validated the use of Brims decision tree, in which Fraser Brims himself was the co-author (Harris et al., 2019).

1.12.7 PROMISE score

In an attempt to find biomarkers for the prediction of survival and pleurodesis success in patients with MPE, Psallidas *et al.* screened 17 potential biomarkers for survival and 7 for pleurodesis success which were prospectively gathered in a total of 502 patients spread over 3 independent datasets (Psallidas et al., 2018). They found no marker that predicts pleurodesis success with statistical significance. They did find 8 biomarkers that were used to create a survival scoring system: Hb, CRP, WBC, ECOG PS, cancer type, previous chemotherapy or radiotherapy and pleural fluid tissue inhibitor of metalloproteinases 1 (TIMP1). The biological PROMISE score includes all 8 variables, whereas the clinical PROMISE score removes TIMP1 from the equation, since this last factor is not routinely available in most patients. The scoring system predicts patient death in 3 months by putting them in 4 groups, ranging from a risk of <25% for group A to a risk of >75% for group D (Table 6). The results were validated by a different cohort of 162 patients in the same study. When comparing the PROMISE with the LENT score, the PROMISE score performed better. Up to this point, the PROMISE scoring system has not been validated by any other research group.

2 AIM OF THE STUDY

Primary aim of this retrospective study of prospectively collected biosamples and corresponding clinical data was to analyse the impact of biomarkers on overall survival of patients with malignant pleural effusion due to primary or secondary pleural malignancies excluding lung cancer.

3 MATERIALS AND METHODS

3.1 Patient collective

Two cohorts of patients were included in this retrospective study. Observation period was July 2016 to March 2020. The first cohort (MPM cohort) consisted of patients with pathologically confirmed MPM presenting with an ipsilateral MPE. The second cohort (extrathoracal malignancy (ETM) cohort) consisted of patients with a MPE of which the primary tumour was any other than lung cancer or MPM. All patients underwent an operative treatment of the MPE in the Department of Thoracic Surgery of the Ruhrlandklinik, Essen (Germany). The study was approved by the Ethics Committee of the Medical Faculty of University Duisburg-Essen (approval number 17-7931-BO) and complied with the Declaration of Helsinki.

3.2 Exclusion criteria

Exclusion criteria of this study were the following: 1) younger than 18 years of age; 2) no history of malignancy other than lung cancer or no newly diagnosed MPM; 3) no samples of the MPE or peripheral blood; 4) no cytological proof of malignant cells in the pleural effusion in the ETM cohort or no histological proof in the pleura in the MPM cohort.

3.3 Collected data

Epidemiological and clinical data of patients (age, gender, histology and primary tumour site, treatment of primary tumour, chest radiograph images, and clinical manifestations), laboratory findings of patient serum (CRP, WBC, LDH, Albumin, Hb, red blood cell count (RBC), haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count and mean platelet volume (MPV)), cytology of the pleural effusion, outcome of pleurodesis, and patient survival (measured in days since the diagnosis) were routinely registered. The mGPS was calculated from the collected data.

The final study population included 116 patients: 71 (61%) in the MPM cohort and 45 (39%) in the ETM cohort.

3.4 Statistical analysis

OS was defined as the number of days passed since the date of diagnosis of the MPE until the day of death or last contact. OS was calculated using the Kaplan-Meier method and a log rank test was used to calculate survival differences between two groups. We also calculated the median OS, hazard ratio (HR) and 95% confidence intervals (CIs). We used the Cox regression model to perform the multivariable survival analyses. For metric variables, normality was tested using the Shapiro-Wilk test. In case of non-normally distributed data, a Spearman correlation was computed. A Kruskal-Wallis test was used to compare each biomarker with the mGPS in case of non-normality, a Brown-Forsythe test was used in case of normality. Results were considered statistically significant if $p<0.05$, two-sided. Softwares GraphPad Prism 8.4.3 (GraphPad Inc.) and SPSS Statistics 23.0 package (SPSS Inc) were used to perform all calculations.

4 RESULTS

4.1 Clinicopathological & histopathological characteristics

In the MPM cohort, 62 (87%) patients were male and 9 (13%) were female. The median age was 75 years (range 53-94 years) at the time of diagnosis. The side distribution was 21 left (30%), 49 right (69%) and 1 bilateral (1%) (Table 7). Fifty-two (73%) cases were diagnosed with epithelioid, 6 (8%) with sarcomatoid and 13 (18%) with biphasic MPM (Table 8).

In the ETM cohort, 17 (38%) patients were male and 28 (62%) were female. The median age was 58 years (range 21-79 years) at the time of diagnosis of the primary cancer, 64 years (range 27-81 years) at the time of MPE diagnosis. The side distribution was 21 left (46.5%), 17 right (38%) and 7 bilateral (15.5%) (Table 7). Most common malignancy in this cohort was breast cancer (n=19, 42%), followed by sarcoma (n=5, 11%). Other malignancies included malignant melanoma, ovarian cancer, head & neck cancer (3 patients in each group, 7%), colorectal cancer, pancreatic cancer, thyroid cancer (2 patients per group, 4%), endometrial cancer, gastric cancer, renal cell cancer, Merkel cell carcinoma, carcinosarcoma and T-cell acute lymphoblastic leukaemia (T-ALL) (each with one patient, 2%). All data is listed in Table 9 and Figure 2.

4.2 Management & treatment

The MPE management was as follows in the MPM cohort: 50 patients (70%) received an IPC, 7 (10%) underwent talc pleurodesis, 1 patient (1%) got both IPC and talc pleurodesis, 13 patients (18%) received a 24 French chest tube (Table 7). Treatment of the MPM consisted of a trimodal therapy (chemotherapy and radiotherapy followed by surgery) in 3 patients (4%), bimodal therapy (chemotherapy followed by surgery) in 14 patients (20%), chemotherapy alone in 34 patients (48%) and surgery alone in 1 patient (1%). Twelve patients (17%) received best supportive care. Eight patients (11%) where lost to follow-up or

received treatment elsewhere. In case surgery was performed, an eP/D was the most frequent procedure (n=9, 50%), followed by P/D (n=4, 22%), eP/D + HITOC (n=2, 11%), EPP (n=2, 11%) and local resection (n=1, 6%) (Table 8).

The MPE management was as follows in the ETM cohort: 31 patients (69%) received an IPC, 6 (13%) underwent talc pleurodesis and 4 patients (9%) received both IPC and talc pleurodesis. Four patients (9%) were treated with a 24 French chest tube (Table 7). The types of treatment of the primary tumour were diverse and are listed in Table 10.

4.3 Survival

The median overall survival in the MPM cohort was 354 days (range 18-1407 days). Thirty-day survival was 96% and 90-day survival was 77% (Table 7).

In the ETM cohort, the median overall survival was 200 days (range 9-1339 days). Thirty-day survival was 89% and 90-day survival was 64% (Table 7).

4.4 Correlations between different biomarkers

We took the most frequently studied biomarkers and looked if there was a correlation between them, namely CRP, WBC, LDH, albumin and platelets.

In the MPM cohort, we found statistically significant correlations between CRP and WBC, albumin and platelets (p-value of 0.011, <0.001 and 0.015, respectively). We further found a correlation between WBC and LDH as well as platelets (p-value of 0.007 and <0.001, respectively). Other correlations were not statistically significant and are shown in Tables 11 & 12.

In the ETM cohort, a statistical significant correlation was found between CRP and WBC and LDH (p-value of 0.025 and 0.015 respectively), as well as between WBC and LDH and platelets (p-value 0.011 and <0.001). Other correlations were not statistically significant and are shown in Tables 13 & 14.

Next, we calculated the composite score mGPS and looked if there was a correlation between mGPS and WBC, LDH or platelets in both cohorts. In the MPM cohort, we found a statistically significant correlation between mGPS and WBC as well as between mGPS and platelets (p=0.012 and 0.026, respectively). When dividing the mGPS in its subgroups and comparing those with each other, mGPS 0 vs 1 had significant results in both WBC and platelet groups (p-value of 0.010 and 0.038 respectively). All data is shown in Table 15. When performing the same analysis in the ETM cohort, no significant correlation was found in any groups, although there was a tendency to significance when comparing mGPS with WBC (p=0.076), more particularly when comparing mGPS 0 vs 1 (p=0.080) (Table 16).

4.5 Prognostic power of circulating biomarkers

We analysed differences in OS for each biomarker. All results are listed in Tables 17 & 18, all statistically significant results are visualized in Figures 3 & 4.

In the MPM cohort, patients who had a serum CRP of lower than 0.5 mg/dl at the day of diagnosis of the MPE had a significantly better survival compared to patients who had a higher serum CRP level (median OS of 690 days vs 304 days respectively, p=0.048). If we did the same comparison with serum CRP of lower than 1.0 ml/dl vs ≥1.0 mg/dl, the results were even more significant (median OS of 585 days vs 249 days, respectively, p=0.005).

In the ETM cohort, patients who had a serum CRP of lower than 0.5 mg/dl at the day of diagnosis of the MPE also had a significantly better survival compared to patients who had a higher serum CRP level (not reached vs 193 days respectively, p=0.021). If we did the same comparison with serum CRP of lower than 1.0 ml/dl vs ≥1.0 mg/dl, the results were again even more significant (median OS of 780 days vs 104 days, respectively, p=0.009).

When we analysed the WBC, a range between 4.0 and 9.2 x 10^9/l was considered normal. In both cohorts, there was no significant difference in OS when comparing

patients with a normal WBC with patients who had either leukopenia or leukocytosis (median OS of 318 days vs 398 days respectively, p=0.892 in the MPM cohort and median OS of 402 days vs 172 days respectively, p=0.058 in the ETM cohort).

A serum LDH of ≤247 U/l was taken as reference, since it is the laboratory reference at our centre. In both MPM and ETM cohorts, there was no significant difference in survival when comparing low with high LDH serum levels (median OS of 387 days vs 206 days respectively, p=0.253 in the MPM cohort and median OS of 290 days vs 98 days, respectively, p=0.297 in the ETM cohort). Based on references in the literature, we took a second cut-off value of 500 U/L. None of the patients in the MPM cohort reached such serum levels. In the ETM cohort, survival was significantly worse in patients with serum LDH > 500 U/L compared to lower values (median OS of 31 vs 270 days, respectively, p=0.009).

Using 3.5 g/dl as reference, hypoalbuminemia associated with a significantly worse outcome in both cohorts with a median OS of 118 days vs 344 days (p=0.003) in the MPM cohort and median OS of 50 days vs 290 days (p=0.016) in the ETM cohort.

When looking at the serum platelet count, OS was not significantly different in patients with platelets between 150-350 x 10^9/l compared with those who had thrombocytopenia or thrombocytosis in the MPM cohort (median OS of 344 days vs 254 days, respectively, p=0.193). However, we did find a significantly better prognosis in the ETM cohort in patients with a normal platelet count (median OS of 76 days vs 444 days respectively, p<0.001).

Using a cut-off of 10 g/dl for Hb, patients in the MPM cohort with a lower value had a significant worse survival than those with higher levels (median OS of 71 days vs 344 days, respectively, p<0.001). We did not find similar results in the ETM cohort (median OS of 63 days vs 270 days, respectively, p=0.249).

The lower the mGPS, the better the OS in both the MPM and the ETM cohorts (p-value of <0.001 and 0.044, respectively). When comparing mGPS 0, 1 and 2 with each other, those values were statistically significant in every group in the MPM cohort. They were only significant when comparing mGPS 0 vs 2 in the ETM cohort. All results are listed in Tables 19 to 22.

Using the Kaplan-Meier method, we also calculated the OS using serum RBC, haematocrit, MCV, MCH, MCHC and MPV. All results are listed in Tables 17 & 18.

Although not a biomarker, we looked in the MPM cohort if patients who underwent curative intended surgery had a better survival than those who did not. As expected, survival was significantly better (p=0.004) with a median survival of 763 days in surgically treated patients vs 274 days in the other group (Table 23).

In order to define independent prognostic factors, hazard ratios and 95% confidence intervals were calculated by a multivariate cox regression model. In the MPM cohort low Hb and high mGPS were statistically significant independent predictors for short survival (Table 24). In the ETM cohort, only abnormal platelet count was a significant independent predictor for poor survival (Table 25).

5 DISCUSSION

In the majority of cases, MPE is caused by metastasis of a distant tumour, which greatly worsens the patient's prognosis. In case of primary malignancy of the pleura, MPM is by far the commonest disease, which also has a bad prognosis. In the last few decades, much research has been performed in the discovery of molecular markers which help in the diagnosis, prognosis and disease follow up for all kinds of malignancy. We looked at biomarkers of the blood which were easily obtainable and are present in the routine blood test that all patients receive at admission in our centre. Since the pathogenesis is different, we divided our patient collective in 2 cohorts: one with primary malignancy of the pleura (the MPM cohort) and one with pleural metastases of any kind of malignancy besides lung cancer or MPM (the ETM cohort).

5.1 Inflammatory markers

Correlation between blood levels of CRP and WBC count in both cohorts confirms earlier findings: in many cases the inflammatory cascade is partially responsible for the development of malignancy, its survival and its spread. Increased CRP and WBC count suggests a chronic inflammation may be present in these patients.

In the literature, both high CRP and low albumin have been shown to be associated with worse outcome in patients with cancer. Moreover, a combination of both markers has a better prognostic value than each marker on its own. Since the creation of the GPS in 2003, it has been the subject of many studies. Its prognostic value has been validated in almost every type of cancer, and has been modified in the mGPS and even HS-mGPS updates of the scoring system. There is unfortunately only 1 paper in recent literature in which Pinato *et al.* prove the prognostic role of mGPS (as for NLR) in patients with MPM (Pinato et al., 2012). In an earlier paper by Kao *et al.* in which the prognostic value of NLR in MPM patients is the main subject, the mGPS is briefly mentioned as it is measured in a small subgroup of patients receiving thalidomide alone or in combination with

chemotherapy (Kao et al., 2010). In these patients, mGPS could not be used as an independent predictive factor for prognosis. Since there was close to no difference between GPS and mGPS in both cohorts in our study, we decided to use the – currently in the literature recommended – mGPS for our calculations. The univariate analysis of our data shows that in both cohorts a higher serum CRP, a hypoalbuminemia, or a higher mGPS are all associated with a shorter OS. Since the mGPS is a combination of CRP and albumin levels in the blood, we used the mGPS instead of both single markers to further examine our data. In the multivariate analysis however, mGPS was a significant independent prognostic factor only in the MPM cohort. Accordingly, our data confirms the one study of Pinato *et al.* that the mGPS can be used as a prognostic factor in patients with MPM. The multivariate analysis of the ETM cohort however does not confirm the mGPS as an independent prognostic factor. A possible explanation is that the ETM cohort is rather heterogeneous. Most patients were diagnosed with MPE several months or years after diagnosis of their primary malignancy. Most of those patients received some kind of therapy during this time. In contrast, in 7 patients in this cohort the MPE was diagnosed at the same time as the primary tumour or less than 6 months after. This means that the blood samples of these patients were either pre-, mid- or post-treatment shortly after treatment termination. In a paper of Chang *et al.* they measure the GPS before and directly after concurrent chemoradiotherapy in patients with advanced head and neck cancer (Chang et al., 2017). Next, they split the patients into three groups according to the change in GPS before and after treatment: improved, conserved and worse. The univariate analysis shows that both the pre- and post-treatment GPS as well as the change in GPS was prognostic for both patients OS and recurrence free survival (RFS) in this patient group. The multivariate analysis, however, showed only a statistical significant result for the GPS after treatment for both OS and RFS. Additionally, the tumour stage showed independent significant results as well, which was not the case for age, gender, tumour site or ECOG PS. Limitations to this study was the relatively small number

of patients and that it was retrospective. Another study, conducted by Nishida *et al.*, calculated the GPS before and during concurrent chemoradiotherapy in patients with cervical cancer (Nishida et al., 2015). The multivariate analysis showed that both pre- and mid-treatment GPS was statistically significant for OS, but not for disease free survival (DFS). Interestingly, the p-value of the GPS mid-treatment was lower and the hazard ratio higher compared to the pre-treatment GPS, suggesting that the mid-treatment values are superior to the pre-treatment ones. The fact that this study was also retrospective with a relatively small number of patients accounts to the limitations of this study. Lastly, in a recent study of Kasahara *et al.* including patients with advanced non-small-cell lung cancer (NSCLC) who had been treated with antibodies to programmed cell death-1 (PD1), they measured the GPS before treatment and 1 month after treatment had begun and looked at both OS and progression free survival (PFS) (Kasahara et al., 2019). They found a significant association between both OS and PFS when using the GPS after treatment had started, as for the NLR at this time. This was not the case for pre-treatment GPS. Once again, limitations to this study were that it was retrospective and included a relatively small number of patients. Moreover, the duration of the immunotherapy was variable, ranging from 1 cycle to as much as 30 cycles. This means that in some patients, the GPS was measured when the treatment was still ongoing, whereas in others the treatment was already stopped.

The GPS and mGPS have been extensively validated in multiple studies when measuring the values prior to therapy. These last three studies, however, suggest that the mid- or early post-treatment (m)GPS are even superior in predicting survival. This means that the (m)GPS can potentially be used for both treatment decision making and treatment outcome prediction. Our data suggests that the timing of the measurement is crucial and that the (m)GPS on its own has limited value when measured at an unspecific time in the course of disease. More research is warranted, but measuring the (m)GPS before and during or shortly

after treatment may provide useful information for both clinicians and patients with malignancy.

5.2 Lactate dehydrogenase

We observed a correlation between LDH and WBC in both cohorts, suggesting an influence of (chronic) inflammation to cell destruction and the following release of LDH in the bloodstream. Since there was, however, no correlation between LDH and albumin in either cohort, there was also no correlation between LDH and the mGPS. A possible explanation is that albumin is not only a negative acute-phase protein, but also partially mirrors the nutritional status of the patient. More research is warranted to dissect the factors leading to albumin changes. The lack of correlation between CRP and LDH in the MPM cohort can be explained by the relatively low levels of LDH overall in the MPM cohort (maximum LDH level of 357 U/L). These were greatly higher in the ETM cohort (2836 U/L), hence the significant correlation between high CRP und LDH in this group.

As stated before, tumour cells change from an aerobic to an anaerobic metabolism and thus they produce more LDH. At this point, LDH levels are being used to help the prognosis for patients with breast or testicular cancer (Forkasiewicz et al., 2020). In Wilms tumour, it is used for both the diagnosis as for monitoring the therapeutic response. Total LDH activity is increased in most tumour tissues, but not all. LDH itself exists in 5 major isoenzymes, numbered LDH-1 through LDH-5. The type of LDH isoenzymes is largely tissue-specific. LDH-5 (also called LDHA) is primarily present in cells with a strong anaerobic metabolism, like skeletal muscle cells, whereas LDH-1 (also called LDHB) is favoured in tissues with a strong aerobic metabolism. In most cancer types, there is an over-expression of LDH-5 (Forkasiewicz et al., 2020; Gallo et al., 2015). Measurements of these isoenzymes have been the focus of many studies, but will not be discussed here.

In a meta-analysis of Zhuo *et al.*, 9 studies were used to determine the predictive value of pre-treatment serum LDH in patients with MPM. With a cut-off of 500 U/l

used in the majority of studies, it was shown that pre-treatment levels higher than this had a poor OS (Zhuo et al., 2016). Since all of our patients in the MPM cohort had a serum LDH of <500 U/l, we could not verify this result. Why LDH levels were this low in our cohort, is unclear. Since we had patients at all disease stages with both high and low tumour load, with and without metastases, this cannot be the explanation. Although there are many papers about using serum levels of LDH in patients with metastatic malignancy, there are surprisingly little studies found about patients with MPE specifically. Most of these studies look at the LDH levels of the pleural effusion, sometimes comparing them with serum LDH levels to create an index. Those studies show that high levels of pleural fluid LDH or a pleural fluid/serum LDH index of >1 may be prognostic for a worse OS, although several studies show contradictory results (Domej et al., 2000; Mehmet & Çakmak, 2010; Nicholas Dias, Yung Peng, 2017). This is why pleural fluid LDH is part of the LENT score (Clive et al., 2014). Since we did not measure pleural fluid LDH in this study, we cannot discuss those results in this dissertation. When looking at papers about metastatic disease in general, the cut-off values of serum LDH vary greatly. Since normal values of LDH are laboratory specific, many authors use the term *upper limit of the normal* (ULN) instead of numeric values. Some use this ULN as cut-off, whereas others use 1.5 or 2 times the ULN. Some authors, however, stick to numeric values, which can range from 180 to 800 U/L as cut-off values. We decided to use both our own ULN - which is 247 U/L - and 2 x ULN, which is roughly 500 U/L. We saw no difference in OS which was statistically significant when comparing levels below and above the ULN. However, we found a significant worse OS in ETM patients with LDH > 500 U/L, but it was not an independent prognostic factor in the multivariate analysis.

5.3 Platelets

A correlation between platelets and WBC or mGPS in the MPM cohort is in line with the finding that platelets may contribute to the inflammatory response. In contrast, in our ETM cohort there was no statistically significant correlation

between platelets and CRP, albumin and mGPS. A possible explanation could be, as we stated before, that the point in time of the mGPS measurement is crucial to its potential use.

As was the case for the prognostic properties of LDH, there are many studies confirming the prognostic value of platelet count in almost every kind of cancer with metastatic disease. Some authors look at platelets only, while others look at platelet-based inflammatory indicators like MPV, platelet to lymphocyte ratio (PLR) or the platelet x C-reactive protein multiplier value (P-CRP). Cut-off values are variable, so we decided to use the normal values of our own laboratory, which was 150-350 x 10^9/l. Although many studies can be found about the prognostic value of platelets in patients with metastatic disease in general, there are very few papers to our knowledge specifically looking at patients with MPE. In a retrospective study of Ghanim *et al.* they studied the programmed death-ligand 1 (PD-L1) tumour proportion score and Ki-67 index in pleural biopsies or cytologies in patients with MPE. In addition, they looked at the impact of CRP and platelet count. Using a cut-off value of 400 g/l, they showed that an elevated platelet count was significantly associated with poor OS. The multivariate analysis, however, could not confirm this as an independent variable. Moreover, they found that patients with metastases outside of the chest wall had even higher platelet values (Ghanim et al., 2020). Although limited research is available about the prognostic value of platelets in patients with MPE in general, several studies have been conducted about MPE patients specifically with lung cancer. A study of Lim *et al.* looks at prognostic factors of patients with stage IV lung cancer and MPE, particularly at a combination of platelet count and lymphocyte-to-monocyte ratio (LMR). They find that age, gender, histology, Hb (13 g/dl), platelet count (300 x 10^9/l), serum protein levels (6.7 g/dl), albumin (3.1 g/dl), CRP (2.68 mg/dl), LMR and the combination of platelet count and LMR (COP-LMR) show statistically significant differences in OS. In the multivariate test, they only look at COP-LMR instead of both separate values, which show significant results, as does age, gender, serum albumin and

CRP as independent prognostic variables (Lim et al., 2018). One year later, the same research group looked again at prognostic factors in a similar patient population. In the univariate analysis of different biomarkers, they find a statistically significant poor OS in patients with serum values of Hb <12 g/dl, platelets ≥300 x 10^9/l, albumin <3.1 g/dl, CRP ≥2.68 mg/dl or PLR ≥181.2. In the multivariate analysis, however, all but platelet count were statistically significant as an independent predictor for OS (Lim et al., 2019). In a study of Ohuchi *et al.* they investigated the involvement of platelet count and MPV in the prognosis and progression of lung cancer patients. They found several prognostic values in the univariate analysis, of which ECOG PS, CRP and platelet count are verified in the multivariate analysis as independent prognostic markers for survival. The cut-off being used for platelets is 244 x 10^9/l. They also found an association between platelet count and metastasis in bone, soft tissue, lymph node and MPE (Ohuchi et al., 2013). The research group of Kasapoglu *et al.* looked at possible prognostic factors in patients with NSCLC and MPE. Using a normal platelet count between 130 and 400 x 10^9/l, they did not find a statistically significant prognostic value of thrombocytosis or thrombocytopenia. They did find an independent negative prognostic value of MPE with additional distant metastasis, absence of chemo- and/or radiotherapy, increased blood CRP, decreased blood albumin and lower pleural fluid protein levels in the multivariate analysis in this patient group (Kasapoglu et al., 2016). Since our ETM cohort excluded patients with lung cancer, we cannot confirm these findings. However, we found an independent prognostic value of poor OS of an abnormal platelet count in all other patients with MPE except MPM.

Although several studies found a prognostic value of platelets in patients with MPM, we could not verify these results in our own MPM cohort. A possible explanation is the low number of patients with abnormal platelet levels in this cohort, i.e. 20, which is less than 30%. For comparison, in the ETM cohort more than 50% of patients had abnormal platelet levels. Another possible cause is the

fact that more than 70% of the patients in the ETM cohort received chemotherapy before the diagnosis of the MPE. Since we used the blood samples at time of the MPE diagnosis, we assume that in some patients the abnormal platelet levels are due to the chemotherapy. Besides other causes of thrombocytopenia in cancer patients like infiltration of the bone marrow, infection, graft versus host disease, liver dysfunction and other medication, chemotherapy is the major cause. Unfortunately, this chemotherapy-induced thrombocytopenia (CIT) is a frequent complication. It may be reversible after pausing or ceasing the chemotherapy, but the thrombocytopenia can be permanent as well. It does not just increase the risk of bleeding, it may lead to postponing the chemotherapy treatment, in dose reduction or in breaking off the chemotherapy altogether. All these factors result in a worse prognosis (Mones & Soff, 2019). On the other hand, thrombocytosis also has a negative effect on the survival prognosis in cancer patients. As mentioned before, there is evidence that platelets contribute in all steps of tumorigenesis and that they can offer protection to tumour cells. They also create a higher risk of DVT and VTE, especially in patients receiving chemotherapy (Haemmerle et al., 2019). Also, the thrombocytosis itself can in rare cases be caused by chemotherapy (Ahmed et al., 2012).

5.4 Haemoglobin

Several of the aforementioned scoring systems for patients with MPM use Hb in their calculation. In the CALGB prognostic score different values of Hb, <11.2 g/dl being the lowest, showed a worse prognostic outcome (Curran et al., 1998). In Brims decision tree analysis score the strongest predictor of poor survival was weight loss. In the group without weight loss, however, they found that the Hb at the time of diagnosis was predictive for survival, using Hb of 12.1 g/dl as the lowest value (Brims et al., 2016). Although not using it in their prognostic index, the EORTC group showed that low Hb (<1 g/dl lower than normal) had a statistically significant effect on OS in patients with MPM (Curran et al., 1998). The relevance of low Hb as prognostic factor in patients with MPM has been confirmed

in several studies. A study of Suzuki *et al.* found that patients with a good PS and Hb >12.1 g/dl had a statistically significant better prognosis (Suzuki et al., 2014). Similar results were found by Berardi *et al.*, showing that Hb levels below 13 g/dl as well as the increased plasma levels of the tumour marker CA 125 had a negative predictive impact on OS (Berardi et al., 2016). Pass *et al.* used the biomarkers of the EORTC prognostic score and added several extra plasma biomarker in an attempt to improve the prognostic results (Pass et al., 2016). They found that besides the EORTC prognostic score, Hb and log-osteopontin were independent predictors of OS. A study of Linton *et al.* verified many of the known prognostic markers, namely age, gender, histological subtype, platelet count (cut off value of 400 x 10^9/l) and haemoglobin level (difference of 1 g/dl from baseline), and added calretinin expression on the immunohistochemistry to the list of independent predictive factors for OS (Linton et al., 2014). Similar results were found by Kao *et al.,* who confirmed formerly validated markers like histological subtype, tumour stage and Hb level difference and added chemotherapy and NLR as the independent predictors of OS (Kao et al., 2013). We decided to use a cut-off value of 10 g/dl in our study, and confirmed in both the univariate and multivariate analysis that low values of Hb are an independent prognostic factor for worse OS in patients with MPM. Although the prognostic use of Hb in patients with MPE had been proven before (e.g. in the PROMISE score), this was not the case in our ETM cohort.

5.5 Prognostic scoring systems

The use of prognostic scoring systems can be useful on a population basis, but are rather imprecise when it comes to the individual patient level. A study of Abisheganaden *et al.* used the LENT score retrospectively on their patient population with lung carcinoma (Abisheganaden et al., 2018). They found that, especially in patients with adenocarcinoma and epidermal growth factor receptor (EGFR) mutation, the LENT score greatly underestimated the patient survival. Indeed, the therapeutic use of the tyrosine kinase inhibitor (TKI) has greatly

improved survival in such patients. The authors suggest that prognostic scores such as the LENT score need constant modification based on the rapid development of novel discoveries like mutational drivers and their corresponding treatments. Targeted and immunooncological therapies further diversify the toolkit needed to estimate prognosis in the future.

5.6 Limitations

The findings of this thesis have to be seen in light of several limitations. First, this study was a single-centre retrospective research, prone to institutional biases. The patient population consists only of patients undergoing diagnostic procedures or treatment for malignant pleural effusion at a thoracic surgical department. Secondly, the patient number was relatively small. This can be explained by the fact that we only used patients of our own institution. Thirdly, the patient population in our ETM cohort was divers, consisting of patients of which diagnosis of the primary malignancy was recent (<6 months) or at the same time as the MPE diagnosis, as well as patients that were diagnosed with the primary cancer a long time ago and received some kind of treatment. With some markers however, the time at which the sample is taken is crucial to be able to use it as a prognostic marker. Because the occurrence and diagnosis of MPE is independent of the primary malignancy in the majority of cases, this problem is difficult to avoid. In order to confirm our findings, prospective and/or multi-centre studies with a larger patient population are needed. In addition, as these biomarkers are often used in prognostic scoring systems, the scoring systems have to be constantly verified and possibly modified in order to keep up with recent development in both diagnostics and treatment. In conclusion, we can say that despite the large amount of data generated in countless studies, prospective studies and analyses of validation cohorts are needed before the use of biomarkers can be applied in everyday clinical practice. The fast pace of recent discoveries keep the subject relevant and exciting.

6 SUMMARY

In the majority of cases, malignant pleural effusion is caused by metastasis of a distant tumour and confers poor prognosis. In case of primary malignancy of the pleura, malignant pleural mesothelioma is by far the commonest disease, which also has a bad prognosis. In the last few decades, much research has been done to identify biomarkers that help the diagnosis, prognosis and disease follow up for all kinds of malignancies. Since the pathogenesis is different, we divided our patient collective in two cohorts: one with primary malignancy of the pleura and one with pleural metastases of extrathoracal malignancies. In this dissertation we comprehensively analysed the associations between several circulating biomarkers and their impact on overall survival.

We demonstrated the prognostic value of several blood based biomarkers, namely C-reactive protein (CRP), albumin, modified Glasgow prognostic score (mGPS) and haemoglobin (Hb) in patients with malignant pleural mesothelioma and confirmed the use of mGPS and Hb as independent prognostic factors for overall survival. These results verify previous studies. In patients with malignant pleural effusion with pleural metastases of extrathoracal malignancies, we demonstrated the prognostic power for overall survival of circulating CRP, albumin, mGPS, lactate dehydrogenase (LDH) and platelet count.

We believe it is important to look at patients with malignant pleural effusion in a carefully grouped manner. After all, the biomarkers being used in patients with malignant pleural mesothelioma are different from those of patients with metastatic disease. Some of the prognostic scoring systems try to incorporate this aspect. Our study indicates that a distinct set of biomarkers show prognostic power in patients with malignant pleural effusion depending on whether it is a primary malignancy of the pleura or pleural dissemination of an extrathoracal primary tumour.

7 ZUSAMMENFASSUNG

Ein maligner Pleuraerguss ist in den häufigsten Fällen durch Metastasen bedingt, welche mit einer deutlich verschlechterten Überlebensprognose einhergeht. Als häufigster primärer Malignom der Pleura gilt das maligne Pleuramesotheliom, welches auch eine schlechte Prognose hat. Zwecks Diagnosestellung, Prognose und Therapieverlauf von Patienten welche an einem malignen Pleuraerguss leiden, wurden in den letzten Jahrzehnten zunehmend laborchemische Biomarker erforscht. Zwecks Stratifizierung entsprechend der Pathogenese wurden im vorliegenden Studienkollektiv zwei Patientengruppen gebildet: eine an primärem Pleuramalignom Erkrankter und eine an pleuralen Metastasen eines von extrathorakalen Malignoms Erkrankter. In dieser Dissertation analysieren wir umfassend die Zusammenhänge zwischen verschiedenen Biomarkern und deren Einfluss auf das Gesamtüberleben.

Der prognostische Wert von verschiedenen aus dem Blut gemessenem Biomarkern (C-reactives Protein (CRP), Albumin, modified Glasgow prognostic score (mGPS) und Hämoglobin (Hb)) wurde in Patienten mit malignem Pleuramesotheliom bemessen, wobei mGPS und Hb als unabhängige Einflussfaktoren für das Gesamtüberleben identifiziert werden konnten. Diese Ergebnisse bestätigen vorangegangene Studien. Bei Patienten mit einem malignen Pleuraerguss eines extrathorakalen Tumors, konnte eine Korrelation mit Gesamtüberleben und zirkulierendem CRP, Albumin, Laktat Dehydrogenase (LDH), Blutplättchen und mGPS festgestellt werden.

Es ist von klinischer Relevanz Patienten mit einem malignem Pleuraerguss ihrer Tumorentität entsprechend zu betrachten, weil sich im Vorhandensein bestimmter Biomarker deutliche Unterschiede zwischen den Gruppen zeigten. Manche prognostischen Modelle versuchen diese Unterschiede zu berücksichtigen, wobei sich in der vorliegenden Auswertung ebenfalls zeigte dass bestimmte Konstellationen abhängig der Ätiologie einen prognostischen Wert haben.

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9 ATTACHMENT

Table 1: European organisation for research and treatment of cancer prognostic score (Curran et al., 1998).

Parameter	Good prognosis group	Poor prognosis group
ECOG PS	0	≥1
Histology	Epithelioid	Non-epithelioid
Gender	Female	Male
Certainty of diagnosis	Definite	Possible
WBC	$<8.3 \times 10^9$/l	$\geq 8.3 \times 10^9$/l

ECOG PS = Eastern Cooperative Oncology Group performance status; WBC = white blood cell count.

Table 2: Cancer and Leukemia Group B prognostic score (Herndon et al., 1998).

Variables	Group	Median survival (months)	95% CI (months)
PS = 0, age <49	1	13.9	11.1-31.4
PS = 0, age ≥49, Hb ≥14.6			
PS = 1-2, WBC <8.7, no chest pain	2	9.5	6.9-10.5
PS = 0, age ≥49, Hb <14.6	3	9.2	75-10.5
PS = 1-2, WBC <15.6, chest pain, no weight loss, Hb ≥ 12.3			
PS = 1-2, WBC 9.8-15.5, chest pain, weight loss, Hb ≥ 11.2			
PS = 1-2, WBC 8.7-15.5, no chest pain	4	6.5	3.7-9.4
PS = 1-2, WBC <15.5, chest pain, no weight loss, Hb <12.3	5	4.4	3.4-5.1
PS = 1-2, WBC 9.8-15.5, chest pain, weight loss, Hb <11.2			
PS = 1-2, WBC <9.8, chest pain, weight loss			
PS = 1-2, WBC ≥15.6	6	1.4	0.5-3.6

The numbers being used are from the original study. CI = confidence interval; PS = Eastern Cooperative Oncology Group performance status; Hb = haemoglobin (g/dl); WBC = white blood cell count (10^9/l).

Table 3: Glasgow prognostic score (McMillan, 2008).

	Points allocated
GPS	
CRP ≤1.0 mg/dl and albumin ≥35g/dl	0
CRP >1.0 mg/dl	1
Albumin <35g/dl	1
CRP >1.0 mg/dl and albumin <35g/dl	2
mGPS	
CRP ≤1.0 mg/dl	0
CRP >1.0 mg/dl and albumin ≥35g/dl	1
CRP >1.0 mg/dl and albumin <35g/dl	2
HS-mGPS	
CRP ≤0.3 mg/dl	0
CRP >0.3 mg/dl and albumin ≥35g/dl	1
CRP >0.3 mg/dl and albumin <35g/dl	2

GPS = Glasgow prognostic score; mGPS = modified Glasgow prognostic score; HS-mGPS = high sensitivity modified Glasgow prognostic score; CRP = C-reactive protein.

Table 4: LENT score (Clive et al., 2014).

Variable		Score
Pleural fluid LDH	<1500 IU/l	0
	>1500 IU/l	1
ECOG PS	0	0
	1	1
	2	2
	3-4	3
NLR	<9	0
	>9	1
Tumour type	Mesothelioma, haematological malignancy	0
	Breast cancer, gynaecological cancer, renal cell carcinoma	1
	Lung cancer, other	2
	Low risk	0-1
	Moderate risk	2-4
	High risk	5-7

LDH = lactate dehydrogenase; ECOG PS = Eastern Cooperative Oncology Group performance status; NLR = neutrophil-to-lymphocyte ratio.

Table 5: Brims score (Brims et al., 2016).

Variable			Group	Median survival (months)
No weight loss	Hb >15.3 g/dl	Albumin >43 g/dl	1	34.0
		Albumin ≤43 g/dl	2	17.7
	Hb 12.1 – 15.3 g/dl	Epithelioid or not defined	2	
		Sarcomatoid or biphasic	4	7.4
	Hb ≤12.1 g/dl		4	
Weight loss	PS ≥2		4	
	PS 0-1	Sarcomatoid	4	
		Non-sarcomatoid	3	12.0

Hb = haemoglobin, PS = Eastern Cooperative Oncology Group performance status.

Table 6: PROMISE score (Psallidas et al., 2018).

Variable		Points (clinical)	Points (biological)
Chemotherapy	No	0	0
	Yes	4	3
Radiotherapy	No	0	0
	Yes	2	2
Hb (g/dl)	>16	0	0
	14-16	1	1
	12-14	2	2
	10-12	3	3
	<10	4	4
WBC (10^9/l)	<4	0	0
	4-6.3	2	2
	3.6-10	4	4
	10-15.8	7	7
	>15.8	10	9
CRP (IU/l)	<3	0	0
	3-10	3	3
	10-32	5	5
	32-100	8	8
	>100	11	10
ECOG PS	0-1	0	0
	2-4	7	7
Cancer type	Mesothelioma	0	0
	All other cancer types	4	5
	Lung cancer	5	6
TIMP1 (ng/mg protein)	<40	/	0
	40-160	/	1

	>160	/	2
Category A	<25% risk	0-20	0-20
Category B	25-49% risk	21-27	21-28
Category C	50-74% risk	28-35	29-37
Category D	>75% risk	>35	>37

Hb = haemoglobin; WBC = white blood cell count; CRP = C-reactive protein; ECOG PS = Eastern Cooperative Oncology Group performance status; TIMP1 = tissue inhibitor of metalloproteinases 1.

Table 7: Epidemiological and clinical data of patients & MPE management.

	MPM	ETM
Patients	71 (61%)	45 (39%)
Age at cancer diagnosis (years)		
Median	75	58
Range	53-94	21-79
Age at pleural effusion (years)		
Median	75	64
Range	54-93	27-81
Gender		
Male	62 (87%)	17 (38%)
Female	9 (13%)	28 (62%)
MPE side		
Left	21 (30%)	21 (46.5%)
Right	49 (69%)	17 (38%)
Both	1 (1%)	7 (15.5%)
MPE Management		
IPC	50 (70%)	31 (69%)
Talc pleurodesis	7 (10%)	6 (13%)
IPC + talc	1 (1%)	4 (9%)
Chest tube	13 (18%)	4 (9%)
Survival (days)		
Median	254	200
Mean	379	291
Range	18-1407	9-1339
30 day survival	68 (96%)	40 (89%)
90 day survival	55 (77%)	29 (64%)

MPM = malignant pleural mesothelioma cohort; ETM = extrathoracal malignancy cohort; MPE = malignant pleural effusion.

Table 8: Histology & therapy in the MPM cohort.

Histology	Number of cases (%)
Epithelioid	52 (73%)
Sarcomatoid	6 (8%)
Biphasic	13 (18%)
Therapy	
Chemotherapy + radiotherapy + surgery	3 (4%)
Chemotherapy + surgery	14 (20%)
Chemotherapy alone	34 (48%)
Surgery alone	1 (1%)
Best supportive care	12 (17%)
Unknown	8 (11%)
Surgery	
Pleurectomy/Decortication	4 (22%)
Extended Pleurectomy/Decortication	9 (50%)
Extended Pleurectomy/Decortication + HITOC	2 (11%)
Extrapleural Pneumonectomy	2 (11%)
Local resection	1 (6%)

MPM = malignant pleural mesothelioma; HITOC = hyperthermic intrathoracic chemotherapy.

Table 9: Histology in the ETM cohort.

Primary malignancy	Number of cases (%)
Breast cancer	19 (42%)
Sarcoma	5 (11%)
Malignant melanoma	3 (7%)
Ovarian cancer	3 (7%)
Head and neck cancer	3 (7%)
Colorectal cancer	2 (4%)
Pancreatic cancer	2 (4%)
Thyroid cancer	2 (4%)
Endometrial cancer	1 (2%)
Gastric cancer	1 (2%)
Merkel cell carcinoma	1 (2%)
T-cell acute lymphoblastic leukaemia (T-ALL)	1 (2%)
Renal cell cancer	1 (2%)
Carcinosarcoma	1 (2%)

ETM = extrathoracal malignancy.

Table 10: Therapy of the primary tumour in the ETM cohort.

	Yes	No	Unknown
Surgery of primary	38 (84.5%)	5 (11%)	2 (4.5%)
Chemotherapy of primary	32 (71%)	9 (20%)	4 (9%)
Radiotherapy of primary	21 (47%)	19 (42%)	5 (11%)
Targeted therapy of primary	22 (49%)	17 (38%)	6 (13%)

ETM = extrathoracal malignancy.

Table 11: Correlation analysis between CRP and WBC, LDH, albumin and platelets in the MPM cohort.

	Correlation analysis		
	CRP <1.0 mg/dl Mean (Std. Deviation)	≥1.0 mg/dl Mean (Std. Deviation)	p-value
WBC	7.345 (2.12)	8.773 (2.65)	**0.011**
LDH	221.8 (47.78)	237.1 (52.35)	0.215
Albumin	4.252 (0.40)	3.863 (0.45)	**<0.001**
Platelets	267.1 (97.40)	337.6 (159.5)	**0.015**

MPM = malignant pleural mesothelioma; CRP = C-reactive protein; WBC = white blood cell count; LDH = lactate dehydrogenase.

Table 12: Correlation analysis between WBC, LDH, albumin and platelets in the MPM cohort.

	Correlation analysis		
	Spearman r	95% CI	p-value
WBC vs LDH	0.320	0.086 - 0.520	**0.007**
WBC vs Albumin	-0.054	-0.290 - 0.188	0.654
WBC vs Platelets	0.402	0.179 - 0.585	**<0.001**
LDH vs Albumin	0.022	-0.219 - 0.260	0.859
LDH vs Platelets	0.178	-0.065 - 0.401	0.138
Albumin vs Platelets	-0.219	-0.436 - 0.224	0.067

MPM = malignant pleural mesothelioma; CI = confidence interval; WBC = white blood cell count; LDH = lactate dehydrogenase.

Table 13: Correlation analysis between CRP and WBC, LDH, albumin and platelets in the ETM cohort.

	Correlation analysis		
	CRP <1.0 mg/dl Mean (Std. Deviation)	≥1.0 mg/dl Mean (Std. Deviation)	p-value
WBC	6.849 (3.60)	9.624 (4.39)	**0.025**
LDH	236.6 (51.15)	460.1 (490.4)	**0.015**
Albumin	4.142 (0.46)	3.794 (0.70)	0.085
Platelets	256.5 (95.82)	342.5 (157.3)	0.087

ETM = extrathoracal malignancy; CRP = C-reactive protein; WBC = white blood cell count; LDH = lactate dehydrogenase.

Table 14: Correlation analysis between WBC, LDH, albumin and platelets in the ETM cohort.

	Correlation analysis		
	Spearman r	95% CI	p-value
WBC vs LDH	0.376	0.084 - 0.609	**0.011**
WBC vs Albumin	-0.136	-0.424 - 0.176	0.378
WBC vs Platelets	0.633	0.410 - 0.785	**<0.001**
LDH vs Albumin	-0.064	-0.362 - 0.246	0.681
LDH vs Platelets	0.223	-0.085 - 0.491	0.141
Albumin vs Platelets	-0.139	-0.426 - 0.174	0.369

ETM = extrathoracal malignancy; CI = confidence interval; WBC = white blood cell count; LDH = lactate dehydrogenase.

Table 15: Correlation analysis between mGPS and WBC, LDH & platelets in the MPM cohort.

		p-value		
		WBC	LDH	Platelets
ANOVA	mGPS	**0.012**	0.251	**0.026**
Multiple comparisons	mGPS 0 vs 1	**0.010**	0.303	**0.038**
	mGPS 0 vs 2	>0.999	>0.999	0.211
	mGPS 1 vs 2	0.701	>0.999	>0.999

MPM = malignant pleural mesothelioma; mGPS = modified Glasgow prognostic scale; WBC = white blood cell count; LDH = lactate dehydrogenase.

Table 16: Correlation analysis between mGPS and WBC, LDH & platelets in the ETM cohort.

		p-value		
		WBC	LDH	Platelets
ANOVA	mGPS	0.076	0.123	0.169
Multiple comparisons	mGPS 0 vs 1	0.080	0.427	0.617
	mGPS 0 vs 2	0.203	0.125	0.156
	mGPS 1 vs 2	>0.999	0.810	0.321

ETM = extrathoracal malignancy; WBC = white blood cell count; LDH = lactate dehydrogenase; ANOVA = analysis of variance; mGPS = modified Glasgow prognostic scale.

Table 17: Univariate analysis of the different biomarkers in the MPM cohort.

		Number of patients (n=71)	Univariate analysis		
			Median overall survival (days)	HR (95% CI)	p-value
CRP	<0.5 mg/dl	13	690	1	**0.048**
	≥0.5 mg/dl	58	304	1.90 (1.08 – 3.35)	
	<1.0 mg/dl	21	585	1	**0.005**
	≥1.0 mg/dl	50	249	2.18 (1.28 – 3.72)	
WBC	4.0-9.2 x 10^9/l	51	318	1	0.892
	<4.0 or >9.2 x 10^9/l	20	398	1.04 (0.57 – 1.90)	
LDH	≤247 U/l	48	387	1	0.253
	>247 U/l	23	206	1.38 (0.76 – 2.53)	
Albumin	<3.5 g/dl	8	118	1	**0.003**
	≥3.5 g/dl	63	344	0.34 (0.11 – 1.09)	
Platelets	150-350 x 10^9/l	51	344	1	0.193
	<150 or >350 x 10^9/l	20	254	1.45 (0.78 – 2.71)	
Hb	<10 g/dl	7	71	1	**<0.001**
	≥10 g/dl	64	344	0.29 (0.08 – 1.10)	

RBC	Reference Outside of reference	20 51	507 304	1 1.59 (0.91 – 2.77)	0.129
Haematocrit	Reference Outside of reference	33 38	344 304	1 1.35 (0.79 – 2.30)	0.272
MCV	80-98 fl <80 or >98 fl	61 10	367 194	1 2.03 (0.84 – 4.93)	**0.037**
MCH	28-32 pg <28 or >32 pg	47 24	387 218	1 2.00 (0.97 – 4.13)	**0.018**
MCHC	32-36 g/dl <32 or >36 g/dl	62 9	318 315	1 1.60 (0.65 – 3.91)	0.213
MPV	<9.7 fl ≥9.7 fl unknown	8 43 20	171 344	1 0.59 (0.22 – 1.57)	0.187

MPM = malignant pleural mesothelioma; HR= hazard ratio; CI = confidence interval; CRP = C-reactive protein; WBC = white blood cell count; LDH = lactate dehydrogenase; Hb = haemoglobin; RBC = red blood cell count; reference RBC: 4.8-5.9 x 10^{12}/l for male & 4.3-5.2 x 10^{12}/l for female; reference haematocrit: 40-52 % for male & 35-47 % for female; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MPV = platelet count and mean platelet volume.

Table 18: Univariate analysis of the different biomarkers in the ETM cohort.

		Number of patients (n=45)	Univariate analysis		
			Median overall survival (days)	HR (95% CI)	p-value
CRP	<0.5 mg/dl	6	NR	1	**0.021**
	≥0.5 mg/dl	39	193	4.51 (2.01 – 10.11)	
	<1.0 mg/dl	12	780	1	**0.009**
	≥1.0 mg/dl	33	104	2.79 (1.42 – 5.47)	
WBC	4.0-9.2 x 10^9/l	23	402	1	0.058
	<4.0 or >9.2 x 10^9/l	22	172	1.87 (0.96 – 3.67)	
LDH	≤247 U/l	13	290	1	0.297
	>247 U/l	32	98	1.43 (0.74 – 2.77)	
	≤500 U/l	39	270	1	**0.009**
	>500 U/l	6	31	3.02 (0.78 – 11.67)	
Albumin	<3.5 g/dl	9	50	1	**0.016**
	≥3.5 g/dl	36	290	0.40 (0.14 – 1.17)	
Platelets	150-350 x 10^9/l	21	444	1	**<0.001**
	<150 or >350 x 10^9/l	24	76	3.03 (1.50 – 6.13)	

Hb	<10 g/dl ≥10 g/dl	8 37	63 270	1 0.62 (0.24 – 1.64)	0.249
RBC	Reference Outside reference	14 31	446 151	1 2.273 (1.14 – 4.53)	**0.038**
Haematocrit	Reference Outside reference	16 29	444 76	1 3.03 (1.56 – 5.88)	**0.002**
MCV	80-98 fl <80 or >98 fl	38 7	172 290	1 1.14 (0.48 – 2.71)	0.747
MCH	28-32 pg <28 or >32 pg	29 16	210 201	1 1.45 (0.70 – 3.01)	0.279
MCHC	32-36 g/dl <32 or >36 g/dl	39 6	270 197	1 1.67 (0.58 – 4.80)	0.246
MPV	<9.7 fl ≥9.7 fl	13 32	200 312	1 0.56 (0.25 – 1.28)	0.124

ETM = extrathoracal malignancy; HR= hazard ratio; CI = confidence interval; CRP = C-reactive protein; NR = not reached; WBC = white blood cell count; LDH = lactate dehydrogenase; Hb = haemoglobin; RBC = red blood cell count; reference RBC: 4.8-5.9 x 10^{12}/l for male & 4.3-5.2 x 10^{12}/l for female; reference haematocrit: 40-52 % for male & 35-47 % for female; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MPV = platelet count and mean platelet volume.

Table 19: Kaplan-Meier method for each mGPS in the MPM cohort.

	Median overall survival (days)	Number of patients	Std. error	95% CI	p-value
mGPS 0	585	26	105	492 – 905	**<0.001**
mGPS 1	274	39	65.61	269 – 526	
mGPS 2	118	6	59	35 – 266	

MPM = malignant pleural mesothelioma; mGPS = modified Glasgow prognostic scale; CI = confidence interval.

Table 20: Kaplan-Meier method for each mGPS-pair in the MPM cohort.

		Multiple comparisons	
		HR (95% CI)	p-value
mGPS	0 1	1 2.02 (1.15 – 3.56)	**0.015**
	0 2	1 4.20 (0.92 – 19.36)	**<0.001**
	1 2	1 2.72 (0.75 – 9.90)	**0.018**

MPM = malignant pleural mesothelioma; HR = hazard ratio; CI = confidence interval; mGPS = modified Glasgow prognostic scale.

Table 21: Kaplan-Meier method for each mGPS in the ETM cohort.

	Median overall survival (days)	Number of patients	Std. error	95% CI	p-value
mGPS 0	780	7	148.35	297 – 879	**0.044**
mGPS 1	201	30	88.26	214 – 560	
mGPS 2	48.5	8	74.25	8 – 299	

ETM = extrathoracal malignancy; mGPS = modified Glasgow prognostic scale; CI = confidence interval.

Table 22: Kaplan-Meier method for each mGPS-pair in the ETM cohort.

		Multiple comparisons	
		HR (95% CI)	p-value
mGPS	0 1	1 1.68 (0.73 – 3.89)	0.274
	0 2	1 3.52 (1.01 – 12.34)	**0.013**
	1 2	1 2.09 (0.71 – 6.12)	0.077

ETM = extrathoracal malignancy; HR = hazard ratio; CI = confidence interval; mGPS = modified Glasgow prognostic scale.

Table 23: Overall survival after curative intended surgery in the MPM cohort.

		Univariate analysis		
		Median overall survival (days)	HR (95% CI)	p-value
Curative intended surgery	Yes	763	1	**0.004**
	No	274	2.425 (1.41 – 4.17)	

MPM = malignant pleural mesothelioma; HR = hazard ratio; CI = confidence interval.

Table 24: Multivariate analysis of the different biomarkers in the MPM cohort.

		Multivariate analysis		
		HR	95% CI	p-value
Hb	<10 g/dl	1	0.145 – 0.799	**0.013**
	≥10 g/dl	0.340		
mGPS	0	1	1,017 – 3.385	**0.044**
	1-2	1,856		

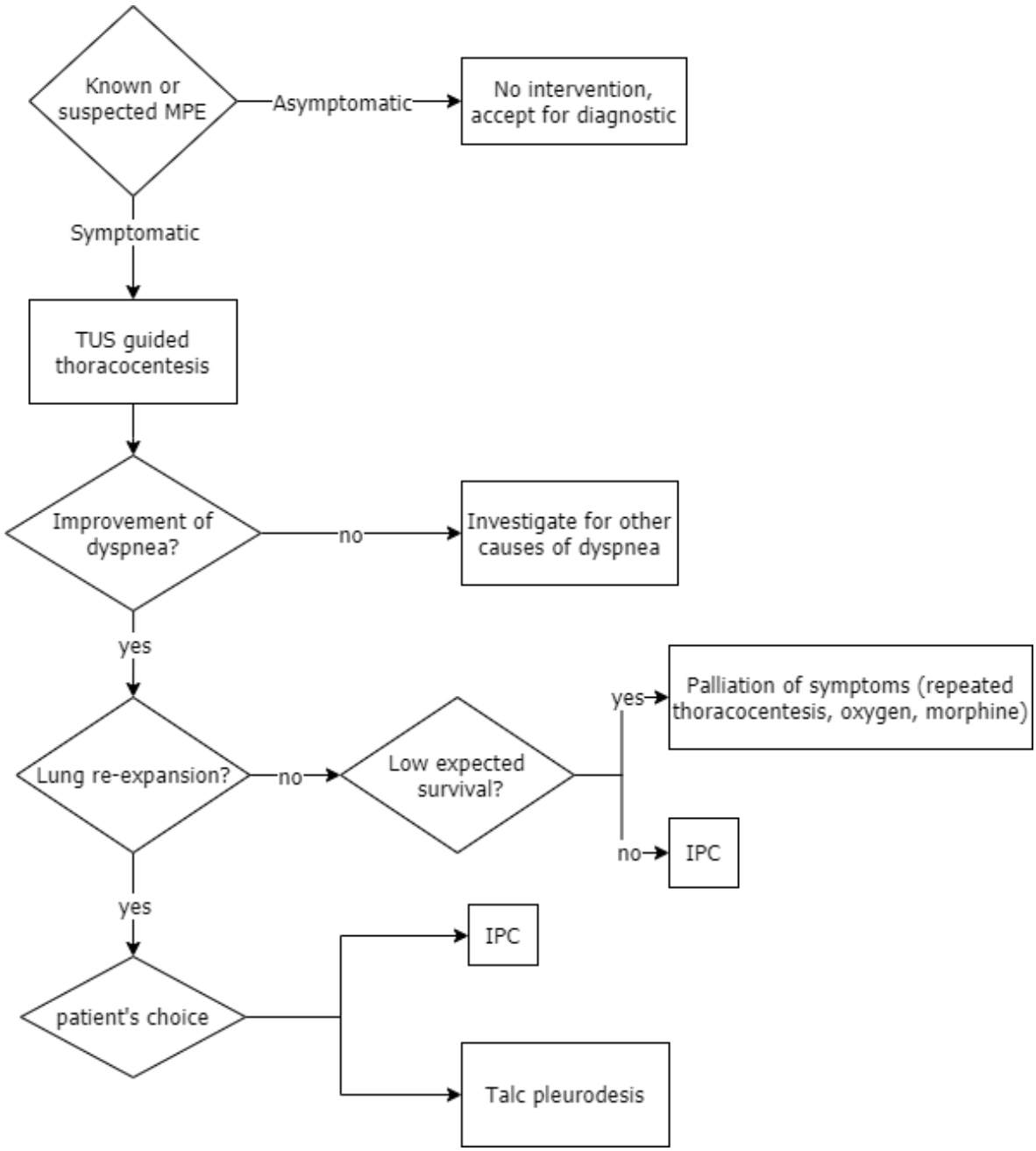
MPM = malignant pleural mesothelioma; HR = hazard ratio; CI = confidence interval; Hb = haemoglobin; mGPS = modified Glasgow prognostic scale.

Table 25: Multivariate analysis of the different biomarkers in the ETM cohort.

		Multivariate analysis		
		HR	95% CI	p-value
LDH	≤500 U/l	1	0.734 – 4.958	0.185
	>500 U/l	1.908		
Platelets	150-350 x 10^9/l	1	1.203 – 6.674	**0.017**
	<150 or >350 x 10^9/l	2.834		
mGPS	0	1	0.585 – 4.240	0.369
	1-2	1.575		

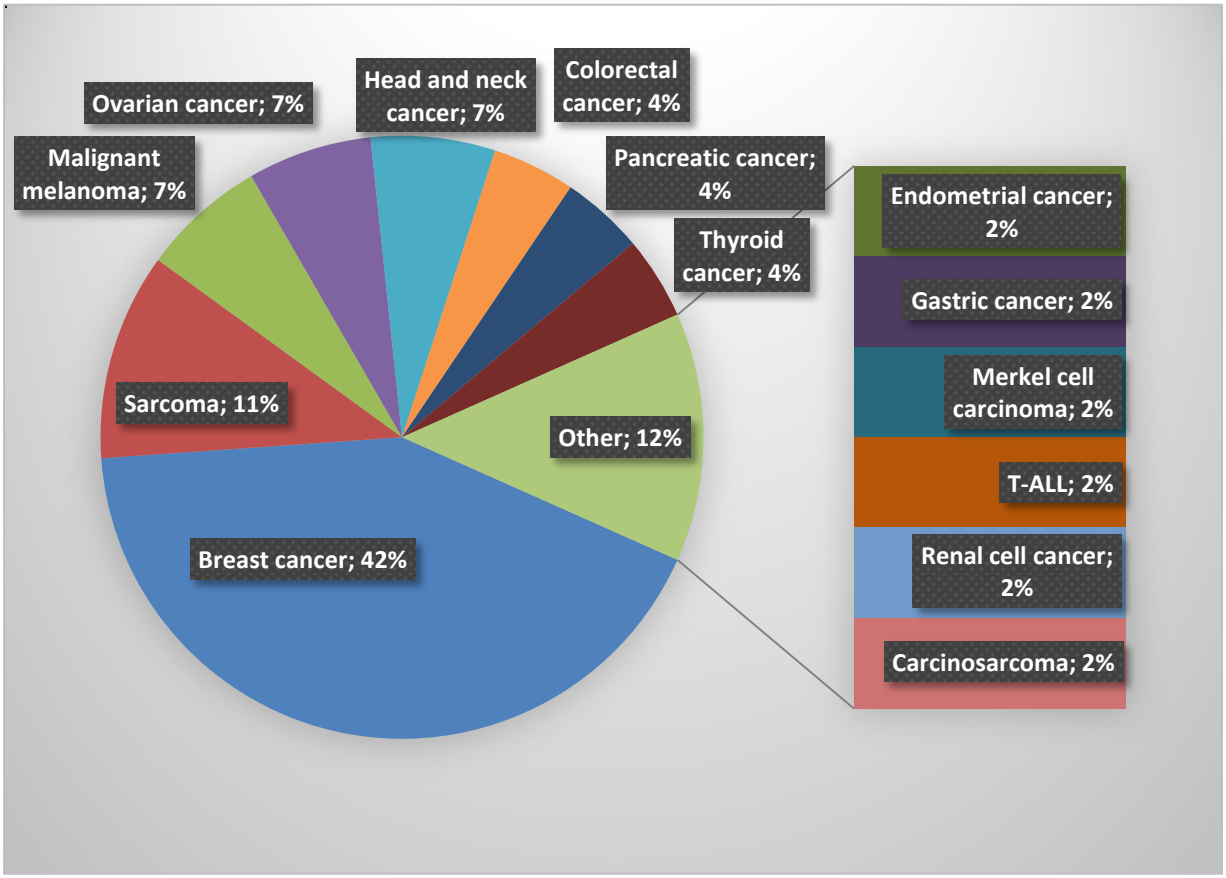
ETM = extrathoracal malignancy; HR = hazard ratio; CI = confidence interval; LDH = lactate dehydrogenase; mGPS = modified Glasgow prognostic scale.

Figure 1: Management of MPE algorithm (adapted from Feller-kopman et al., 2018).



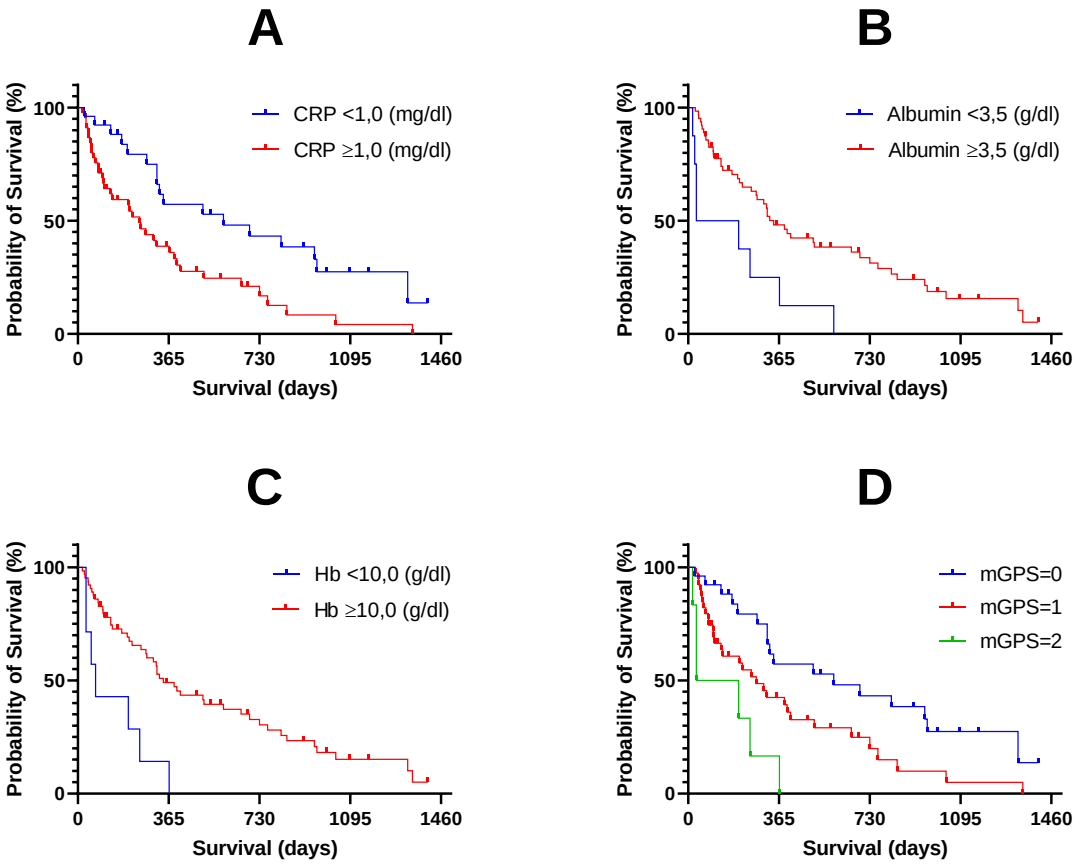
MPE = malignant pleural effusion, TUS = thoracic ultrasound, IPC = indwelling pleural catheter.

Figure 2: Histology in the ETM cohort.



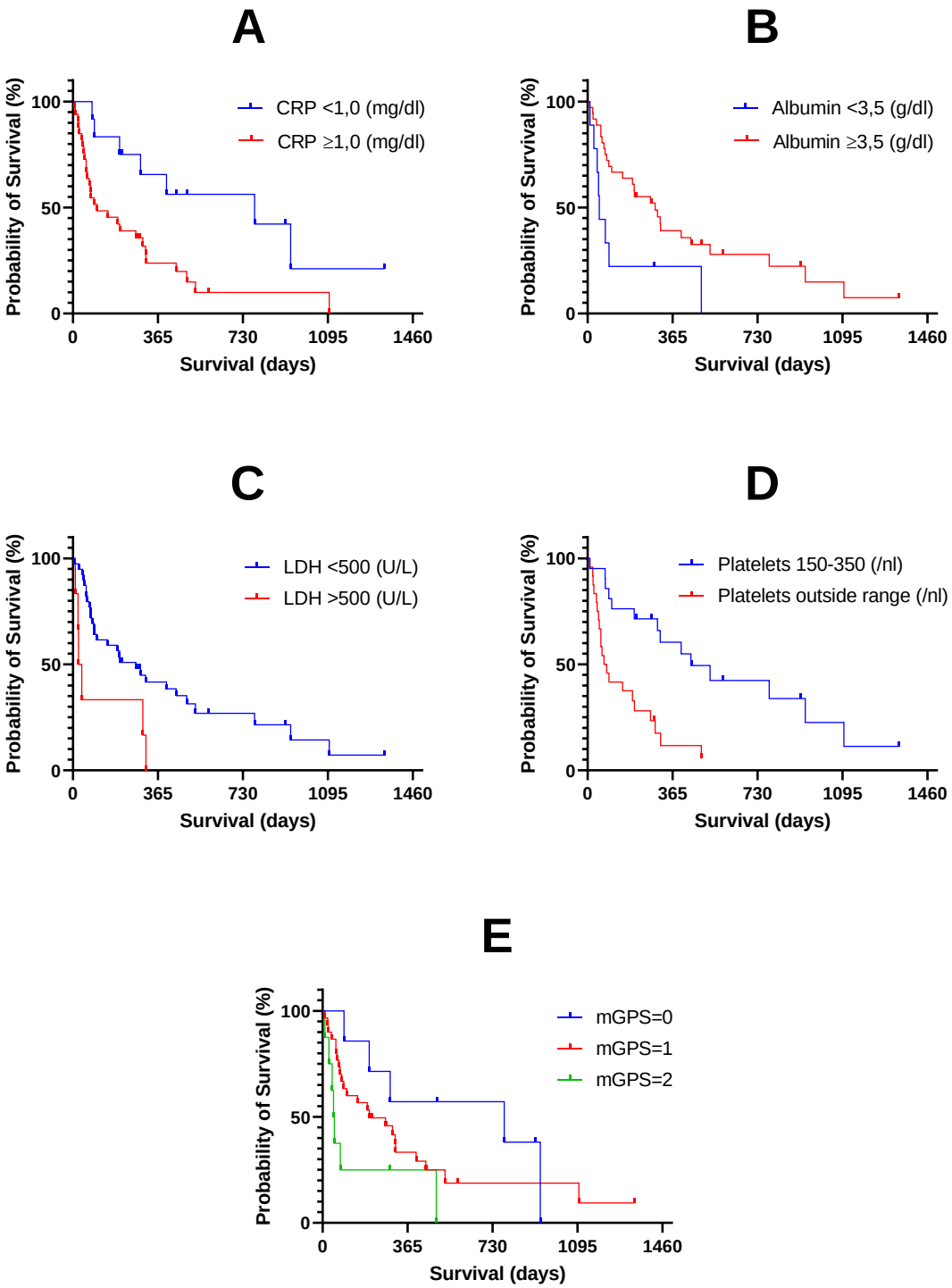
ETM = extrathoracal malignancy; T-ALL = T-cell acute lymphoblastic leukaemia.

Figure 3: Overall survival in the MPM cohort.



OS = overall survival; MPM = malignant pleural mesothelioma; (A): OS based on CRP (p=0.005); (B): OS based on albumin (p=0.003); (C): OS based on Hb (p<0.001); (D): OS based on mGPS (p<0.001)

Figure 4: Overall survival in the ETM cohort.



OS = overall survival; ETM = extrathoracal malignancy; (A): OS based on CRP (p=0.009), (B): OS based on albumin (p=0.016); (C): OS based on LDH (p=0.009); (D): OS based on platelets (p<0.001); (E): OS based on mGPS (p=0.044).

10 LIST OF ABBREVIATIONS

ANG-1 = angiopoietin 1

ANG-2 = angiopoietin 2

ARDS = acute respiratory distress syndrome

ATS = American Thoracic Society

BAL = bronchoalveolar lavage

BAP-1 = breast cancer-1 associated protein-1

BRCA-1 = breast cancer-1

BW = body weight

CALGB = the Cancer and Leukemia Group B

CART = classification and regression tree

CI = confidence interval

CIT = chemotherapy-induced thrombocytopenia

COP-LMR = combination of platelet count and lymphocyte to monocyte ratio

CRP = C-reactive protein

CT = computed tomography

DFS = disease free survival

DVT = deep vein thrombosis

e.g. = for example

EACTS = the European Association for Cardio-Thoracic Surgery

ECOG = Eastern Cooperative Oncology Group

EGFR = epidermal growth factor receptor

EORTC = the European Organisation for Research and Treatment of Cancer

eP/D = extended pleurectomy/decortication

EPO = erythropoietin

EPP = extrapleural pneumonectomy

ERS = European Respiratory Society

ESTRO = the European Society for Radiotherapy and Oncology

ESTS = the European Society of Thoracic Surgeons

et al. = and others

ETM = extrathoracal malignancy

Fb-3 = fibulin-3

FDG = F-18 fluorodeoxy-glucose

GPS = Glasgow prognostic score

Hb = haemoglobin

HITOC = hyperthermic intrathoracic chemotherapy

HR = hazard ratio

HS-mGPS = high-sensitivity mGPS

i.a. = among others

i.e. = that is

IL-2 = interleukin-2

INF = interferon

IPC = indwelling pleural catheter

LDH = lactate dehydrogenase

LENT = LDH, ECOG PS, NLR and tumour type

LMR = lymphocyte-to-monocyte ratio

MAPS = mesothelioma avastin cisplatin pemetrexed study

MCH = mean corpuscular haemoglobin

MCHC = mean corpuscular haemoglobin concentration

MCV = mean corpuscular volume

mGPS = modified Glasgow prognostic score

MMP = matrix metalloproteinases

MPE = malignant pleural effusion

MPF = megakaryocyte potentiating factor

MPM = malignant pleural mesothelioma

MPV = mean platelet volume

MRI = magnetic resonance imaging

NLR = neutrophil-to-lymphocyte ratio

NSCLC = non-small-cell lung cancer

OPN = osteopontin

OS = overall survival

P/D = pleurectomy/decortication

PA = posterior-anterior

P-CRP = platelet x C-reactive protein multiplier value

PD1 = programmed cell death-1

PD-L1 = programmed death-ligand 1

PET = positron-emission

PFS = progression free survival

PLR = platelet-to-lymphocyte ratio

PS = performance status

RBC = red blood cell

RECIST = Response Evaluation Criteria in Solid Tumours

RFS = recurrence free survival

RNA = ribonucleic acid

SFTP = solitary fibrous tumour of the pleura

STR = Society of Thoracic Radiology

STS = Society of Thoracic Surgeons

T-ALL = T-cell acute lymphoblastic leukaemia

TGF-β = transforming growth factor-beta

TIMP1 = tissue inhibitor of metalloproteinases 1

TKI = tyrosine kinase inhibitor

TNF = tumour necrosis factor

TNM = tumour – node – metastasis

TUS = thoracic ultrasound

UICC = the Union for International Cancer Control

ULN = upper limit of the normal

VATS = video assisted thoracoscopic surgery

VEGF = vascular endothelial growth factor

vs = versus

VTE = venous thromboembolism

WBC = white blood cell

WHO = World Health Organisation

11 NOTE OF THANKS

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12 CURRICULUM VITAE

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