DOI:10.14753/SE.2020.2356

IDENTIFICATION OF PROGNOSTIC AND DIAGNOSTIC MARKERS IN THYMIC EPITHELIAL TUMORS

PhD thesis

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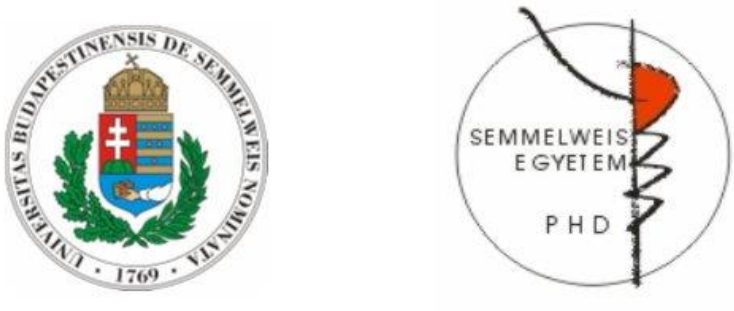

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2019

DOI:10.14753/SE.2020.2356

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1. Abbreviations

AJCC	American Joint Committee on Cancer
ARDS	acute respiratory distress syndrome
AUC	area under the curve
CCS	Cause-specific survival
ChART	Chinese Alliance for Research in Thymomas
Cht	Chemotherapy
CI	Confidence interval
CIR	Cumulative incidence of recurrence
CR	complete response
CRP	C-reactive protein
CT	Computed tomography
DFS	Disease-free survival
dNLR	derived Neutrophil-to-Lymphocyte ratio
ECG	electrocardiography
ELISA	Enzyme linked immunosorbent assay
EPD	Extended Pleurectomy/Decortication
EPP	Extrapleural pneumonectomy
ESMO	European Society for Medical Oncology
esRAGE	endogenous secretory Receptor for Advanced Glycation Endprod-ucts
ESTS	European Society of Thoracic Surgeons
f:m ratio	female:male ratio
FDG-PET	^{18}Fluorine-fluorodeoxyglucose–positron emission tomography
FFR	Freedom from recurrence
HMGB1	high mobility group box 1
HR	hazard ratio
HSP	Heat shock protein
IARC	International agency for research on cancer
IASLC	International Association for the Study of Lung Cancer
ICD-O	International Classification of Diseases for Oncology

ITMIG	International Thymic Malignancies Interest Group
JART	Japanese Association for Research on the Thymus
LP	Local pleurectomy
MEN 1	multiple endocrine neoplasia syndrome type 1
MG	Myasthenia gravis
MGFA	Myasthenia Gravis Foundation of America
MODS	multi organ dysfunction syndrome
MRI	Magnetic resonance imaging
MRI	Magnetic resonance imaging
NLR	Neutrophil-to-Lymphocyte ratio
NPV	Negative Predictive Value
OS	Overall survival
P/D	Pleurectomy/Decortication
PLR	Platelet-to-Lymphocyte ratio
PORT	postoperative radiation therapy
PPV	Positive Predictive Value
PR	partial response
R0	no residual tumor
R1	microscopic residual tumor
R2	macroscopic residual tumor
RAGE	Receptor for Advanced Glycation Endproducts
RATS	robotic-assisted thoracic Surgery
RECIST criteria	Response Evaluation Criteria in Solid Tumor
RFS	Recurrence-free survival
ROC	Receiver operating characteristic curves
RT	Radiotherapy
SD	standard deviation
SEER	Surveillance, Epidemiology, and End Results
SEM	standard error of the mean
sRAGE	Soluble Receptor for Advanced Glycation Endproducts
SST	somatostatin
SUV_{max}	maximum standard uptake value

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TAMG	Thymoma-associated MG
TETs	Thymic epithelial tumors
TNET	Thymic neuroendocrine tumor
TNM	Tumor Node Metastasis
TP	Total pleurectomy
UICC	Union for International Cancer control
VATET	Video-assisted thorascopic extended thymectomy
VATS	Video-assisted thoracic surgery
WHO	World Health Organization

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2. Introduction to Thymic epithelial tumors

2.1 Epidemiology

In the European Union non-communicable diseases that affect less than five in 10,000 people are considered rare (European Commission). In a population-based study using two nationwide databases in the Netherlands the incidence of thymic epithelial tumors (TETs) was reported to be 3.2/1,000,000 (de Jong, Blaauwgeers et al. 2008). The overall annual incidence in the United States was reported as 0.15 per 100,000 inhabitants (Engels and Pfeiffer 2003). TETs are thus an orphan disease.

Two distinct forms of TETs can be distinguished: thymomas and thymic carcinomas (TC). In the Netherlands (1994-2003) thymoma incidence was 2.2/1,000,000 and TC incidence 0.3/1,000,000 with equal incidence rates for men and women (de Jong, Blaauwgeers et al. 2008). In the Netherlands the median age at diagnosis of TETs was 59 years (de Jong, Blaauwgeers et al. 2008).

Among the rarest forms of TETs are thymic neuroendocrine tumors (TNETs). They accounted for 0.4% of carcinoid tumors in the Surveillance, Epidemiology, and End Results (SEER) database of the United States National Cancer Institute (Yao, Hassan et al. 2008, Gaur, Leary et al. 2010) and constitute about 4-7% of all anterior mediastinal tumors (Gaur, Leary et al. 2010).

2.2 Etiology and heredity

To date no risk factors for the development of TETs have been discovered. Regarding the existence of genetic variants a higher risk of thymomas in diverse Asian and Pacific Islanders was reported. So far no evidence for the contribution of alcohol, tobacco, occupational or environmental hazards, dietary factors or ionizing radiation could be detected. The incidence of TETs seems not to be higher in immunosuppressed HIV patients or organ transplant recipients (Engels and Pfeiffer 2003). Epstein Barr virus (EBV) was detected in TCs of lymphoepithelial histology (Mann, Wu et al. 1992, Engels and Pfeiffer 2003).

The familial occurrence of thymomas is a very rare event. In one family thymomas occurred in three family members (next to autoimmune diseases in 4 other family members,

such as Grave`s disease, pernicious anemia, Sjögren`s diease and autoimmune pancytopenia). Of the 27 tested family members 11 had a constitutional translocation t(14;20)(q24;p12) which was present in all the thymoma patients of this family. The DNA strand break 14q24 was in a tumor suppressor gene (RAD5I family) known to be involved in tumors such as uterine leiomyoma or pulmonary chondroid hamartoma). The DNA strand break 20p12 was in close proximity (100kb) to BMP2, a TGFβ-family member involved in the differentiation of thymocytes (Nicodeme, Geffroy et al. 2005).

2.3 Histology

2.3.1 Thymoma and TC

In 1999 the WHO defined histopathological criteria for thymomas, namely types A, AB, B1, B2, B3 and for the different TC subtypes collectively as type C (J.). In the year 2002 the new WHO Histologic Classification of TETs was published (Chen, Marx et al. 2002). In the fourth edition of the WHO Classification of Thymic Tumors (TETs, germ cell tumors, lymphomas, dendritic cell and myeloid neoplasms, and soft tissue tumors of the thymus and mediastinum) a comprehensive overview of newly defined tumor entities and their variants as well as refined criteria for the diagnosis of thymomas and thymic squamous cell carcinoma (see Table 2; and reference (Marx, Chan et al. 2015)).

Table 1: WHO classification of TETs. Adapted from Table 1: Epithelial Tumors: The 2015 World Health Organization Classification of Tumors of the Thymus: Continuity and Changes (Marx, Chan et al. 2015). Behavior is coded /0 for benign tumors; /1 for unspecified, borderline, or uncertain behavior; /2 for carcinoma in situ and grade III intraepithelial neoplasia; and /3 for malignant tumors. NUT, nuclear protein in testis. [a]These new codes were approved by the IARC/WHO Committee for ICD-0.

Thymoma	**ICD-O**
Type A thymoma, including atypical variant	8581/3[a]
Type AB thymoma	8582/3[a]
Type B1 thymoma	8583/3[a]
Type B2 thymoma	8584/3[a]
Type B3 thymoma	8585/3[a]
Micronodular thymoma with lymphoid stroma	8580/1[a]
Metaplastic thymoma Other rare thymomas	8580/3
Microscopic thymoma	8580/0
Sclerosing thymoma	8580/3
Lipofibroadenoma	9010/0[a]
Thymic carcinoma	
Squamous cell carcinoma	8070/3
Basaloid carcinoma	8123/3

Mucoepidermoid carcinoma	8430/3
Lymphoepithelioma-like carcinoma	8082/3
Clear cell carcinoma	8310/3
Sarcomatoid carcinoma	8033/3
Adenocarcinomas	
Papillary adenocarcinoma	8260/3
Thymic carcinoma with adenoid cystic carcinoma-like features	8200/3[a]
Mucinous adenocarcinoma	8480/3
Adenocarcinoma, NOS	8140/3
NUT carcinoma	8023/3[a]
Undifferentiated carcinoma	8020/3
Other rare thymic carcinomas	
Adenosquamous carcinoma	8560/3
Hepatoid carcinoma	8576/3
Thymic carcinoma, NOS	8586/3
Thymic neuroendocrine tumors	
Carcinoid tumors	
Typical carcinoid	8240/3
Atypical carcinoid	8249/3
Large-cell neuroendocrine carcinoma	8013/3
Combined large-cell neuroendocrine carcinoma	8013/3
Small-cell carcinoma (SCC)	8041/3
Combined SCC	8045/3
Combined thymic carcinomas	

Table 2: WHO classification of thymic tumors: refined diagnostic criteria. From: The 2015 WHO Classification of Tumors of the Thymus: Continuity and Changes (Marx, Chan et al. 2015). [a]Paucity versus abundance: any area of crowded immature T cells or moderate numbers of immature T cells in >10% of the investigated tumor are indicative of "abundance";

Thymoma subtype	**Obligatory criteria**	**Optional criteria**
Type A	Occurrence of bland, spindle shaped epithelial cells (at least focally); paucity[a] or absence of immature (TdT+) T cells throughout the tumor	Polygonal epithelial cells CD20+ epithelial cells
Atypical type A variant	Criteria of type A thymoma; in addition: comedotype tumor necrosis; increased mitotic count (>4/2mm2); nuclear crowding	Polygonal epithelial cells CD20+ epithelial cells
Type AB	Occurrence of bland, spindle shaped epithelial cells (at least focally); abundance*a* of immature (TdT+) T cells focally or throughout tumor	Polygonal epithelial cells CD20+epithelial cells
Type B1	Thymus-like architecture and cytology: abundance of immature T cells, areas of medullary differentiation (medullary islands); paucity of polygonal or dendritic epithelia cells without clustering (i.e.<3 contiguous epithelial cells)	Hassall's corpuscles; perivascular spaces
Type B2	Increased numbers of single or clustered polygonal or dendritic epithelial cells intermingled with abundant immature T cells	Medullary islands; Hassall's corpuscles; perivascular spaces
Type B3	Sheets of polygonal slightly to moderately atypical epithelial cells; absent or rare intercellular bridges; paucity or absence of intermingled TdT+ T cells	Hassall's corpuscles; perivascular spaces
Micronodular thymomas (MNT) with lymphoid stroma;	Nodules of bland spindle or oval epithelial cells surrounded by an epithelial cell-free lymphoid stroma	Lymphoid follicles; monoclonal B cells and/or plasma cells (rare)

Metaplastic thymoma	Biphasic tumor composed of solid areas of epithelial cells in a background of bland-looking spindle cells; absence of immature T cells	Pleomorphism of epithelial cells; actin, keratin, or EMA-positive spindle cells
Rare other: Microscopic thymoma; sclerosing thymoma, lipofibroadenoma		

Outcome related to WHO histology

In a study on 200 Chinese patients undergoing surgery for TETs (only 55 patients received adjuvnat radiotherapy and 8 patients adjuvant chemotherapy) OS was reported as follows: none of the Type A and AB thymomas patients died of tumor; there was one patient with type B1 thymoma who died at 22 months; type B2, B3, and TC (formerly type C thymomas) patients had a significantly worse prognosis: 5-year OS 75.0%, 70.0%, and 48.0%, respectively (see Figure 1). Masaoka-Koga stage was a statistically significant predictor of survival. There was a statistically significant association between WHO histologic subtype and stage. WHO histology was an independent predictive factor of OS in stage I and II TETs: type B2, B3, and TC had a worse prognosis than type A, AB, and B1 thymomas ($p<0.003$) (Chen, Marx et al. 2002).

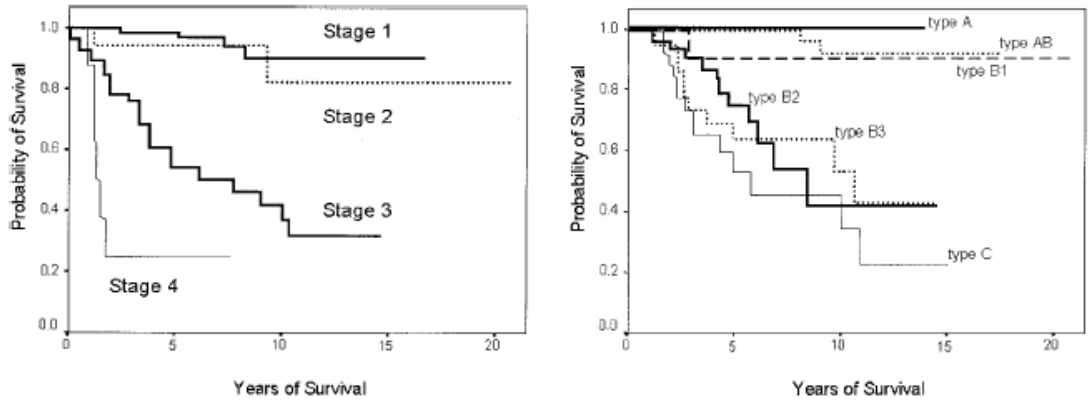


Figure 1: OS by Stage and WHO histology. Patients with higher stage had a significantly ($p<0.001$) increased risk of death from tumor. Type B2-3 and C thymomas (TCs) had a significantly ($p<0.01$) increased risk of death from tumor. From (Chen, Marx et al. 2002).

Our group has recently proposed a new subtype of TC: primary thymic adenocarcinoma of enteric type (Moser, Schiefer et al. 2015). The inclusion of this new TC subtype may help prevent misdiagnosis of metastatic disease from an extrathymic primary cancer, particularly metastatic disease from the gastrointestinal tract Figure 2.

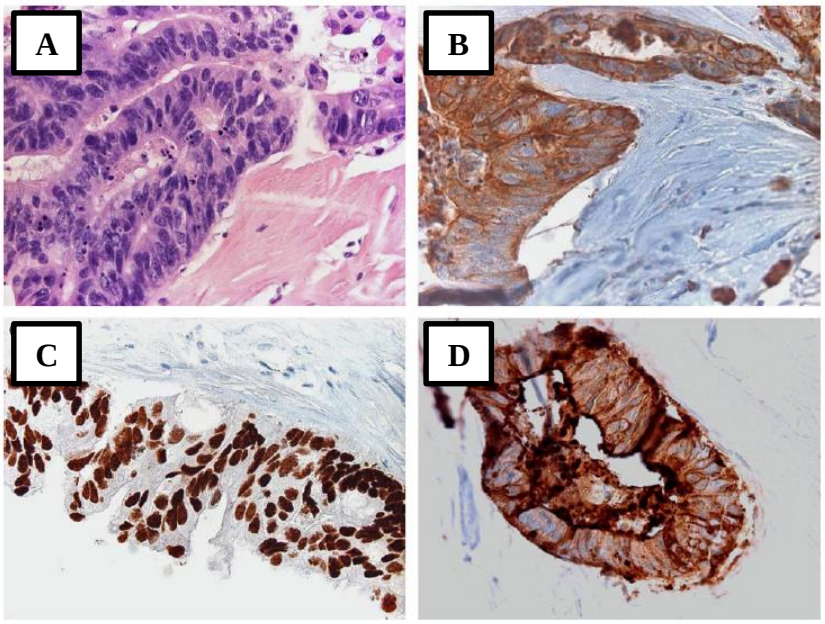


Figure 2: Primary thymic adenocarcinoma of enteric type. Hematoxylin and eosin staining (A). Immunohistochemistry for CK20 (B), CDX2 (C), and CEA (D). From (Moser, Schiefer et al. 2015).

2.3.2 TNETs

The current nomenclature of TNETs distinguishes: thymic typical (<2 mitoses/2mm^2; no necrosis) and atypical (<2 mitoses/2mm^2; with necrosis; or 2–10 mitoses/2mm^2; + or − necrosis) carcinoids, large cell neuroendocrine carcinoma (LCNEC; >10 mitoses/2mm^2; no small cell features) and small cell carcinoma; and the combination of LCNEC or small cell carcinoma with thymoma of TC (Marx, Chan et al. 2015). Pathognomonic for TNETs is their high biologic aggressiveness and poor prognosis due to high recurrences rates and tumor related deaths (Strobel, Zettl et al. 2014, Filosso, Yao et al. 2015). OS of patients with TNETs in the SEER database at 1-, 3- and 5 years was 89%, 66% and 53% with poorer survival of patients in advanced stages (Gaur, Leary et al. 2010). 5 year OS for localized disease (tumor confined to organ) was 80%, regional disease (local invasion or metastasis to regional lymph nodes) 48% and distant disease 31% (Gaur, Leary et al. 2010). OS was reported to be significantly better in patients undergoing macroscopic complete resection (Ose, Maeda et al. 2018). TNETs can be associated with paraneoplastic syndromes, e.g. Cushing`s syndrome or multiple endocrine neoplasia syndrome type 1 (MEN 1).

2.3.3 Thymic hyperplasia

Thymic hyperplasia (TH) is caused by non-malignant thymic changes with an increase in constituent cells (Castleman 1955). Pathology distinguishes two inherently different types of TH. True thymic hyperplasia (TTH) is diagnosed if the thymus is of regular microscopic histologic architecture but is marked by increased weight and size. Follicular (or lymphoid) TH (FTH) is characterized by the presence of lymphoid follicles with germinal centers in the thymic medulla (Rosai and Levine 1976). The diagnosis benign enlargement of the thymus :TTH and FTH can solely be made from the pathological specimen. To date there are no established risk factors (Engels 2010) nor biomarkers that can distinguish TH from malignant TETs.

2.4 Paraneoplastic syndromes:

There is a strong association between thymomas and autoimmune diseases: systemic lupus erythematosus, autoimmune cytopenias (pure red cell aplasia (PRCA), aplastic anemia (AA), autoimmune hemolytic anemia (AIHA), immune thrombocytopenia, autoimmune neutropenia, thrombotic thrombocytopenic purpura, agranulocytosis, polymyositis, Good`s syndrome, hypogammaglobulinemia, autoimmune thyroid diseases, autoimmune hepatitis, cutaneous autoimmune diseases, paraneoplastic pemphigus or Lichen planus (Bernard, Frih et al. 2016). Many of the observed paraneoplastic disease are neurological: Myasthenia gravis, Lambert Eaton syndrome, Myositis, Isaac`s syndrome, encephalitis, Morvan`s syndrome, Autoimmune Autonomic Neuropathy, Paraneoplastic cerebellar degeneration, Stiff person syndrome (Evoli and Lancaster 2014). The most frequent paraneoplastic autoimmune neurological disorder is MG. TETs are diagnosed in up to 15% of patients with MG. Conversely, about 30% of patients with TETs experience symptoms of MG at the time of diagnosis (Marx, Willcox et al. 2010).

2.4.1 Myasthenia gravis

Myasthenia gravis (MG) is a neurological disorder with a prevalence of 10/100,000 people (Phillips 2003). MG is a B-cell mediated autoimmune disorder (Gilhus and Verschuuren 2015) characterized by autoantibodies against the nicotinic acetylcholine receptor and less common against other proteins of the neuromuscular junction. The autoimmune destruction of acetylcholine receptors results in impaired transmission at the

neuromuscular junction and leads to the patients' pathognomonic fluctuating muscle weakness (Vincent 2002). MG is more prevalent in patients with B-type thymomas than types A or AB and is not observed in patients with TCs (Chen, Marx et al. 2002, Radovich, Pickering et al. 2018) Table 3.

Table 3: Frequency of the different histological TET subtypes in relation to MG and stage Adopted from Thymic epithelial tumours: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up (Girard, Ruffini et al. 2015). The statistics were derived from four publications (Kojima, Ito et al. 2006, Ruffini, Detterbeck et al. 2014, Omasa, Date et al. 2015, Weis, Yao et al. 2015).

	Relative frequency	**Myasthenia gravis**	**Masaoka stage**				
			I	**II**	**III**	**IVA**	**IVB**
Type A	12 (3–26)	15 (0–35)	60	31	8	<1	<1
Type AB	28 (15–43)	20 (5–42)	67	26	6	1	1
Type B1	18 (6–53)	40 (5–69)	50	37	9	3	1
Type B2	26 (8–41)	50 (23–73)	32	29	28	8	3
Type B3	16 (3–35)	50 (25–65)	19	36	27	1	3
Carcinoma	18 (1–28)	<5	10	10	45	15	20

Intrathymic pathogenesis of MG

MG is a well described autoimmune disease with well identified autoimmune targets and autoimmune effector mechanisms (Levinson 2013). Evidence for the role of thymus in pathogenesis of MG was collected: pathological evidence (germinal center hyperplasia and thymomas), clinical evidence (treatment benefits of surgical thymectomy), immunological evidence (thymic B cells and $CD4^+$ T cells reactive to nicotinic acetylcholine receptors (AChRα), decreased function and number of thymic regulatory T cells, increased chemokine expression (CXCL13 and CCL21) of hyperplastic thymi of MG patients) and structural evidence (nicotinic AChR subunits expressed on myoid cells and thymic epithelial cells). Despite the increase in knowledge on MG the events leading to a loss of self tolerance to AChRs remain elusive (Levinson 2013).

Recently, the first randomized controlled trial of thymectomy in non-thymomatous MG confirmed the clinical improvement of patients undergoing thymectomy (Wolfe, Kaminski et al. 2016).

Thymoma-associated MG (TAMG)

Multi-omic analysis of TAMG as part of the "The Cancer Genome Atlas" project observed a higher prevalence of aneuploidy in thymomas of MG patients (Radovich,

Pickering et al. 2018). Interestingly, the presence of MG was not associated with mutations in single genes or methylation patterns in TETs compared to MG negative TETs (e.g. *GTF2I)*. Tumors of TAMG patients revealed an overexpression of genes with limited (*NEFM*) or extensive *(RYR3)* sequence similarity of autoimmune targets. Thus thymomas and MG were linked by the expression of known autoimmune targets (muscle autoantigens) and increased aneuploidy (Radovich, Pickering et al. 2018).

2.5 Molecular biology

Molecular profiling utilizing next generation gene panel sequencing combined with FISH and immunohistochemistry can be utilized in the search for novel therapeutic targets (Enkner, Pichlhofer et al. 2017). Nonsynonymous mutations were identified in TCs by cancer gene sequencing of ALK, ATM, CDKN2A, ERBB4, FGFR3, KIT, NRAS and TP53. Fluorescence in situ hybridization (FISH) detected gene deletions of CDKN2A, TP53 and ATM in TCs, but not in type A thymomas. Differences between TCs and type A thyomomas in total microRNA were detected by sequencing (e.g.C19MC microRNA cluster highly expressed in type, but silenced in TC). Immunohistochemistry showed increased PDGFRA in TCs and PD-L1 in type B3 and TCs. The identified differences in cancer gene mutations and differential microRNA expression between type B3 and A thymomas and TCs may spark new developments in drug development (Enkner, Pichlhofer et al. 2017).

The genome of TETs was explored in with a muli-omic platform participating in the "The Cancer Genome Atlas". The gene *GTF2I* showed a high mutational frequency (39%) in WHO type A ad AB thymomas. Other recurrent somatic mutations at a lower frequency were described for the genes of *HRAS, TP53* and *NRAS.* The clonality of all four significantly mutated genes suggested their involvement in early thymic cancer development (Radovich, Pickering et al. 2018). The multi-omics approach could define four robust molecular types of TETs associated with differing survival outcome and revealed that thymomas have the lowest mutational burden of all investigated cancers in adults. The authors suggested that future drug development based on the advances in genomic knowledge of the project will have an impact on TET management (Radovich, Pickering et al. 2018).

2.6 Screening, symptoms, diagnosis and prognosis

2.6.1 Screening

In patients with paraneoplastic syndromes, particularly MG, screening for thymomas is recommended by chest CT (followed by FDG-PET) or integrated FDG-PET/CT (Titulaer, Soffietti et al. 2011).

2.6.2 Symptoms

TETS can present as an incidental finding in asymptomatic people undergoing screening investigations or during radiological workup (Chest X-ray (CXR) or computed tomography (CT)) for unrelated symptoms or disorders. TETs can present symptoms due to local compression of (thoracic) organs or systemic symptoms from TET associated paraneoplastic diseases.

Local symptoms arising from TETs that are typically located in the bed of the thymus from compression and/or invasion of adjacent thoracic organs (e.g. cough, dyspnea, chest pain). Rare symptoms are superior vena cava syndrome from direct mediastinal mass compression of the superior caval vein or diaphragmatic paralysis due to phrenic nerve invasion/compression.

Thymomas may manifest with a characteristic growth pattern along the serous membranes in the chest cavity, the pleura and the pericardium. Pleural and pericardial effusions cause local symptoms and are a sign of more advanced disease.

2.6.3 Diagnosis

A preoperative biopsy is obtained in cases of suspicion of lymphoma, germ cell tumors, mediastinal metastasis and patients with suspicion of advanced TETs with infiltration on potentially resectable structures for planning of neoadjuvant therapy. In MG patients with a high suspicion for thymomas surgery is planned without the need for a biopsy.

2.6.4 Imaging

Chest X-rays (CXR)

Forty-five to 80% of thymomas were reported to be visible on CXR (Marom 2010) (See Figure 3). Any anterior mediastinal mass identified on CXR has to be further characterized by CT. Patients with clinical suspicion for thymoma and normal CXR will have a CT scan because of higher sensitivity (Marom 2010).

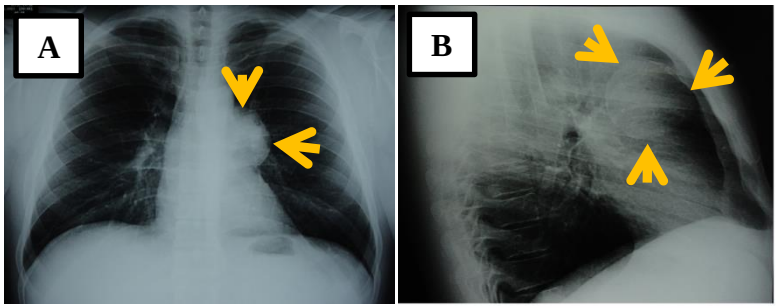


Figure 3: Chest X-ray of ADC of the thymus. Adopted from (Moser, Schiefer et al. 2015).

Computed tomography (CT)

CT is the preferred imaging modality for diagnosis and assessment of resectability for patients with TETs (See Figure 4). Intravenous contrast agents are essential for preoperative staging and assessment of resectability of invasive tumors (e.g. vessel infiltration) (Marom 2010). Standard report terms for chest CT findings of anterior mediastinal masses suspicious for thymomas were defined by ITMIG (Marom, Rosado-de-Christenson et al. 2011).

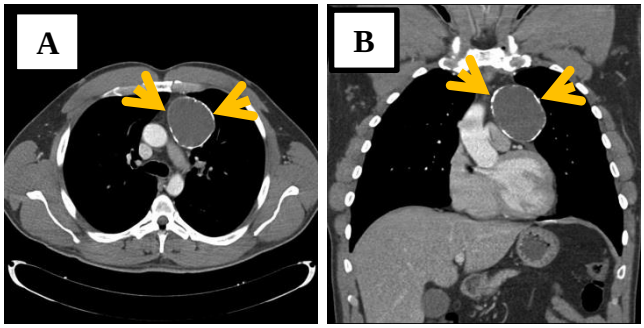


Figure 4: Computed tomography of ADC of the thymus. Adapted from (Moser, Schiefer et al. 2015).

In a retrospective study of 133 patients with thymoma who underwent surgical resection (1997-2010) 23 patients (17%) had an incomplete surgical resection. Several preoperative CT imaging characteristics predicting respectability were identified: lobulated tumor contour, ≥50% abutment of vessel circumference, thoracic lymphadenopathy, lung changes, pleural nodularity, larger tumor size (mean 9.7 cm). On multivariable analysis only degree of vessel abutment and pleural nodularity remained independent prognosticators of

incomplete resection. The authors concluded that CT can predict the probability of complete surgical resection and might help identify patients benefiting from neoadjuvant treatments (Hayes, Huang et al. 2014).

In a retrospective study on 84 patients with thymoma (1986-2007) associations between computed tomography features of thymomas and their pathological classification (Masaoka-Koga) were reported (Ozawa, Hara et al. 2016). Stage III-IV thymomas were of larger size, displayed a more irregular shape or contour, and necrosis and calcification were more prevalent than in stages I-II. WHO B2 an B3 type thymomas showed irregular contour and shape, invasion of: mediastinal fat, great vessel, pericardium or lung more often than WHO type A, AB or B1 thymomas.

Magnetic resonance imaging (MRI)

MRI has no radiation toxicity but is not recommended for mediastinal masses with unknown etiology because it provides poor resolution of pulmonary parenchyma. It may be employed to assess vessel invasion with or without intravenous contrast agents (Marom 2010).

^{18}Fluorine-fluorodeoxyglucose–positron emission tomography (FDG-PET)

In a retrospective study on 94 patients with anterior mediastinal nodules or masses PET/CT maximum standard uptake value (SUV_{max}) was found to discriminate thymomas from TC, diffuse large cell B cell lymphoma and Hodgkin lymphoma. The authors suggested that a tumor tissue biopsy should be obtained in cases of $SUV_{max} \geq 7.5$ to possibly select patients with TC for neoadjuvant therapy and to avoid futile resections in patients with lymphomas (see Figure 5; (Watanabe, Shimomura et al. 2019)).

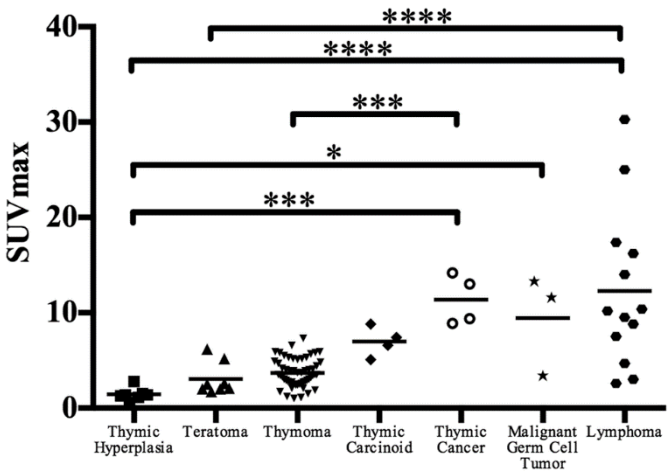


Figure 5 SUVmax of anterior mediastinal tumors. Distribution of SUVmax (FDG-PET CT) of patients with anterior mediastinal tumors. From (Watanabe, Shimomura et al. 2019). *$p < 0.05$, ***$p < 0.005$, ****$p < 0.001$

Conflicting data regarding the use of SUV_{max} to distinguish thymoma WHO types were reported (Shibata, Nomori et al. 2009, Otsuka 2012, Watanabe, Shimomura et al. 2019). FDG-PET CT SUV_{max} may aide in detecting local invasiveness of thymomas but SUV_{max} cut-offs for Masaoka-Koga or TNM stage could not be defined (Luzzi, Campione et al. 2009, Otsuka 2012, Watanabe, Shimomura et al. 2019). In an ITMIG prospective database with FDG-PET data of 154 patients SUV_{max} was reported to predict histologic type and pathologic Masaoka-Koga stage; ROC analysis: area under curve: 0.79; $p < 0.001$ and 0.81; $p < 0.001$, respectively (Korst, Fernando et al. 2017).

In a study of 27 patients with advanced or recurrent TETs response to chemotherapy was assessed by 18F-FDG PET-CT according to RECIST criteria (Response Evaluation Criteria in Solid Tumor). Percent change of SUVmax in before and after treatment 18F-FDG PET-CT correlated with morphovolumetric response ($r = 0.64$, $p = 0.001$). Percent change of SUVmax of -25% was reported to discriminate responders from non-responders (sensitivity of 88% and a specificity of 80%) (Segreto, Fonti et al. 2017).

Of additional value are ^{68}Gallium labeled **somatostatin** (SST) analogues and somatostatin receptor scintigraphies to evaluate further therapeutic modalities in patients not responding to therapy (Imbimbo, Ottaviano et al. 2018). ^{68}Ga-SST-analogues PET/CT and 18F-FDG-PET/CT showed concordance in 43% of 39 patients with metastasized TETs. In only 5% of patients there was additional information when ^{68}Ga-SST-analogues PET/CT was positive and 18F-FDG-PET/CT was negative (Sollini, Erba et al. 2014).

2.7 Tumor staging systems

Over time diffenent staging systems for patients with TETs have evolved (International Association for the Study of Lung Cancer 2016). A widely used staging system that was used for all studies in this thesis is the Koga modification (Koga, Matsuno et al. 1994) of the Masaoka (Masaoka, Monden et al. 1981) stage classification. Stage definitions for the Koga modification of the Masaoka system (from now on termed Masaoka-Koga) are depicted in Table 4 (Detterbeck, Nicholson et al. 2011).

Table 4: Masaoka-Koga staging sytem. Adapted from Koga et al. and Detterbeck et al (Koga, Matsuno et al. 1994, Detterbeck, Nicholson et al. 2011).

I	Grossly and microscopically completely encapsulated tumor
IIa	Microscopic transcapsular invasion
IIb	Macroscopic invasion into thymic or surrounding fatty tissue, or grossly adherent to but not breaking through mediastinal pleura or pericardium
III	Macroscopic invasion into neighboring organ (i.e., pericardium, great vessel, or lung)
IVa	Pleural or pericardial metastases
IVb	Lymphogenous or hematogenous metastasis

Although the Masaoka-Koga staging system was the most widely used staging system for TETs, more than 15 different classification systems were in use to stage patients with TETs (Filosso, Ruffini et al. 2014). In an effort to create one official stage classification for TETs, the Union for International Cancer control (UICC) and the American Joint Committee on Cancer (AJCC) have reviewed a retrospective database including data of 10,808 patients collected by the International Thymic Malignancies Interest Group (ITMIG) and International Association for the Study of Lung Cancer (IASLC) in order to develop a new TNM Clinical Classification for TETs (see

Table 5) (Detterbeck, Stratton et al. 2014, Filosso, Ruffini et al. 2014, Kondo, Van Schil et al. 2014, Nicholson, Detterbeck et al. 2014, Carter, Benveniste et al. 2017).

Table 5: IASLC/ITMIG TNM (8th edition) categories and stage. Adapted from the Staging Manual in Thoracic Oncology, Second Edition, An International Association for the Study of Lung Cancer Publication (International Association for the Study of Lung Cancer 2016).

TX	Primary tumor cannot be assessed.
T0	No evidence of primary tumor
T1	Tumor encapsulated or extending into the mediastinal fat, may involve the mediastinal pleura.
T1a	No mediastinal pleural involvement
T1b	Direct invasion of the mediastinal pleura
T2	Tumor with direct involvement of the pericardium (partial or full thickness).
T3	Tumor with direct invasion into any of the following; lung, brachiocephalic vein, superior vena cava, phrenic nerve, chest wall, or extrapericardial pulmonary artery or vein.
T4	Tumor with direct invasion into any of the following; aorta (ascending, arch or descending), arch vessels, intrapericardial pulmonary artery, myocardium, trachea, or oesophagus
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in anterior (perithymic) lymph nodes
N2	Metastasis in deep intrathoracic or cervical lymph nodes
M0	No pleural, pericardial or distant metastasis
M1	Distant metastasis
M1a	Separate pleural or pericardial nodule(s)
M1b	Distant metastasis beyond the pleura or pericardium

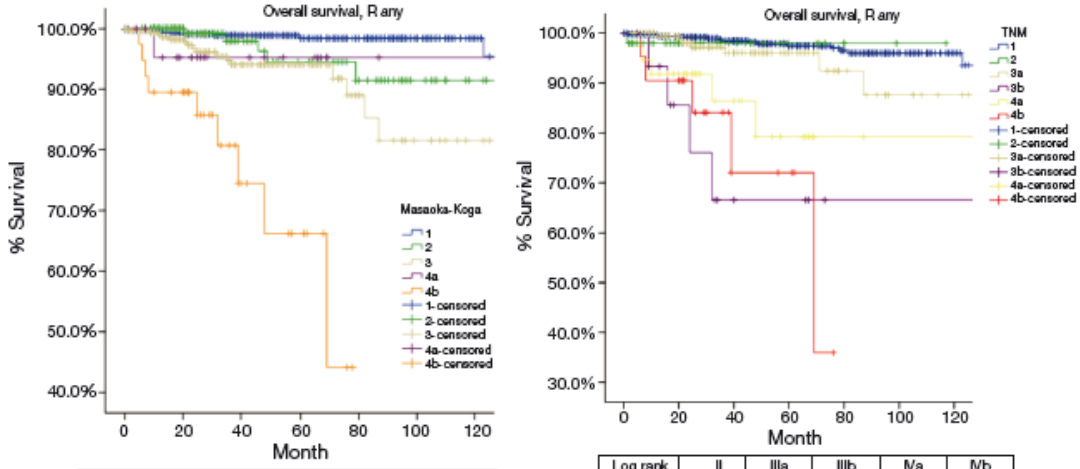
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage IIIA	T3	N0	M0
Stage IIIB	T4	N0	M0
Stage IVA	Any T	N1	M0

	Any T	N0, N1	M1a
Stage IVB	Any T	N2	M0, M1a
	Any T	Any N	M1b

A lymph node map was proposed by ITMIG (Detterbeck, Stratton et al. 2014, Carter, Benveniste et al. 2017). The new N descriptor distinguishes the anterior region (N1), including prevascular, para-aortic, ascending aorta, superior and inferior phrenic, supradiaphragmatic, and low anterior cervical lymphnodes from the deep region (N2), including internal mammary, upper and lower paratracheal, subaortic, subcarinal, hilar, lower jugular and supraclavicular lymph nodes.

The ESMO Clinical Practice Guidelines 2015 recommend the routine removal of N1 nodes (anterior mediastinal and anterior cervical). A systematic lymphadenectomy of N1 and N2 nodes is only recommended for TC histology which presents with higher rates of lymphatic metastases than thymomas (20% vs. 3%) (Girard, Ruffini et al. 2015).

The comparison of OS staged according to the Masaoka-Koga and TNM system is depicted in Figure 6.



Log rank	II	III	IVa	IVb
I	0.111	0.000	0.194	0.000
II		0.103	0.642	0.000
III			0.864	0.000
IVa				0.043

Log rank	II	IIIa	IIIb	IVa	IVb
I	0.694	0.072	0.000	0.000	0.000
II		0.718	0.006	0.069	0.004
IIIa			0.000	0.008	0.000
IIIb				0.312	0.901
IVa					0.315

Figure 6: OS of patients with any R resection in different stage by the Masaoka-Koga or the 8th edition TNM staging (Kaplan-Meier survival curves: log-rank test). Adopted from (Liang, Gu et al. 2016).

The Masaoka-Koga and IASLC/ITMIG TNM (8th edition) are displayed side by side in order to hightlight differences (see Table 6).

Table 6: Relationship between IASLC/ITMIG TNM (8th edition) categories and Masaoka-Koga staging system. Adopted from (Liang, Gu et al. 2016).

TNM Stage	**TNM**	**Definition (involvement of)**	**Masaoka-Koga**
Stage I	T1aN0M0	Encapsulated or unencapsulated, with or without extension into mediastinal fat	Stage I and II
	T1bN0M0	Extension into mediastinal pleura	Stage III (partial-pleura)
Stage II	T2N0M0	Pericardium	Stage III (partial-pericardium)
Stage IIIa	T3N0M0	Lung, brachiocephalic vein, superior vena cava, chest wall, phrenic nerve, hilar (extrapericardial) pulmonary vessels	Stage III (partial-completeness of resection)
Stage IIIb	T4N0M0	Aorta, arch vessels, main pulmonary artery, myocardium, trachea, or esophagus	Stage III (partial-incompleteness of resection)
Stage IVa	TxN1M0	Anterior (perithymic) nodes	Stage IVb
	TxN0M1a	Separate pleural or pericardial nodule(s)	Stage IVa
	TxN1M1a	Anterior (perithymic) nodes, Separate pleural or pericardial nodule(s)	Stage IVb
Stage IVb	TxN2M0	Deep intrathoracic or cervical nodes	Stage IVb
	TxN2M1a	Deep intrathoracic or cervical nodes, Separate pleural or pericardial nodule(s)	Stage IVb
	TxNxM1b	Pulmonary intraparenchymal nodule or distant organ metastasis	Stage IVb

2.8 Definition of TET recurrences

Recurrences of TETs can occur after all disease was eradicated (after R0 Resection or Radiographic Complete Response). Classification of recurrences in local, regional and distant was followed as recommended by ITMIG (see

Table 7) (Huang, Detterbeck et al. 2011).

Table 7: ITMIG Definitions of TET recurrence. Adopted from (Huang, Detterbeck et al. 2011).

Local recurrence—anterior mediastinum

- TET occurring in bed of thymus or previously resected TET
- Includes pericardial, pleural, or pulmonary tumor that is immediately adjacent to the thymus or previously resected TET
- Lymph nodes immediately adjacent to the thymus or previously resected TET (including nodes in the neck immediately adjacent to the upper poles of the thymus)
- Recurrence at the site of a previous noncontiguous metastasis (stage IVa)

Regional recurrence—intrathoracic recurrence not contiguous with thymus or previous thymoma

- Parietal or visceral pleural nodules
- Pericardial nodules
- Mediastinal lymph nodes not adjacent to the normal thymus or the previous TET

Distant recurrence

- Extrathoracic recurrence
- Intraparenchymal pulmonary nodules (with rim of normal lung between the nodule and the visceral pleura)

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2.9 Treatment of TETs

The following descriptions on TET treatment are focused but not limited to the treatment of patients undergoing surgery. All studies in this thesis were performed on TET patients who underwent surgical resection with or without multimodal therapies.

2.9.1 National and international Consensus statements

Different countries have formulated national expert consensus guidelines for the treatment of patients with TETs. Exemplary, the French and Italian efforts are briefly explained. In 2012 in France a nationwide network named RYTHMIC (**R**éseau tumeurs T**HYM**iques et **C**ancer) for the management and research on patients with TETs was initiated by the French National Cancer Institute. A central pathologic review of all TET specimens as well as a tumor board discussion of all patients are central components of the French network (Hadoux, Girard et al. 2012). In the recent past many national efforts directed at improving patient care for those diagnosed with TETs emerged. In 2014 in Italy a network called TYME (**T**h**Y**mic **M**alignanci**E**s was founded (Imbimbo, Ottaviano et al. 2018). An expert consensus of 66 multi-disciplinary specialists from 27 Italian centres for the management (diagnosis and treatment) of TETs in Italy was published (Imbimbo, Ottaviano et al. 2018).

Other national efforts include the Chinese Alliance for Research in Thymomas (ChART) registry (Wang, Pang et al. 2016) or the Japanese Association for Research on the Thymus (JART) (Okuda, Yano et al. 2014).

International efforts of the ESTS Thymic working group (Ruffini, Falcoz et al. 2018) and ITMIG (Detterbeck 2013) have established large thymic databases necessary to establish guidelines based on higher quality evidence.

2.9.2 Management algorithm: resectable disease

The 2015 ESMO (European Society for Medical Oncology) clinical practice guidelines for patients with resectable TETs (European Society for Medical Oncology) is depicted in Figure 7.

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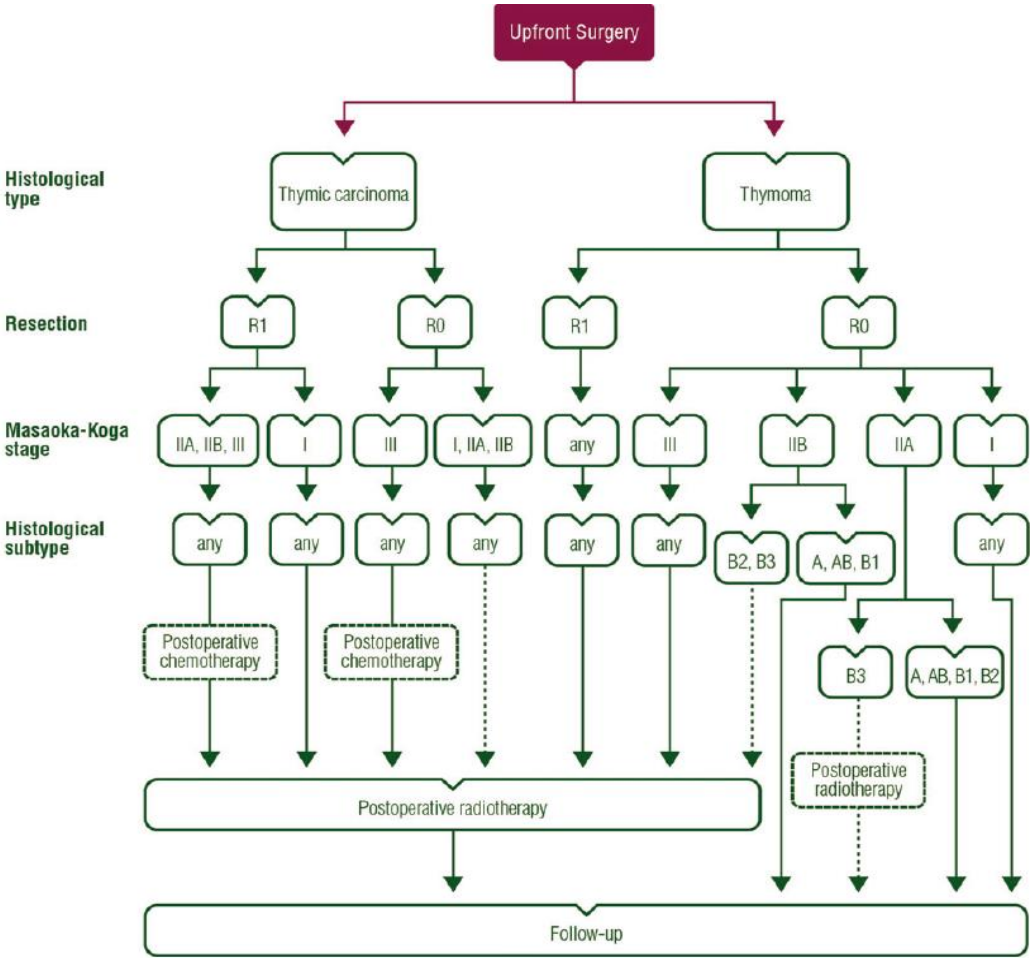

Figure 7: Treatment algorithm for resectable thymic tumour (Masaoka-Koga stage I–III, TNM stage I–IIIA). From: Thymic epithelial tumours: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up (14).

2.9.3 Institutional treatment specifics

There are no randomized controlled clinical multicenter trials on the treatment of TETs. The current medical evidence is mostly based on retrospective single center experiences and population studies. Thus the peer-reviewed literature cannot give definitive recommendations for patient care. We recommend that all cases undergo multidisciplinary tumor board review. The treatment scheme applied for TET patients is based on the experience gained from our thoracic surgery center, a multidisciplinary consensus of thoracic surgeons, oncologists, radiotherapists, and pathologists (see

Table 8).

Table 8: Therapeutic algorithm at the Division of Thoracic Surgery, Medical University Vienna (Moser, Scharitzer et al. 2014).

WHO type stage[a]	A, AB, B1	B2, B3	Thymic carcinoma
I	-	-	-
II	-	-	adjuvant therapy?
III	nCRT	nCRT	nCRT
IV	nCRT	nCRT	nCRT

nCRT neoadjuvant Chemo- and/or radiotherapy
[a]*Masaoka-Koga stage*

The institutional treatment algorithm stipulates a multimodal therapy regimen for patients in Masaoka–Koga stages III and IV. For TCs neoadjuvant or adjuvant therapy is discussed also in stage II patients. Standard chemotherapy for either neoadjuvant or adjuvant chemotherapy consists of three cycles of cisplatin 50 mg/m^2, doxorubicin 50 mg/m^2, and cyclophosphamide 500 mg/m^2 (PAC chemotherapy). Individualized radiation therapy in an adjuvant or neoadjuvant setting is applied with doses up to 50 to 60 Gray. For definitive staging and histology the histopathological examination of the operative TET specimen is reqired. All TET specimens are reviewed by the reference pathology at the Faculty of Mannheim, University of Heidelberg, Germany (second opinion). Decisions on postoperative RT (PORT) or adjuvant ChT are based on pathological staging.

2.9.4 Surgery

If a complete TET resection (R0) is deemed feasible upfront surgery is indicated. This is usually the case in Masaoka-Koga stages I and II, as well as stage III with invasion of structures that are readily resectable (e.g. pericardium, adjacent mediastinal pleura or lung). In this Chapter different surgical approaches to TETs, the rationale for extended thymectomy and surgery for TETs with pleural involvement are detailed.

2.9.4.1 Surgical approach: open vs. minimally invasive

A variety of different open and minimally-invasive surgical approaches for the resection of TETs are in use. The open approaches are: thoracotomy, sternotomy, hemiclamshell and clamshell and cervical incisions. Minimally-invasive techniques include various modifications of VATS and robotic surgery (RATS) (Figure 8). Minimally-invasive techniques are established as the treatment standard for early-stage TETs (Masaoka-Koga stages I and II) by specialized centers experienced in thymic surgery (Liu, Lin et al. 2014,

Manoly, Whistance et al. 2014, Friedant, Handorf et al. 2016). All the oncological principles that are followed in open standard thymic surgery have to be met. There are no signs that minimally-invasive procedures are inferior to open surgery considering complications, recurrence rates or survival. The reported advantages of minimally-invasive surgery are shorter length of hospital stay, less intraoperative blood loss and improved cosmetic results (Ruffini, Filosso et al. 2018). The state of the art of thymic minimally invasive surgery across Europe (Matilla, Klepetko et al. 2017) as well as our initial experience with a combined sequential left-sided and subxiphoid video-assisted thoracic surgery approach for resection of large anterior mediastinal tumors (Matilla JR 2018) was recently reviewed by our group.

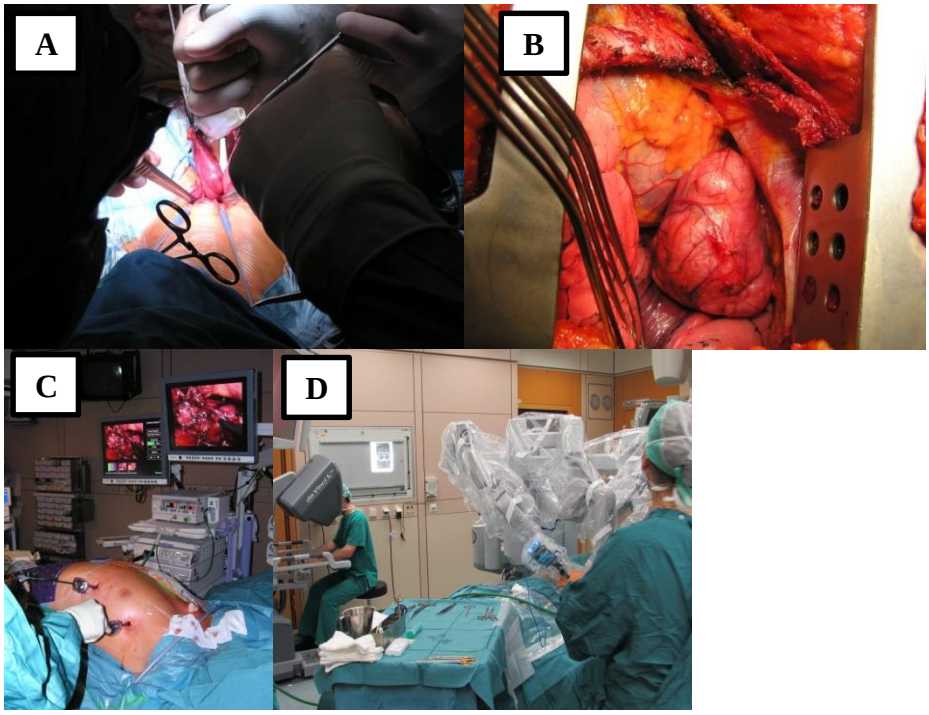

Figure 8: Surgical approach. Open surgery: through (A) cervical incision for basic thymectomy. Cervical thymectomy: The two thymic horns are developed through a cervical incision. The part of the surgical thymus shown in the photograph is called basic thymectomy. Left-sided thoracotomy for resection of thymoma ((B) arrow heads), VATS thymectomy (C), Robotic thymectomy (D). Photographs A-D from the division of thoracic surgery, Medical University Vienna;

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Rationale for extended thymectomy

Basic thymectomy is the surgical removal of the thymic horns (see Figure 8A and Figure 9).

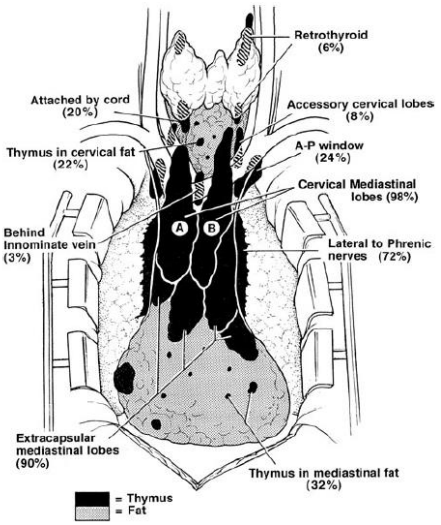

Figure 9: Surgical anatomy of the thymus. *(black,thymus; gray, fat, which may contain islands of thymus and microscopic thymus).* From (Sonett and Jaretzki 2008).

Thymomectomy is the removal of the thymoma/TC without removal of the thymus. Extended thymectomy is the removal of all mediastinal fatty tissue between the phrenic nerves along with basic thymectomy. While extended thymectomy is the treatment of choice for patients with myasthenia gravis its role in thoracic surgery for patients with thymomas without MG is still a matter of debate. The rationale to perform extended thymectomy in patients with MG with and without TETs is to completely remove all thymic tissue that is dispersed within the mediastinal fatty tissue (see Figure 9 and Figure 10) (Masaoka, Nagaoka et al. 1975, Sonett and Jaretzki 2008). Extended thymectomy for MG is supported by improvements in MG disease activity in patients with residual thymus undergoing extended thymectomy after failure to improve after basic or non-radical thymectomy (Masaoka and Monden 1981, Jaretzki, Penn et al. 1988). In cases of patients with TETs without MG current recommendations advocate an extended thymectomy in conjunction with the resection of the TET (Ruffini, Filosso et al. 2018). In support of this more radical approach are the risk of multiple TETs, risk of local recurrences, risk of postoperative newly developed MG, the difficulty to pre- and intraoperatively decide

whether the TET is encapsulated and the difficulty to achieve tumor-free resection margins in stage II tumors (Ruffini, Filosso et al. 2018).

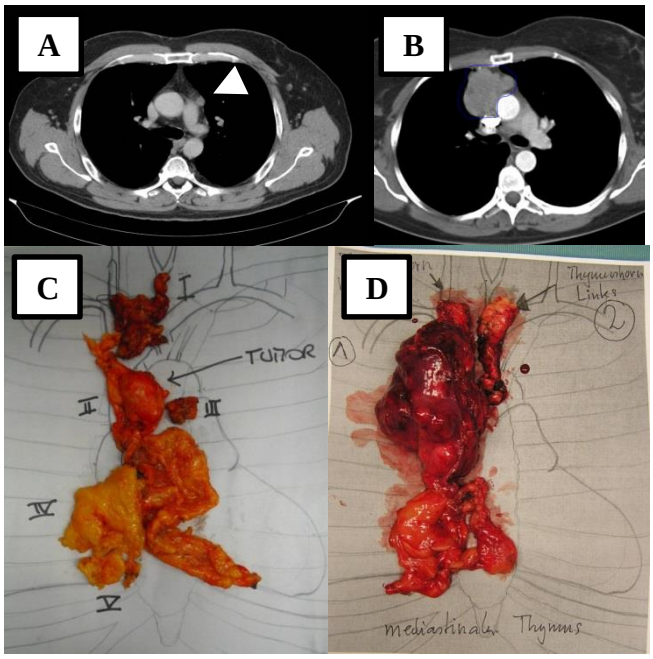

Figure 10: Extended thymectomy. Representative CT sections with the corresponding operative specimens of extended thymectomies (picture from the division of thoracic surgery, Medical University Vienna) of (A and C) a patient with MG (Osserman II-b): cervikal & left VATS approach; thymoma - WHO type B2 Masaoka II-1, R0 (B) (B and D) Incidental finding during preoperative radiological workup strumectomy); thoracotomy; thymoma WHO type AB, Masaoka II-1, R0, no MG; follicular thymitis;

2.9.4.2 Treatment of TETs with pleural involvement

For lower stages of TETs complete surgical resection has become the accepted treatment standard. Patients with advanced-stage TETs presenting with pleural or pericardial dissemination (Masaoka-Koga Stage IVA (Koga, Matsuno et al. 1994)) are encountered in only 6.8% of all patients with TETs (Koga, Matsuno et al. 1994, Kondo and Monden 2003, Murakawa, Karasaki et al. 2015). Because of the scarcity of existing data in cases with TETs with pleural disease the value of surgical resection remains in question. There are several reasons for low case numbers: TETs with pleural involvement are usually treated in single institutions. Also, there is a wide array of different forms of clinical presentation. While some patients are diagnosed with one or few well defined and localized pleural lesions , others present with a diffuse pattern of pleural involvement. A subset

of patients presents the combination of pleural and pulmonary tumor spread. Depending on the disease distribution several surgical techniques were developed for resection: extrapleural pneumonectomy (EPP), total pleurectomy (TP) or local pleurectomy (LP). All of the surgical approaches are frequently combined with ChT and/or RT (Ishikawa, Matsuguma et al. 2009, Fabre, Fadel et al. 2011). EPP will be performed for numerous visceral pleural, parietal pleural and pericardial implants (and pulmonary nodules) that cannot be locally resected (Wright 2011). TP removes all parietal (Figure 11), mediastinal and diaphragmatic pleural surfaces and pericardium with or without resection of the diaphragm. Therefore TP is performed when visceral pleura and lung are not affected by malignant disease. LP is the local resection of pleural implants without removal of all pleural surfaces (metastasectomy) and is performed for mono- or oligometastatic disease. Pleurectomy/decortications (P/D) is a lung sparing procedure with the intent of removing all macroscopic disease in order to prolong patient survival. It is a TP of the parietal and visceral pleural surfaces (Imanishi, Nabe et al. 2018). Extended P/D (EPD) includes resection of diaphragm and pericardium in addition to P/D (Bilancia, Nardini et al. 2018, Imanishi, Nabe et al. 2018). In patients with Masaoka-Koga stage IVa (pleural or pericardial metastases) current recommendations include major pleural surgery with curative intent such as P/D or EPP usually performed as part of multimodal therapy (Ruffini, Filosso et al. 2018).

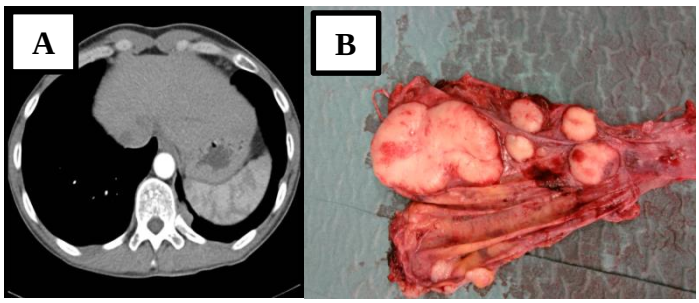

Figure 11: Pleural metastases of WHO type B2 thymoma. Representative CT scan section of the regional recurrence (A) with part of the operative specimen of total pleurectomy showing several pleural implants (B).

Debulking surgery

There is an ongoing debate on wheter debulking (reduction in tumor volume) for not completely resectable tumors has a role in TET surgery. Debulking surgery may be indicated in patients where resection can alleviate cardiopulmonary compromise (see Figure 12) in order to facilitate systemic treatments or RT. Debulking may reduce the number of local recurrences (Mornex, Resbeut et al. 1995) and reduce radiation field sizes of adjuvant RT (Attaran, Acharya et al. 2012). In cases of reoperation for recurrent thymomas debulking surgery should be limited to selected patients with no other available treatment options (Dai, Song et al. 2015).

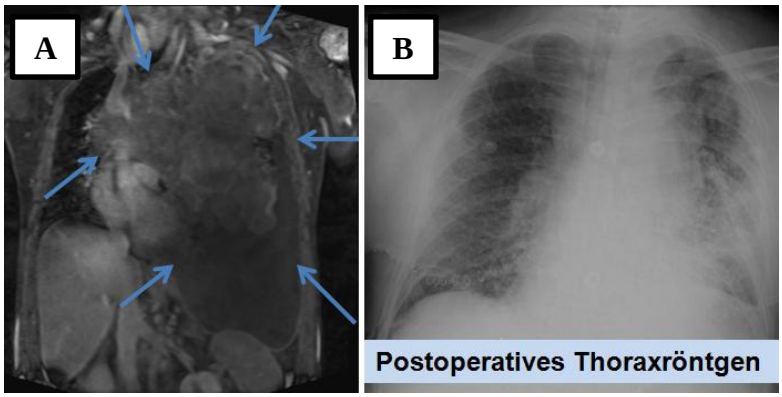

Figure 12: Magnetic resonance image (A) of a not completely resectable TC. Because of upper inflow occlusion and severe compromise of cardiorespiratory function the patient was not amenable to ChT. After incomplete resection of the TC regular cardiopulmonary conditions allowed adjuvant ChT (postoperative chest X-ray (B)). From (Moser and Klepetko 2013).

2.9.5 Systemic therapy

In patients with *unresectable* disease (distant metastases or technical unresectability, e.g. extensive infiltration of cardiac ventricles) or those unfit for surgery or anasthesia because of comorbidities may undergo individualized therapy. The patients`performance status, tumor stage or symptoms will influence decisions regarding the use RT, chemo- or chemoradiotherapy, or other systemic treatments. As all studies in this thesis were done on patients undergoing surgical resection the following sections will focus on systemic or radiation therapy with regard to patients undergoing surgery for TETs.

2.9.5.1 Chemotherapy (Cht)

ChT has a role in patients with unresectable disease, as neoadjuvant or adjuvant therapy and in metastatic and recurrent disease.

In cases of TET invasion of *potentially resectable* structures (e.g. superior vena cava, aortic arch) when upfront surgery is in doubt to achieve R0 resection borders neoadjuvant therapy should be considered in order to increase the probability for a complete resection. In a metaanalysis evaluating the effect of induction therapy (cisplatin based ChT) and surgery on OS of patients with advanced TETs reported a pooled response rate of induction therapy of 59%, a pooled rate of complete resection of 73% and pooled 5- and 10-year OS following induction therapy confirming favorable outcomes of this approach (Hamaji, Ali et al. 2015). The highest response rates (70-81%) with neoadjuvant ChT were reported with platinum-based ChT combined with anthracycline (Loehrer, Chen et al. 1997, Berruti, Borasio et al. 1999, Kim, Putnam et al. 2004, Chau, Kim et al. 2010).

PAC ChT for 29 patients with metastatic or recurrent thymoma (intergroup trial) showed three complete responses (CRs) and 12 partial responses (PRs): rate of combined CR+PR: 50% (Loehrer, Kim et al. 1994). Combinations of platinum based ChT without anthracycline were reported with inferior response rates: 32-56% (Giaccone, Ardizzoni et al. 1996, Loehrer, Jiroutek et al. 2001, Chau, Kim et al. 2010, Lemma, Lee et al. 2011) but are an option for patients who cannot undergo the most aggressive regimens. ChT with single agents was inferior to the combinations of several chemotherapeutic agents (Chau, Kim et al. 2010). A vast array of second-line chemotherapeutic approaches were undertaken for progression of disease with first line ChT (e.g. pemetrexed (Gbolahan, Porter et al. 2018), ocreotide (Loehrer, Wang et al. 2004)). The role of chemotherapy in advanced TETs was recently reviewed (Schmitt and Loehrer 2010).

2.9.5.2 Novel systemic therapies: targeted therapy and immunotherapy

The recent experience with immune checkpoint inhibitor therapy (Brown, Dorfman et al. 2003, Merveilleux du Vignaux, Maury et al. 2017, Badiyan, Roach et al. 2018, Saleh, Khalifeh-Saleh et al. 2018, Yokoyama and Miyoshi 2018) and the use of targeted therapies for patients with TETs was comprehensively reviewed (Berardi, De Lisa et al. 2014, Ried, Marx et al. 2016).

KIT is a potential target in TCs

KIT mutations are present in up to 12% of TCs (Schirosi, Nannini et al. 2012) . In patients with TCs with reactivity of CD117 (product of proto-oncogene c-kit) immunostaining

testing for c-kit is recommended. Several therapeutic possibilities were described as for example imatinib for mutated exons 9 and 11, sunitinib for mutated exons 13, 14 or sorafenib for mutated exon 17 (Schirosi, Nannini et al. 2012).

Immune checkpoint inhibitor therapy for TCs

Forty patients with recurrent TC progressing after ChT were treated with pembrolizumab, monoclonal antibody with specificity for PD-1 (single-arm phase 2 study), showed 22.5% objective reponses, including one complete reponse. Despite the observation that TC patients are not typically affected by paraneoplastic autoimmune disorders, a high rate of immune-related events (e.g. myocarditis) was reported (Giaccone, Kim et al. 2018).

2.9.6 Radiation therapy (RT)

Treatment recommendations regarding RT for TETs are mostly based on data of small patient cohorts collected over long periods of time and population-based studies (Fuller, Ramahi et al. 2010). The indications for RT as part of a multimodal approach as well as the optimal protocols for different treatment situations remain a matter of debate. All cases should be discussed in a multidisciplinary tumor board.

In the following treatment situations of patients with TETs there is a role for RT:

- unresectable disease (also progressive disease during neoadjuvant ChT)
- following incomplete resections of TETs (R1 or R2 resections).

The role of RT with regards to tumor stage was recently reviewed (Fuller, Ramahi et al. 2010). Postoperative radiation therapy (PORT) should be considered for patients after complete surgical resection of Masaoka-Koga stage II-IV TETs (ESMO guidelines) (Girard, Ruffini et al. 2015). RT is currently not recommended in Masaoka-Koga stage I after complete resection. One reason is the low frequency of recurrences. In stage I thymomas undergoing complete resections and followed-up for 32 years recurrence rates were reported with 2-3% (Awad, Symmans et al. 1998). Another reason is the lack of benefit of PORT in stage I studies. In a Chinese prospective study on 29 patients that were randomized to postoperative radiotherapy for stage I thymomas no survival benefit for either group was detected: 10-year OS: resection plus RT 88% vs. resection alone 92% (Zhang, Lu et al. 1999). The role of PORT after complete resection in stage II thymoma is still a matter of debate (See also Figure 7: Treatment algorithm for resectable thymic

tumour (Masaoka-Koga stage I–III, TNM stage I–IIIA). Several retrospective center experiences do not recommend PORT in this situation (Mangi, Wright et al. 2002, Rena, Papalia et al. 2007, Chen, Feng et al. 2010, Berman, Litzky et al. 2011). A meta-analysis including seven retrospective studies (1724 patients with primary thymoma) found a potential OS benefit of PORT for patients with locally advanced thymomas (Masaoka-Koga stages III and IV) after macroscopically complete resection compared to surgery alone. In this analysis PORT did not convey a survival benefit in stage II patients (Lim, Kim et al. 2016). TCs present in higher stages than thymomas and are accompanyied by lymphatic or hematogeneous metastases in 10-30% (Kondo and Monden 2003, Ruffini, Detterbeck et al. 2014, Hamaji, Shah et al. 2017). A Meta-Analysis of PORT for TC (7 retrospective observational studies, 786 patients) supports PORT for TC (recommendations regarding stage and resection status could not be provided) (Hamaji, Shah et al. 2017).

2.10 Prognostic factors for patients with TETs

2.10.1 Outcome measures for TETs

Overall survival (OS) is an adequate standard outcome measure for many cancers, especially when survival after recurrence is short and the cause of death is the particular cancer. In the case of thymomas many patients die of causes other than thymomas (see Figure 13) and many patients experiencing a thymoma recurrence have a long life expectancy (Huang, Detterbeck et al. 2011). Because of the indolent behavior of especially early stage thymomas, freedom from recurrence (FFR) reflects best the biology of these tumors after curative treatment. In addition to OS, CSS should be reported because it reflects death from thymoma/TC. The analysis of Time-to-progression (TTP) is recommended for patients with residual disease, although the outcome of this patient population is well reflected in the analysis of OS (Huang, Detterbeck et al. 2011). Another issue concerning survival analysis is the increased risk of thymoma patients to develop and die from extrathymic malignancies (Filosso, Galassi et al. 2013).

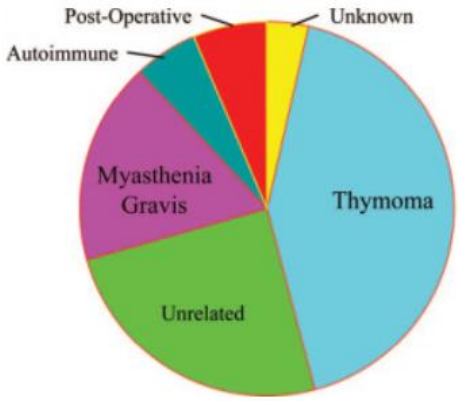

Figure 13: Overall cause of death after resection of thymomas. From (Huang, Detterbeck et al. 2011).

This is a brief description of the outcome measures recommended by ITMIG (Huang, Detterbeck et al. 2011) and ESTS Thymic Working group (Ruffini, Detterbeck et al. 2014, Ruffini, Detterbeck et al. 2014) that were used in this thesis. OS was calculated as the primary outcome from the date of surgery to the date of death of any cause (censored observations: patients at the last time point known to be alive). The end-point of interest for Cause-specific survival (CSS) was defined as death from TET (censored observations: unrelated deaths and unknown cause of death) (Huang, Detterbeck et al. 2011). FFR was calculated only in patients after complete surgical resection (R0) from the date of first pleural surgery to the date of recurrence in patients with full information on recurrence status (Ruffini, Detterbeck et al. 2014). Disease-free survival (DFS) was analyzed from the date of surgery to the date of recurrence or death from any cause (Ruffini, Detterbeck et al. 2014). For the determination of time to progression only patients after incomplete resection (R1 or R2) or patients with partial remission or stable disease after chemotherapy and/or radiotherapy were included (Huang, Detterbeck et al. 2011). Figure 14 depicts estimated outcomes for stage III thymomas when different outcome parameter definitions are applied (Huang, Detterbeck et al. 2011).

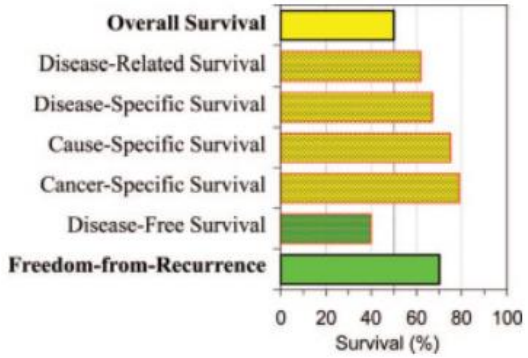

Figure 14: Comparison of different oucome measures after resection of stage III thymomas. From (Huang, Detterbeck et al. 2011).

2.10.2 Pathological predictors of treatment outcome

The most widely accepted prognostic factors for the treatment of patients with TETs are completeness of resection, Masaoka–Koga stage (discussed in section 2.7), and WHO histological type (discussed in section 2.3) (Chen, Marx et al. 2002, Detterbeck and Parsons 2011, Ruffini, Filosso et al. 2011, Venuta, Rendina et al. 2011, Ruffini, Detterbeck et al. 2014, Ruffini, Detterbeck et al. 2014). The WHO histological classification for TETs with regard to the different thymoma types is still a matter of intense discussion with respect to prognosis (Guerrera, Rendina et al. 2015).

In a Korean study including 1597 patients (2000-2013) undergoing resection of TETs with 446 patients undergoing "intentional" lymphnode dissection, lymph node metastasis was identified in 20 (6.7%) of 298 patients with thymoma and 47 (31.7%) of 148 patients with TC. TC (HR:19.2, $p<0.001$) and tumor size (HR:1.09, $p=0.02$) were significant predictive factors for lymph node metastasis at multivariable analysis. The 10-year FFR of pN1/pN2 was significantly worse than that of pN0 ($p<0.001$), but 10-year FFR for intentional lymph node dissection was not superior to the group without intention lymph node dissection (82.4% versus 80.9%, $p=0.46$ in thymoma; 45.7% versus 44.0%, $p=0.42$ in TC) (Hwang, Kang et al. 2018).

2.10.3 Experimental biomarkers for TETs

Sparked by our findings of the involvement of the receptor for advanced glycation endproducts (RAGE) in antigen-specific T cell expansion (Moser, Desai et al. 2007) we identified both soluble (s) receptors, sRAGE and endogenous secretory (es)RAGE to be significantly reduced in serum of patients with MG without TETs (Moser, Bekos et al. 2012). Further work identified a role of the experimental molecules RAGE and its ligand high mobility group box 1 (HMGB1) in thymic physiology and pathology of TETs. Tumor tissue expression of RAGE was most intense in WHO type B2 thymomas and TCs. HMGB1 nuclear staining was strongest in types A and AB, conversely to its cytoplasmic staining intensities: A and AB (none), B1 (strong), B2 (moderate), B3 and TC (weak). Serum concentrations of soluble RAGE were significantly reduced in patients with TETs; particularly invasive tumors. Whereas RAGE serum concentrations were equally reduced

in TH and TETs, HMGB1 was specifically elevated in TETs indicating diagnostic potential. (Moser, Janik et al. 2014).

Also, our group recently revealed a prognostic role of heat shock proteins (HSPs) for patients with TETs. HSPs were differentially expressed in WHO histologic types and pathological Masaoka-Koga tumor stages. Weak HSP tumor expression correlated with worse FFR. HSP serum concentrations were elevated in TETs and MG, correlated with clinical tumor stage and histologic subtype and decreased significantly after complete tumor resection (Janik, Schiefer et al. 2016).

Also recently described was the expression profile for three long non-coding RNAs (AFAP1-AS1, LINC00324, and VLDLR-AS1) to independently predict RFS among thymoma patients (Gong, Jin et al. 2018). Overexpression of cytotoxic T Lymphocyte Antigen 4 (CTLA-4 or CD152) significantly correlated with reduced OS in thymoma patients and in atypical thymomas (Santoni, Amantini et al. 2018).

2.11 Introduction to the biomarkers of the thesis

There is a growing body of evidence for the concept of inflammation being a significant part of tumor progression. Chronic inflammation in tumor microenvironments is thought to propagate tumor survival and proliferation (Coussens and Werb 2002, Korniluk, Koper et al. 2017).

Circulating biomarkers that are modulated in response to cancer-related inflammation may serve as powerful diagnostic and/or prognostic tools for TETs. For this PhD thesis we investigated a series of serum/plasma components and indices from peripheral blood derived cells of the innate and adaptive immune system or platelets: CRP, fibrinogen, neutrophil-to-lymphocyte ratio (NLR), and the platelet-to-lymphocyte ratio (PLR) in patients with TETs.

2.11.1 C-reactive protein (CRP)

CRP is a pentraxin family member with pentameric structure (see Figure 14) (Volanakis 2001, Pepys and Hirschfield 2003).

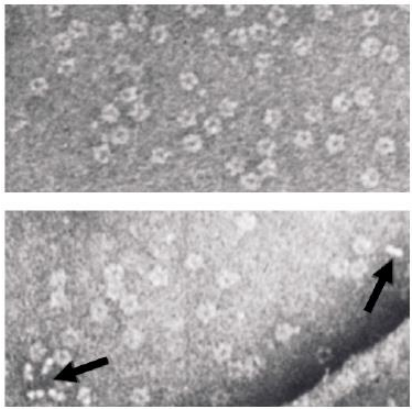

Figure 15: Human CRP. The pentameric disc-like structure face-on and side-on (arrows) in a negatively stained electron micrograph. From (Pepys and Hirschfield 2003).

Triggered by proinflammatory cytokines: interleukin (IL)-6, IL-1β and tumor necrosis factor alpha (TNFα-), it is produced by hepatocytes during the acute phase response (Gabay and Kushner 1999). With a constant half-life of 19 hours in plasma in health and disease, CRP plasma concentrations are a direct reflection of its synthesis rate (Vigushin, Pepys et al. 1993). Therefore the concentration circulating CRP in plasma (or serum) correlates directly with the intensity of the physiological or pathological stimuli driving CRP production (Pepys and Hirschfield 2003). CRP responses can be observed in a number of different disease processes: infections, allergic complications of infection, inflammatory disease, necrosis, trauma or malignancies. Also physiological processes influence circulating CRP concentrations. We have recently described the CRP changes in non-professional marathon runners (Bekos, Zimmermann et al. 2016). It is therefore very clear that CRP plasma (or serum) concentrations can only be interpreted in the clinical context (Pepys and Hirschfield 2003). CRP is a well established clinical marker indicating inflammation, infection and tissue damage (Pepys and Hirschfield 2003).

More recent is the interest in CRP serum concentrations in patients with cancer. Studies on the prognostic value of circulating CRP in patients with solid organ malignancies are accumulating (exemplary): gall bladder cancer (Saqib, Pathak et al. 2018), malignant pleural mesothelioma (Ghanim, Hoda et al. 2012), pancreatic cancer (Szkandera, Stotz et al. 2014), non-small cell lung cancer (O'Dowd, McRae et al. 2010), hepatocellular carcinoma (Nishikawa, Arimoto et al. 2013), nasopharyngeal carcinoma (Fang, Xu et al. 2017) and breast cancer (Villasenor, Flatt et al. 2014).

2.11.2 Fibrinogen

Fibrinogen is a heterodimeric molecule consisting of two parts with different polypeptide chains: Aα, Bβ, γ linked by disulphide bridges. It is produced by hepatocytes in response to proinflammatory cytokines (e.g. IL-6) and a major plasma component (Tennent, Brennan et al. 2007). One of its main functions pertains to coagulation. Thrombin cleaves Aα and Bβ to release fibrinopeptides A and B which polymerize to an insoluble fibrin clot. The endogenous fibrinolytic system can subsequently form fibrin degradation products (Lowe, Rumley et al. 2004). Further fibrinogen was shown to bind activated platelet glycoprotein receptor IIb/IIIa on the surface of activated platelets leading to platelet aggregation (Lowe, Rumley et al. 2004). Plasma fibrinogen concentrations are influenced by physiological, pathological and life style factors: inceased fibrinogen (genetic factors, age, gender, season, pregnancy and contraception, menopause, smoking, exercise, acute phase reactions) vs. decreased fibrinogen (A-, hypo- or Dysfibrinogenaemina; liver disease, disseminated intravascular coagulation, thrombolytic therapy, hemodilution), respectively (Mackie, Kitchen et al. 2003, Lowe, Rumley et al. 2004).

A prognostic role for plasma fibrinogen concentrations in thoracic: breast (Wen, Yang et al. 2015), non-small cell lung (Jiang, Li et al. 2014) and esophageal cancer (Wakatsuki, Matsumoto et al. 2017); and extra-thoracic malignancies: gall bladder (Shu, Weng et al. 2014), gastric (Lee, Lee et al. 2012), colorectal (Yamashita, Kitayama et al. 2009) and hepatocellular cancer (Huang, Jiang et al. 2018) is emerging (selected publications).

2.11.3 NLR and PLR: the role of neutrophils, lymphocytes and platelets in cancer

If a malignancy progresses protumorigenic inflammatory and immune mechanisms are stronger than their antitumorigenic counterparts. Cells of the innate and adaptive immune system have roles in different stages of tumor development: tumor initiation, promotion, invasion and metastasis (Grivennikov, Greten et al. 2010). Indices formed from platelets and immune/inflammatory cells such as neutrophils and lymphocytes are currently investigated for their prognostic potential regarding outcomes of cancer treatment (Ong, Garcea et al. 2008, Wu, Shi et al. 2014, Zhang, Jiang et al. 2015, Yodying, Matsuda et al. 2016, Hsueh, Tao et al. 2017, Pedrazzani, Mantovani et al. 2017, Turri-Zanoni, Salzano et al. 2017, Saqib, Pathak et al. 2018).

Neutrophil subpopulations are important in cancer development. Their granule proteins released following neutrophil activation are involved in cancer progression. Also, neutrophils can promote tumor cell proliferation, angiogenesis, matrix remodelling or interfere with T cell dependent anti-tumor immunity in part by direct interation of neutrophils with tumor cells (Mollinedo 2019).

Lymphocytes can exert tumor-suppressive or tumor-promoting effects (Grivennikov, Greten et al. 2010). Their effects are related to the type of lymphocyte: B cells or T cells and their respective subtypes. Lymphocytes exert their effects via direct cytotoxicity or the production of cytokines (Grivennikov, Greten et al. 2010).

Platelets have a role in the tumor microenvironment. Evidence for tumor induced platelet activation nurturing cancer progression by mechanisms of tumor cell induced platelet aggregation were described (Gasic, Gasic et al. 1968). The release of ADP and thrombin causes platelet activaton involved in tumor metastasis (Asghar, Parvaiz et al. 2019). Further platelets can reduce NKG2D expression in natural killer cells and form an platelet-fibrin-tumor aggregate for immune evasion (Erpenbeck and Schon 2010, Asghar, Parvaiz et al. 2019). Platelets were also found to mediate drug resistance of cancer cells (Huong, Nguyen et al. 2019).

DOI:10.14753/SE.2020.2356

3. Objectives

Patients with the rare disease TETs are being treated worldwide in multiple institutions in small numbers only. High quality evidence from randomized controlled trials regarding surgical or multimodal therapies is lacking. The overall ***aim*** of this PhD thesis was to increase scientific knowledge on surgically treated patients with TETs with particular emphasis on prognostic information.

3.1 Prognostic factors and multi-modal management of TETs treated in a single European thoracic surgery center

The ***aim*** of this study was to determine quality of care at the institutional thoracic surgery Division by assessing survival and recurrence outcomes and confirmation of the value of (well) accepted pathological prognostic information. This study set the ground for further studies more experimental studies on prognostic predictors.

The ***primary objective*** of the study was to determine prognostic factors in patients with TETs undergoing resection and multimodal treatment in a single central European thoracic surgery unit. The ***secondary objective*** was to perform a thorough analysis and documentation of all aspects of surgical treatment, such as surgical approach, extent of resection, and the treatment of recurrences.

3.2 Prognostic factors for TETs with pleural involvement: an ESTS Thymic Working Group Project

The status of surgical resection with or without ChT and/or RT for primary or recurrent TETs with pleural involvement is not sufficiently defined yet, due to limitations in the available data. There are no large-scale studies dedicated to describing the value of parietal pleurectomy and/or EPP in patients with pleural and/or pericardial involvement or dissemination of TETs. That is why we were interested in retrospectively collecting data about survival, recurrence or progression and multimodal therapy in patients treated with parietal pleurectomy or EPP. Because the number of thoracic surgery cases for thymic tumors with pleural involvement is extraordinarily low even in larger thoracic surgery

institutions the value of surgical treatment of this disease at primary presentation and its recurrences has not been explored sufficiently.

The ***aim*** of this multi-institutional effort was to gain scientific data in the rare disease of TETs with pleural involvement with a special emphasis on prognostic information.

The ***primary objective*** of the study was to determine prognostic factors in patients with TETs with pleural involvement undergoing resection and multimodal therapy at multiple institutions participating in ESTS Thymic Working Group projects. The ***secondary objective*** was to perform a thorough analysis and documentation of all aspects of surgical treatment, such as surgical approach, extent of resection, and the treatment of recurrences.

3.3 CRP as a prognostic marker for TETs

The study ***aim*** was to test for pretreatment and follow-up biomarkers valuable in estimating diagnosis, prognosis and surveillance of patients with TETs.

The ***primary objective*** of the study was to evaluate CRP serum concentrations as pretreatment prognostic factors in patients with TETs. The ***secondary objective*** was to investigate CRP as a biomarker for oncological follow-up of patients with TETs. As a **tertiary objective** this study also attempted to elucidate the possible source of CRP in patients with TETs.

3.4 Fibrinogen, NLR, and PLR as prognostic markers for TETs

The study ***aim*** was to develop pretreatment and follow-up biomarkers in patients with TETs.

The ***primary objective*** of the study was to determine the value of fibrinogen serum concentrations, NLR and PLR as pretreatment prognostic factors in patients with TETs. The ***secondary objective*** was to evaluate fibrinogen serum concentrations, NLR and PLR as biomarkers for oncological follow-up of patients with TETs.

DOI:10.14753/SE.2020.2356

4. Methods

4.1 Ethical considerations

Ethical approval for all the studies described in this PhD thesis was obtained from the institutional ethics committee of the Medical University Vienna. Written informed consent was obtained from all patients and volunteers who donated blood for study purposes. All serum samples were drawn at the Division of Thoracic Surgery, Division of Surgery, Medical University Vienna. Tumor tissues were retrieved from the Clinical Institute of Pathology, Medical University Vienna. All experiments were performed in accordance with the approved ethical guidelines. All data of patients and volunteers enrolled in this study were handled carefully and will not be passed to third persons. Every participant was given a unique code to ensure protection of personal data and sample evaluation. In addition, for the multi-institutional study "Surgical therapy of thymic tumours with pleural involvement: an ESTS Thymic Working Group Project" each participating institution obtained ethical approval from its ethics committee.

4.2 Research facility

All the laboratory work was performed at the Department of Surgery (ARGE Moser and ARGE Ankersmit, FOLAB - Surgical research facilities), Medical University Vienna. Infrastructure needed for the project like ELISA Reader, laminar flow, fridge, freezer, centrifuge, etc. was already present.

4.3 Prognostic factors and multi-modal management of TETs: a single center experience

4.3.1 Study design

The study was designed as a retrospective observational study (case series study) in order to describe the disease characteristics and outcome of patients treated for the orphan disease TETs at the Division of Thoracic Surgery, Department of Surgery, Medical University Vienna. The study period ranged from January 1, 2001 to June 1, 2010.

DOI:10.14753/SE.2020.2356

4.3.2 Patients, diagnostic workup and treatment decisions, tumor staging and histology

Patients undergoing thoracic surgery for TETs during the indicated time period at the thoracic surgery division were included in the study. Only one patient had primary surgery for a TET before this period. A total of 79 patients were identified. We excluded seven patients undergoing treatment for recurrences or progressions referred from other institutions because of lack of stage information at diagnosis and primary treatment at the referring institutions. Disease and treatment-specific documentation as well as follow-up information was complete for 72 patients.

The preoperative diagnostic workup of patients with suspected TETs consisted of blood testing, ECG, pulmonary function tests, neurological examination (to diagnose of exclude paraneoplastic disease, especially MG), CT scan of the chest and in the more recent past PET or PET CT in more advanced cases.

In patients with anterior mediastinal tumors where a clinical suspicion of lymphoma was raised preoperative biopsies were obtained to avoid futile surgery for lymphoma. Preoperative histological confirmation via core-needle biopsy was also obtained in cases where induction chemo- and/or radiotherapy were favored to primary resection of a suspected TET. Anterior mediastinal tumor biopsies were performed without traversing the pleura in order to avoid pleural spread of the suspected TET. Decisions on multimodal therapy were decided on by a dedicated panel of experts in thoracic surgery, oncology, radiotherapy, neurology and pathology.

The Masaoka-Koga staging system was used as the standard to document tumor stage (see tumor staging 2.7). Pathologists employed the WHO histological classification system (combined TETs are specifically analyzed in this report; see histology 2.3) and recurrences were reported as recommended by ITMIG (see 2.8). Only patients after R0 resection were included in the analysis of freedom-from recurrence. Time-to-progression was analyzed in patients after R1 or R2 resection with partial remission or stable disease after chemotherapy and/or radiotherapy.

Survival of patients undergoing surgery with different outcomes (remission, recurrence, progression) and different treatment modalities: surgery with or without chemo- and/or radiotherapy was investigated. The prognostic significance of stage, histology, residual

tumor classification, preoperative biopsies, multimodal therapy, MG, age and gender was explored.

4.3.3 Statistical analyses

SPSS software (version 17; SPSS Inc., Illinois, United States) was employed for statistical analysis. GraphPad Prism6 (GraphPad Software Inc., California, USA) was used for graphical display of all box plots and Kaplan-Meier curves. The probability of making a type I error was set at an α value of 0.05. The null hypothesis was rejected if the *p*-value was less than α . Two-tailed *p*-values were employed. Statistical independence of demographic data was computed with the Pearson χ^2 test. Kaplan-Meier survival analysis and log-rank test were employed to assess outcome measures such as OS, CSS and FFR for TETs. Recurrences and outcome measures were reported as recommended by ITMIG (see Chapter 2.10.1).

4.4 Surgical therapy of thymic tumours with pleural involvement: an ESTS Thymic Working Group Project

4.4.1 Study design

The order the achieve the objectives an international multi-institutional study under the patronage of the ESTS Thymic Working Group was initiated to collect a large enough patients cohort for outcome analysis. On behalf of the ESTS Thymic Working Group emails were sent to ESTS members to recruit thoracic surgery centers for participation on the project. Participating institutions received a detailed standardized questionnaire to obtain standardized retrospective data sets. Ten European and two Canadian institutions provided retrospective data from 152 patients of thymomas and TCs with pleural disease (see Table 9).

DOI:10.14753/SE.2020.2356

Table 9: Participating institutions and patient characteristics. Basic demographics and clinical parameters of all reported cases of each collaborating ESTS Thoracic Surgery Center. f:m ratio, female:male ratio; CHU Marseille, Centre Hospitalier Universitaire Marseille; UZ Leuven, Universitair Ziekenhuis Leuven; MUW, Medical University Vienna;

Basic demographic and clinical parameters	**n**	**%**
Total number of cases	152	100
f:m ratio	76:76	50:50
Age in years mean/median *[range]*	50.4 / 51.0 *[22-75]*	
Continent *Country* Institution		
Europe	125	82
France, Centre chirurgical Marie Lannelongue, Paris	38	25
France, CHU Marseille	18	12
Belgium, UZ Leuven	17	11
Austria, MUW Vienna	16	11
Great Britain, Guy's & St Thomas' Hospital, London	13	9
Switzerland, Universitätsspital Zürich	9	6
Italy, University of Torino	6	4
Spain, Madrid HU Puerta de Hierro Majadahonda	4	3
Spain, Unviersitario de Salamanca	2	1.5
Great Britain, Papworth Hospital, Cambridge	2	1.5
North America *Canada*		
Canada, Toronto	23	15
Canada, Montreal	4	3
Decade of diagnosis	152	100
1977-1990	2	1.3
1991-2000	12	7.9
2001-2010	86	56.6
2011-2014	52	34.2
Pleural Surgery for TETs	152	100
Surgery for pleural recurrence	45	29.6
Primary pleural surgery	107	70.4
Diagnosis	152	100
Thymoma	135	88.8
Thymic Carcinoma	17	11.2

Data stem from specialized thoracic surgery centers routinely performing surgery on patients with TETs. The study period was defined by the reported patients: thoracic surgery cases from February 1977 and November 2014. Only few cases were reported before January 2001 (90% of patients had surgery between January 1, 2001 and November 30, 2014). Survival analysis was performed on all 152 patients. Analysis of FFR was done on 115 patients with complete surgical resection (R0; See
Figure 16). The calculated median follow-up time of the entire patient cohort was 52 months [95% confidence interval: 32.0–72.0].

DOI:10.14753/SE.2020.2356

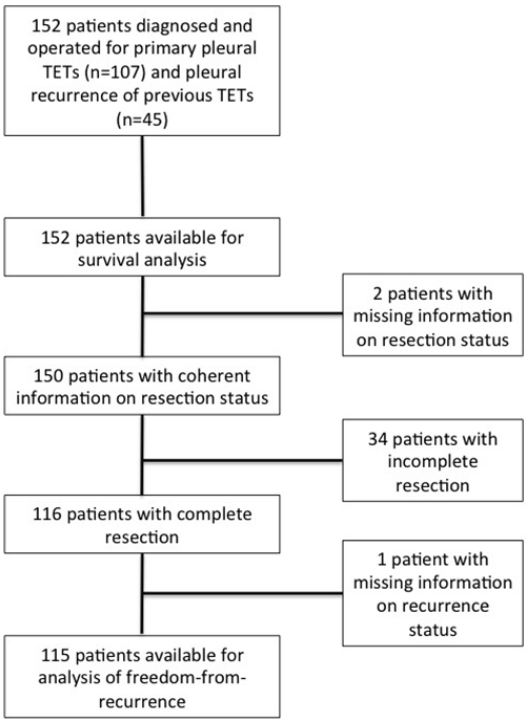

Figure 16: Flow chart illustrating the assignment of patients to different endpoints.

Two types of clinical scenarios were analysed and described in this effort:

Patients with pleural disease of TETs at primary diagnosis (70.4%) as well as patients with pleural disease who had previous surgery for a TET (without involvement of the pleura [29.6%]). Figure 17 illustrates the two scenarios.

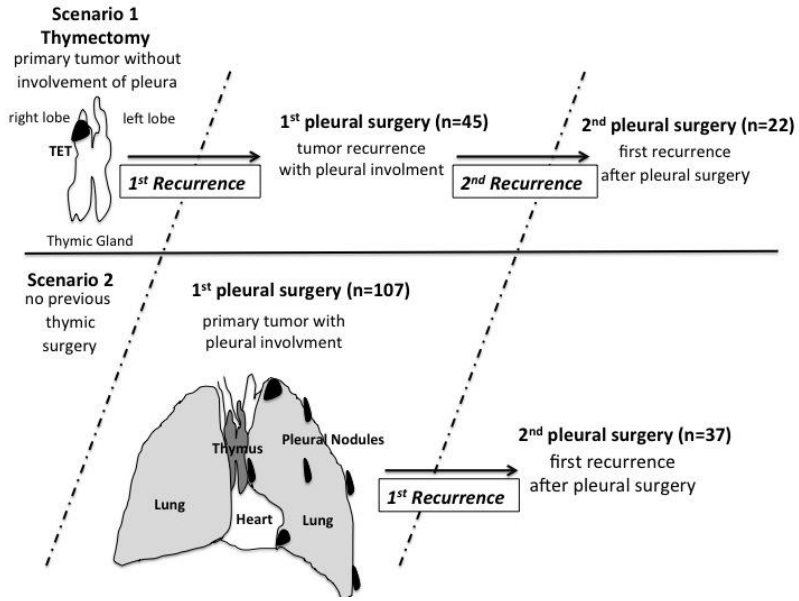

Figure 17: Primary pleural surgery and surgery for recurrent pleural disease. Schematic to clarify terms for recurrent disease: In scenario 1 patients underwent radical surgery for TETs without pleural involvement. TETs with pleural disease occurred at recurrence. In scenario 2 patients TETs involving the pleural at first presentation were treated by surgery.

4.4.2 Statistical analyses

Statistical analysis of data was computed using SPSS software (version 21; IBM SPSS Inc., IL, USA). All plots were created using GraphPad Prism6 (GraphPad Software Inc., California, USA). The probability of making a type I error was set at an α value of 0.05. The null hypothesis was rejected if the p-value was less than α. Two-tailed p-values were employed. Kaplan-Meier product-limit method was used to estimate outcome measures for TETs (OS, CSS, DFS and FFR). The log-rank test was used to test differences between survival rates. Univariate and multivariate Cox proportional hazard models were used to evaluate prognostic factors for the same outcomes: OS, CSS and FFR. The following potential predictors were tested: age (continuous), sex, MG, thymoma vs. TC, resections status (complete vs. incomplete), type of surgery for pleural disease (EPP, TP or LP), primary pleural surgery vs. pleural surgery for recurrence, preoperative therapy, and post-operative therapy. Recurrences and outcome measures were reported as recommended by ITMIG (Huang, Detterbeck et al. 2011)**.** OS was calculated as the primary outcome from the date of surgery (first pleural surgery, see
Figure 16 and Figure 17) to the date of death of any cause (censored observations: patients at the last time point known to be alive). The end-point of interest for CSS was defined

as death from TET (censored observations: unrelated deaths and unknown cause of death) (Huang, Detterbeck et al. 2011). DFS was analyzed from the date of first pleural surgery to the date of recurrence or death from any cause (Ruffini, Detterbeck et al. 2014). FFR was calculated only in patients after complete surgical resection (R0) from the date of first pleural surgery to the date of recurrence in patients with full information on recurrence status (Ruffini, Detterbeck et al. 2014). Log-rank test was used to compare Kaplan–Meier curves. The differences at 1, 3, 5 and 10 years were reported descriptively.

4.5 Evaluation of CRP as a prognostic marker for TETs

4.5.1 Study cohort

At the institutional division of thoracic surgery of the Medical University Vienna 149 patients who underwent surgical tumor resection between June 1990 and May 2015 were included. Nintety percent of patients were treated from 2002 to2015 with a follow-up time ranging from 1–249 months (median 35 months). Eighty patients (53.7%) received multimodal treatment, consisting of surgery with neoadjuvant or adjuvant Cht and/or RT. Sixty-nine patients (46.3%) underwent solely surgical tumor resection. Recurrences after treatment were observed in 27 patients (18.1%). The recurrence types were as follows: local 7 patients (25.9%), regional 10 patients (37.0%) and distant 10 patients (37.0%).

Routine follow-up

Preoperative staging was performed by Chest CT scans and in cases of advanced tumor stage with PET-CT. All patients were followed-up with periodic chest CT-scans. A neurologist was consulted for the diagnosis or exclusion of MG in all cases.

Measurement of CRP serum concentrations

CRP serum concentrations were measured usinga latex-enhanced immunoturbidimetric assay (Roche, Mannheim, Germany). Measurements were performed at the institutional department of laboratory medicine of the Medical University of Vienna.

In-/Exclusion criteria of the study

Since CRP serum concentrations are possibly altered by Cht or surgery there had to be at least 4 weeks between last applications of ChT or surgery and the blood draw to generate serum. A number of clinical conditions were regarded as exclusion criteria for the study:

pneumonia (4 patients), urinary tract infections (3 patients), COPD exacerbation and acute cardiac insufficiency.

Preoperative CRP serum concentrations were missing in 14 out of 149 patients with TETs. One-hundred and twenty-eight patients with TETs were available for CRP analysis.

The collection of postoperative follow-up serum samples started in 2012. For 68 patients pre- and postoperative CRP serum concentrations with median follow-up of 14.8 months [range 0.13–105.6 months] were available (recurrences: 16; no recurrence: 52 patients). CRP serum concentrations of 64 healthy volunteers matched for sex- and age served as controls.

4.5.2 Tumor samples, Immunohistochemical analysis

We performed immunohistochemical staining according to standard protocols [16, 24]. Sections 3-µm thick (formalin-fixed and paraffin embedded tissue) specimens of TETs were deparaffinized. Citrate buffer (pH 6.0, Target Retrieval Solution, DAKO, Glostrup, Denmark) was employed for antigen retrieva by boiling slides at 600 watt 3 × 5 min in a microwave oven. Endogenous peroxidase was quenched with 0.3% hydrogen peroxide. Sections were incubated with monoclonal rabbit anti-human CRP antibody (Clone Y284, Abcam, Cambridge, UK) for 1 h at room temperature and goat anti-rabbit antibody was used as secondary antibody (Vectastatin ABC kit, Vector Laboratories, Burlingame, USA). Immunoreactivity was amplified by using biotin-avidin peroxidase conjugates (Vectastatin ABC kit, Vector Laboratories, Burlingame, USA) and 3,3´-diaminobenzidine was used as chromogen (DAB Peroxidase substrate kit, Vector Laboratories, Burlingame USA). Sections were counterstained with hematoxylin. Liver specimens were used as positive control. The monoclonal rabbit anti-human CRP antibody (Clone Y284, Abcam, Cambridge, UK) was previously used to analyze CRP expression in hepatocellular carcinomas [30, 47].

4.5.3 Statistical analyses

Statistical analysis of data was performed by using SPSS software (version 21; IBM SPSS Inc., IL, USA). All plots were created using GraphPad Prism6 (GraphPad Software Inc., California, USA). Non-normal distributed variables were analyzed with Whitney U-Test and Kruskal-Wallis rank test. Means of two or more than two independent groups with

normal distribution were compard with unpaired student's t test and one-way ANOVA. Post hoc comparisons were computed with Tukey's-B. Nominal variables were compard with Chi-square test. Pearson`s correlation coefficient (r) computed to describe linear relationships between two numerical variables. Kaplan-Meier survival analysis and Log-rank test were used to analyze OS, CSS and FFR. Cox regression analysis was employed to assess prognostic factors and calculate Hazard Ratios (HR) with 95% confidence intervals (CI). Univariable and multivariable analysis of gender (male vs. female), age (continuous), MG (pos. vs. neg.), tumor histology (TC vs. Thymoma), completeness of resection (R0 vs. R1+2), Masaoka-Koga stage (I+II vs. III–IV), tumor size (continuous) and pretreatment CRP (low vs. high) for OS, CSS and FFR was performed. The median 0.22 mg/dL was used to dichotomize pretreatment CRP values into high and low CRP groups for survival analysis. The institutional laboratory cut-off value of 0.5 mg/dL was used to test the diagnostic accuracy (sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV)) of CRP. The probability of making a type I error was set at an α value of 0.05. The null hypothesis was rejected if the p-value was less than α. Two-tailed p-values were employed. We followed the definitions of recurrence and outcome as recommended by ITMIG and ESTS Thymic Working Group (see 2.10).

4.6 Evaluation of fibrinogen, NLR, and PLR as prognostic markers for TETs

4.6.1 Study cohort

We analyzed 122 patients (57.4% females) with TETs who underwent surgical tumor resection at the Division of Thoracic Surgery, Medical University of Vienna between September 1999 and June 2015. Eighty percent of patients were treated within the past 10 years. Mean patient age was 56.5±16.1 years. There were 92 thymomas (75.4%) and 30 TCs (24.6%). Fifty-four percent of patients underwent multimodal treatment regimens combining surgery with radiotherapy and/or chemotherapy. A complete tumor resection (R0) was achieved in 89.3% of cases. Preoperative staging included chest CT scans, and in advanced cases PET-CT scans. Surgical resection alone was performed in 45.9% of cases. Fifty-four percent of patients underwent multimodal therapy including surgery. The median follow-up time was 30.8 months (range: 1-174 months). Oncologic follow-

up was routinely performed according to recommendations of the European Society of Thoracic Surgery (ESTS) (Ruffini, Detterbeck et al. 2014): chest CT scans every 3 to 6 months for the first three years after surgery, followed by annual CT scans.

4.6.2 Blood work and immunohistochemistry on tumor tissue

Cell counts and fibrinogen measurements

Fibrinogen, platelet count and the following cell counts: white blood cells, neutrophils and lymphocytes were measured at the institutional department of laboratory medicine during routine preoperative work-up one day before surgery to exclude coagulation disorders or presence of acute infection. Measurements were repeated 3 to 7 days postoperatively, and at 6 to 12 months after the initial therapy. Fibrinogen plasma concentrations were evaluated using the Clauss method (Clauss 1957). Pre- and postoperative as well as further follow-up fibrinogen plasma concentrations were available from 112, 98, and 27 patients, respectively. Longitudinal NLR values were available in 101, 95, and 36 patients, respectively. Longitudinal PLR values were available for 96, 95, and 36 patients, respectively. Fibrinogen, NLR, and PLR measurements from 51 healthy sex- (24 male, 27 female) and age-matched (54.6 ± 1.4 years) volunteers served as controls.

Immunohistochemistry

Immunohistochemistry was performed using the automated Ventana Benchmark® platform (Ventana Medical Systems, Tuscon, AZ, USA). The following antibodies were used: monoclonal mouse anti-human CD45 (LCA; 2B11& PD7/26; Cell Marque, California, USA,) polyclonal rabbit anti-human Fibrinogen/FITC (Dako, Denmark). For CD45 staining (identify cells of the hematopoietic lineage), heat pre-treatment was performed for 56 min in Ultra cell conditioner Nr 1 buffer (Ultra CC1; pH 6). For Fibrinogen staining, Protease 1 was applied for 8 min. Samples were incubated with primary antibodies for 30 min, and then development was performed using the Ultraview Universal Detection DAB-kit.

4.6.3 Statistical analyses

Statistical analysis of data was performed by using SPSS software (version 22; IBM SPSS Inc., IL, USA). All plots were created using GraphPad Prism6 (GraphPad Software Inc.,

California, USA). Non-normal distributed variables were analyzed with Whitney U-Test and Kruskal-Wallis rank test. Means of two or more than two independent groups with normal distribution were compard with unpaired student's t test and one-way ANOVA. The Chi-square test was employed to assess associations between nominal variables. Survival analyses were performed using Kaplan-Meier analysis and the Log-rank test. We followed the definitions of recurrence and outcome (CSS and FFR) as recommended by ITMIG and ESTS Thymic Working Group (see 2.10).

The receiver operating characteristic (ROC) curve was plotted to calculate the Youden Index (optimal cut-off value) for fibrinogen of 452.5 mg/dL to dichotomize into high and low fibrinogen groups of patients. An empiric cut-off value of 4.0 was employed to differentiate between high and low NLR cohorts [32, 62]; and a median PLR of 136.5 dichotomized into high and low PLR cohorts.

We evaluated the prognostic significance of histology (TC vs. Thymoma), Masaoka-Koga tumor stage (I–II vs. III– IV), Fibrinogen (high vs. low), NLR (high vs. low), and PLR (high vs. low) on CSS and FF by univariable and multivariable Cox regression analyses.

Binary logistic regression was employed to evaluate the predictive values of fibrinogen, NLR, and PLR for detection of tumor recurrence within follow-up. R2 values are indicated as markers of goodness-of-fit of our models.

DOI:10.14753/SE.2020.2356

5. Results

5.1 Prognostic factors and multi-modal management of TETs: a single center experience

5.1.1 Survival is dependent upon tumor stage and resection status but not histology

Seventy-two patients undergoing 84 surgeries (including surgery for recurrences) were included in the study. There were 44 females and 28 males. The mean patient age at surgery was 58.2 [age range: 22 to 86] years. None of the patients died during thoracic surgery or 30-days thereafter (no perioperative mortality). The median follow-up time calculated from OS data (see Figure 18A) was 47.2 months. OS and CSS data from the entire patient cohort are listed for comparison in Table 10 (See Figure 18B).

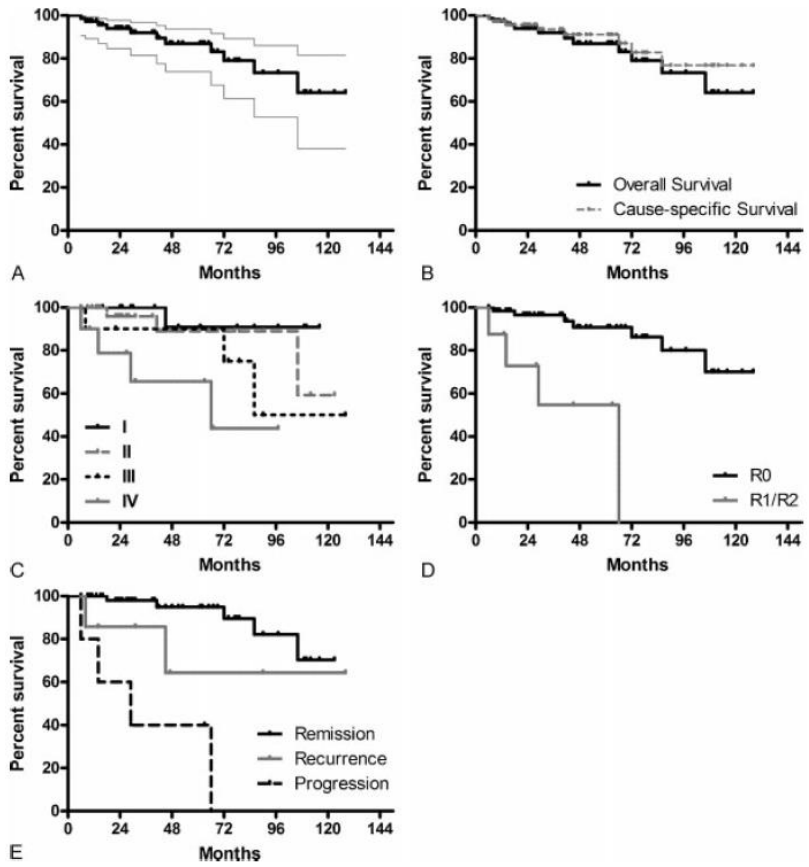

Figure 18: Overall and Cause-specific Survival in patients with TETs. OS is displayed by Kaplan–Meier curve with 95% confidence intervals (A). An overlay of OS and CSS (B), survival according to Masaoka–Koga stage (C), survival and residual tumor status (D) are shown. A comparison of survival of patients in remission or with recurrent or progressive disease is displayed (E).

Table 10: OS and CSS of the entire patient cohort

Survival	90%	80%	70%
OS [months]	41.9	72.4	106.6
CSS [months]	66.1	86.8	

Tumor stage

There was decreased OS survival with more advanced disease as categorized with Masaoka-Koga stage (log rank test: p=0.017, Figure 18C). Stage presentation was as follows: stage I (29.2%), stage II (43.1%), stage III (13.9%), and stage IV (13.9%) (Table 11). In stage I 90% of patients were alive at 116.6 months (end of follow-up period). In stage II OS was 90% at 41.9 months and 60% at 106.6 months. In stage III 80% survival was 72.4 months and 60% survival was 86.6 months. The worst survival was observed in stage IV with 90% at 14.5 months and 50% at 66.12 months.

Residual tumor status

Residual tumor classification was a predictor of survival after resection of TETs. OS was significantly worse following incomplete resection (R1+R2: 90% survival at 6.9 months, 80% survival at 14.5 months, and 60% survival at 29.1 months; R0: 90% survival at 72.4 months; Figure 18D; log-rank test: p<0.001).

Histology

WHO histological subtypes A, AB, B1, B2, B3 and TC did not display differences in survival (log rank test: p=0.136). Formation of subgroups such as group1 (A, AB, B1) and group 2 (B2, B3, TC) did not reveal a significant survival benefit (p=0.151). The distribution of WHO histological types was: A (19.4%), AB (5.6%), B1 (6.9%), B2 (22.2%), B3 (12.5%), TC (19.4%), combined (12.5%), and micronodular thymomas (1.4%). The majority of A and AB thymomas were in stages I (61.1%) and II (22.2%) and only sporadically in higher stages: one case of type AB thymoma in stage III (infiltration of the lung and pericardium) and two cases of type A in stage IVb (lymphogenous metastases). B2 components were present in all combined thymomas (66.7% type B2/B3). See also Cross-Table 11 for Masaoka–Koga stage and WHO histology.

DOI:10.14753/SE.2020.2356

Table 11: Masaoka-Koga stage and WHO histology

WHO Type	Masaoka–Koga stage, *n* (%)				Total no.
	I	II	III	IV	
A	9 (64.3)	3 (21.4)	–	2 (14.3)	14
AB	2 (50.0)	1 (25.0)	1 (25.0)	–	4
B1	1 (20.0)	4 (80.0)	–	–	5
B2	5 (31.3)	9 (56.3)	1 (6.3)	1 (6.3)	16
B3	3 (33.3)	4 (44.4)	1 (11.1)	1 (11.1)	9
Thymic carcinoma	–	3 (21.4)	6 (42.9)	5 (35.7)	14
Combined thymoma	1 (11.1)	6 (66.7)	1 (11.1)	1 (11.1)	9
Micronodular thymoma	–	1 (100.0)	–	–	1
Total no.	21	31	10	10	72

5.1.2 Worse survival in advanced cases: recurrences, progressions, and multimodal therapy

Recurrences and Progressions

Survival was significantly better in patients without recurrences or progressions (log-rank test: Figure 18E; $p<0.001$).

Freedom-from-recurrence

Eighty-nine percent (64 out of 72) patients had an R0 resection. Around 90% of patients after complete resection were free from a recurrence at 28.5 months (median follow-up time 48.2 months; Figure 19A).

Time-to-progression

Eight patients (after R1 or R2 resection) showed the following time to progression: 90% of patients free from progression at 4.2 months, 70% at 6.3 months, 60% at 6.8 months, and 40% at 16.2 months (median follow-up time 28 months; Figure 19B).

DOI:10.14753/SE.2020.2356

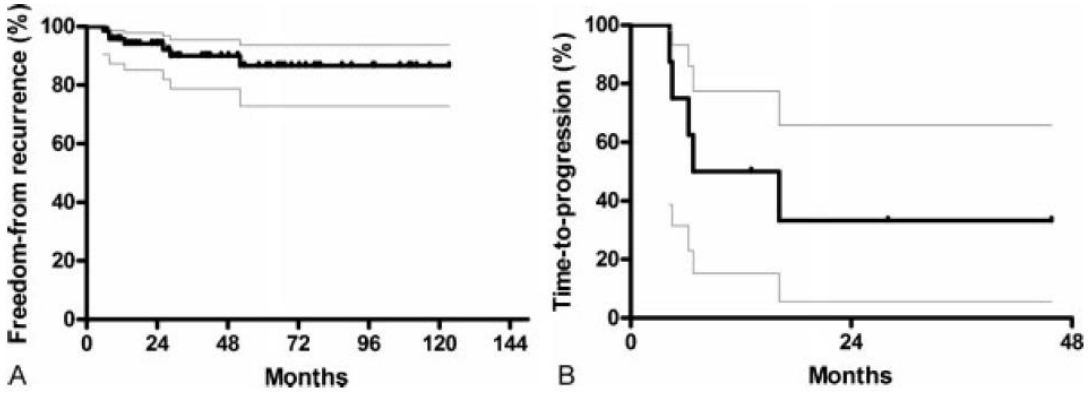

Figure 19: Outcome measures for recurrence and progression. Freedom-from recurrence (A) and time-to-progression (B) in patients undergoing thoracic surgery for TETs (including patients with multimodality treatments). Kaplan–Meier curves with 95% confidence intervals are displayed.

During the oncological follow-up 60 patients (83.3%) were free from recurrence or progression. There were seven (6.9%) recurrences and five (9.7%) progressions (two were referrals from other institutions). There were 10.9% recurrences after R0 resection compared to 50% recurrences (3 patients) after R1 resection and all patients following R2 resection (2 patients) developing a progression. Recurrence according to stage was as follows: I (1 patient, 4.8%), II (3 patients, 9.7%), III (2 patients, 20%), and IV (1 patient, 10%). All five stage IV patients with incomplete resections (R1+R2) developed progressions (50% of stage IV patients). Stage and recurrence or progression were significantly correlated (Pearson $\chi 2$ test: p=0.001). Recurrences in different WHO types were as follows: A (1 patient, 7.1%), AB (0%), B1 (0%), B2 (1 patient, 6.3%), and micronodular thymoma (0%), B3 (2 patients, 22.2%), thymic carcinoma (2 patients, 14.3%), and combined thymomas (1 patient, 11.1%). One patient with WHO type B3 (11.1%), three patients with TC (21.4%), and one patient with combined thymoma (11.1%) experienced progressions. One patient had two and another had three consecutive recurrences.

Table 12 lists different synchronous recurrences separately.

Table 12: Local, regional and distant recurrences.

Recurrence	*n*
Local	1
Regional	3
Distant	3
Local and regional	1
Regional and distant	1
Local, regional, and distant	1

One out of 34 patients treated with surgery alone had a recurrence. In comparison, of 38 patients undergoing multimodal therapy, 11 patients experienced recurrences or progressions. Recurrences were treated by different medical or surgical specialties (Table 13).

Table 13: Treatment of recurrences

Localization of recurrence	Total no.	Treatment	No. of patients
Mediastinum	3	Radiotherapy	1
		Resection	2
Pleural dissemination	3	Resection	3
Brain	1	Microsurgical tumor resection	1
Bone	2	Radiotherapy	1
		Resection and chemotherapy	1
Lymph nodes	4	Chemotherapy	1
		Resection	1
		Resection and chemotherapy	1
		Resection and radiotherapy	1

Multimodal treatment of TETs: Surgery alone vs. Surgery plus Chemo- and/or Radiotherapy

The frequency of surgery plus multimodal therapy was significantly higher in patients in higher Masaoka–Koga stages (see also Figure 20). The survival analysis of surgical therapy alone revealed 80% survival at the end of the follow-up period. Patients that were treated with surgery in conjunction with chemo and/or radiotherapy displayed the following survival: 90% at 29.1 months, 80% at 45.9 months, 70% at 72.4 months, and 60% at 86.8 months (log-rank test: p=0.018).

Thirty-nine patients (54.2%) underwent combined treatment protocols consisting of surgery with chemotherapy and/or radiotherapy in a neoadjuvant or adjuvant setting while 33 patients (45.8%) were treated with surgery alone. Multimodal therapy regimens were more frequently employed in patients with higher stages (Pearson $\chi 2$ test: $p < 0.001$; Figure 20).

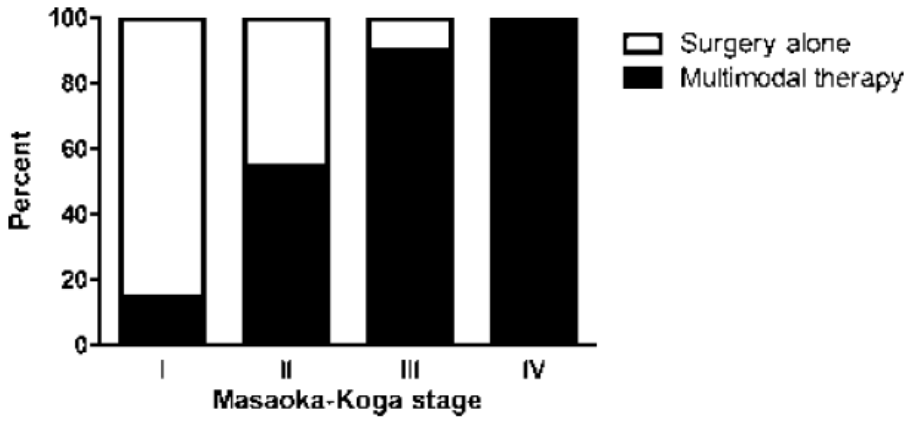

Figure 20: Treatment modality with respect to pathological stage. Patients with stage I TETs were primarily treated by thoracic surgery alone. The use of multimodal therapy regimens increased with higher Masaoka-Koga stage and was the case in 100% of stage IV patients.

The decision for neoadjuvant treatment was influenced by tumor size (e.g. two patients in stage I with tumors >10 cm, and radiological suspicion of invasion of adjacent structures in CT and MRI scans (final pathological stage yI after neoadjuvant therapy and resection was reported). Twelve patients with TC (85.7%) received multimodal therapy. Completeness of resection was the most important determinant for decision making on adjuvant therapy. Surgical treatment alone was only performed in patients after histologically confirmed R0 resections. After R0 resections, 31 patients (51.6%) received adjuvant therapy. All patients with R1 (6 patients) and R2 resections (2 patients) on final pathologic analysis received adjuvant therapy.

Radiotherapy

Thirty-seven radiotherapies were administered to 31 (out of 72) patients (radiotherapy for recurrence or progression included). Two patients were excluded from this analysis (no information on radiation dose and selective internal radiotherapy for liver metastases).

The mean cumulative dose for neoadjuvant treatment was 51.2 Gray (6 patients) and the mean cumulative dose adjuvant therapy was 49.4 Gray (25 patients), respectively. Mean

cumulative radiotherapy doses for progressive (3 patients) and recurrent disease (3 patients) were 40.7 and 42.7 Gray, respectively. None of the six patients treated with neoadjuvant radiotherapy received it in an adjuvant or recurrence/ progression setting.

Chemotherapy

Twenty (out of 72) patients underwent chemotherapy: neoadjuvant (10 patients; 1 patient received additional chemotherapy for a recurrence), adjuvant (5 patients), for recurrence only (1 patient), for progression only (2 patients). Cytotoxic platinum-based therapy was employed in 13 (65%) patients. In two patients (10%), protocols without platinum were used (chemotherapy protocols was not available in 5 patients).

5.1.3 The role of biopsies and surgery

Survival analysis of age (log-rank test: p=0.779), sex (p=0.646), or the use of preoperative biopsies (p=0.833) revealed no statistically significant differences.

Biopsy

Preoperative biopsies in to confirm the histological entity of the tumor were obtained in 31 patients (43.1%). The biopsies were performed on tumors in different pathological stages: I (10 patients, 47.6%), II (8 patients, 25.8%), III (7 patients, 70%), and IV (6 patients, 60%). The risk for recurrences was not increased in patients undergoing preoperative biopsies of thymomas/TC (log-rank test: p=0.571). There were five (16.1%) recurrences in patients who had a biopsy and two (4.9%) recurrences in patients without preoperative biopsies. Postoperative progressions occurred in two patients (6.5%) who had a biopsy and in three (7.3%) without a biopsy.

Surgical approach

The frequency of surgical incisions applied was: sternotomy (45.8%), thoracotomy (23.6%), or hemiclamshell incision (13.9%)

Table 14**.** In a stage IV patient with excellent response after neoadjuvant treatment a cervical incision was used to remove lymphogenous metastases in the neck. In patients undergoing neoadjuvant treatments for primary tumor with suspicion of invasiveness and 12 cm and 10 cm in their greatest dimensions hemiclamshell and clamshell incisions were once used (pathological stage yI;

Table 14).

DOI:10.14753/SE.2020.2356

Table 14: Surgical approach to TETs in different stages

Surgical approach	Masaoka–Koga stage, *n* (%)			
	I	II	III	IV
VATS/VATET	–	4 (12.9)	–	–
Robotic surgery	1 (4.8)	5 (16.1)	–	–
Cervical	–	–	–	1 (10.0)
Thoracotomy	7 (33.3)	7 (22.6)	–	3 (30.0)
Sternotomy	11 (52.4)	13 (41.9)	8 (80.0)	1 (10.0)
Hemiclamshell	1 (4.8)	2 (6.5)	2 (20.0)	5 (50.0)
Clamshell	1 (4.8)	–	–	–
Total no.	21	31	10	10

Extent of resection

Data on the extent of resection were analyzed for 83 resections (missing data for one surgery). Pericardial resections were performed in 26 cases (31.3%). The following pulmonary resections were performed in 22 (26.5%) cases: 17 segmental resections, three lobectomies, one pneumonectomy, and one lobectomy plus a segmental resection, one extrapleural pneumonectomy and 11 (13.3%) partial pleural resections. Unilateral resections of the phrenic nerve, recurrent laryngeal nerve and vagal nerve en bloc with the directly infiltrating tumors were performed in 16, 1 and 2 cases. Resection of diaphragm (3 cases, 3.6%), ribs (2 cases, 2.4%), clavicle (1 case, 1.2%), chest wall (1 case, 1.2%), and part of the liver (1 case, 1.2%) was performed. Vascular resection with reconstruction was done in 11 cases (13.3%): anonymous vein (7 cases), superior vena cava (7 cases), and subclavian artery (1 case). Tangential adventitial resections of the aortic arch and pulmonary trunk were feasible in two cases.

5.1.4 Myasthenia Gravis was not a prognostic factor

The presence or absence of MG was not a relevant factor for survival analysis (log-rank test: p=0.235). The diagnosis of MG was present at the time of surgery in 21.4% of male and 29.6% of female patients. MG was associated with 55% of B3, 37.5% of B2, 33.3% of combined and 21.4% of type A thymomas (only one case in B1 and TC), respectively. Thymoma patients with MG were encountered in 38.7% of stage II, 20% of stage III, 19% of stage I, and 10% of stage IV patients.

DOI:10.14753/SE.2020.2356

5.2 Surgical therapy of TETs with pleural involvement: an ESTS Thymic Working Group Project

Patients undergoing primary surgery for pleural disease of TETs had a mean age of 47.3 years and 54.6 years for patients with surgery for their first recurrent disease to the pleura. Forty patients (26.3%) had surgery for a TET before the first surgery of pleural disease.

5.2.1 TC and incomplete resection predict worse survival

OS, CSS, DFS and FFR of the entire patient cohort at 1, 3, 5 and 10 years is depicted in

Table 15 and with Kaplan-Meier curves in Figure 21, respectively.

Table 15: OS, CSS, DFS and FFR of the entire patient cohort

Survival	1 year	3 year	5 year	10 year
OS % (n=152)	96.4	91.0	87.2	62.7
CSS % (n=152)	97.8	94.9	92.3	79.7
DFS % (n=152)	84.3	60.9	44.9	15.2
FFR % (n=115)	83.1	57.9	43.0	22.2

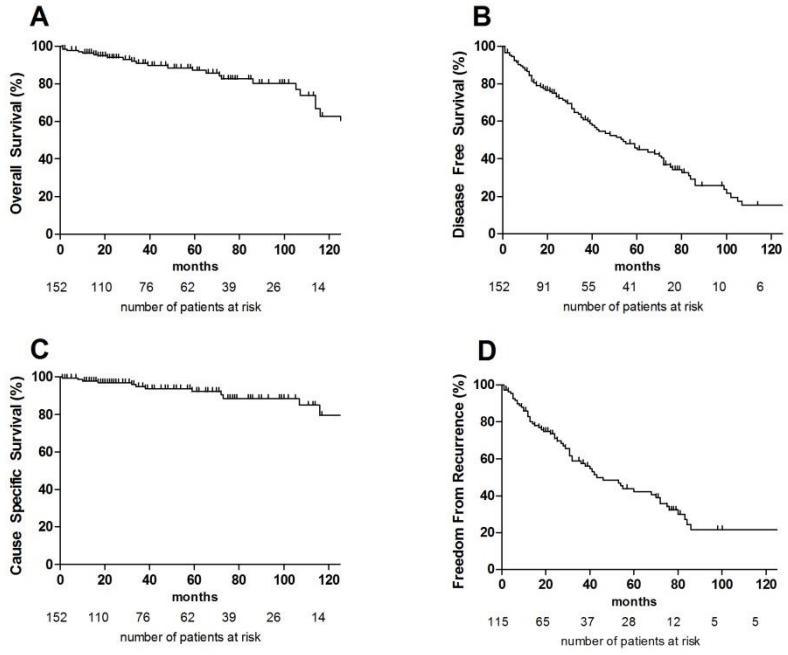

Figure 21: Survival analysis: entire patient cohort. Overall survival (A), disease-free survival (B), cause-specific survival (C) and freedom from recurrence (D).

Mortality

Perioperative mortality (30 days) after pleural surgery for TETs: one patient with TC suffered from pneumonia and acute respiratory distress syndrome after EPP and another patient with TC undergoing total pleurectomy died due to acute pulmonary embolism and prolonged air leak. Mortality between 30 and 90 days postoperatively: One patient with B2 thymoma undergoing EPP had a non-tumor related cause of death. Mortality between 90 days and 1 year: A TC patient who underwent EPP died related to progression of metastatic disease at 8 months. Another patient with B2 thymoma died 10 months after EPP from pneumonia resulting in acute respiratory distress syndrome.

Survival analysis for prognostic factors

Analysis at 3, 5 and 10 years revealed statistically significant better OS in patients undergoing surgery for pleural recurrence compared to primary pleural surgery (p=0.028, p=0.023, p=0.027, respectively). As expected the analysis at 3, 5 and 10 years revealed better OS in patients after complete compared to incomplete resections: R0 vs. R1/R2 (p=0.032, p=0.003, p=0.001, respectively). The type of surgery: EPP vs. TP vs. LP was not associated with differences in FFR, but with differences in OS [1-year: p=0.010], DFS [3-year: p=0.021 and 5-year: p=0.037] and CSS [1-year: p=0.012 and 3-year: p=0.041]. TC compared to thymoma was associated with worse survival for all computed outcomes: OS, DFS, CSS and FFR at 1, 3, 5 and 10 years. There was a statistically significant survival advantage for patients with MG (10-year OS: p=0.010; the 5 and 10-year CSS: p=0.047 and p=0.014, respectively). See Figure 22 for the respective Kaplan-Meier curves.

DOI:10.14753/SE.2020.2356

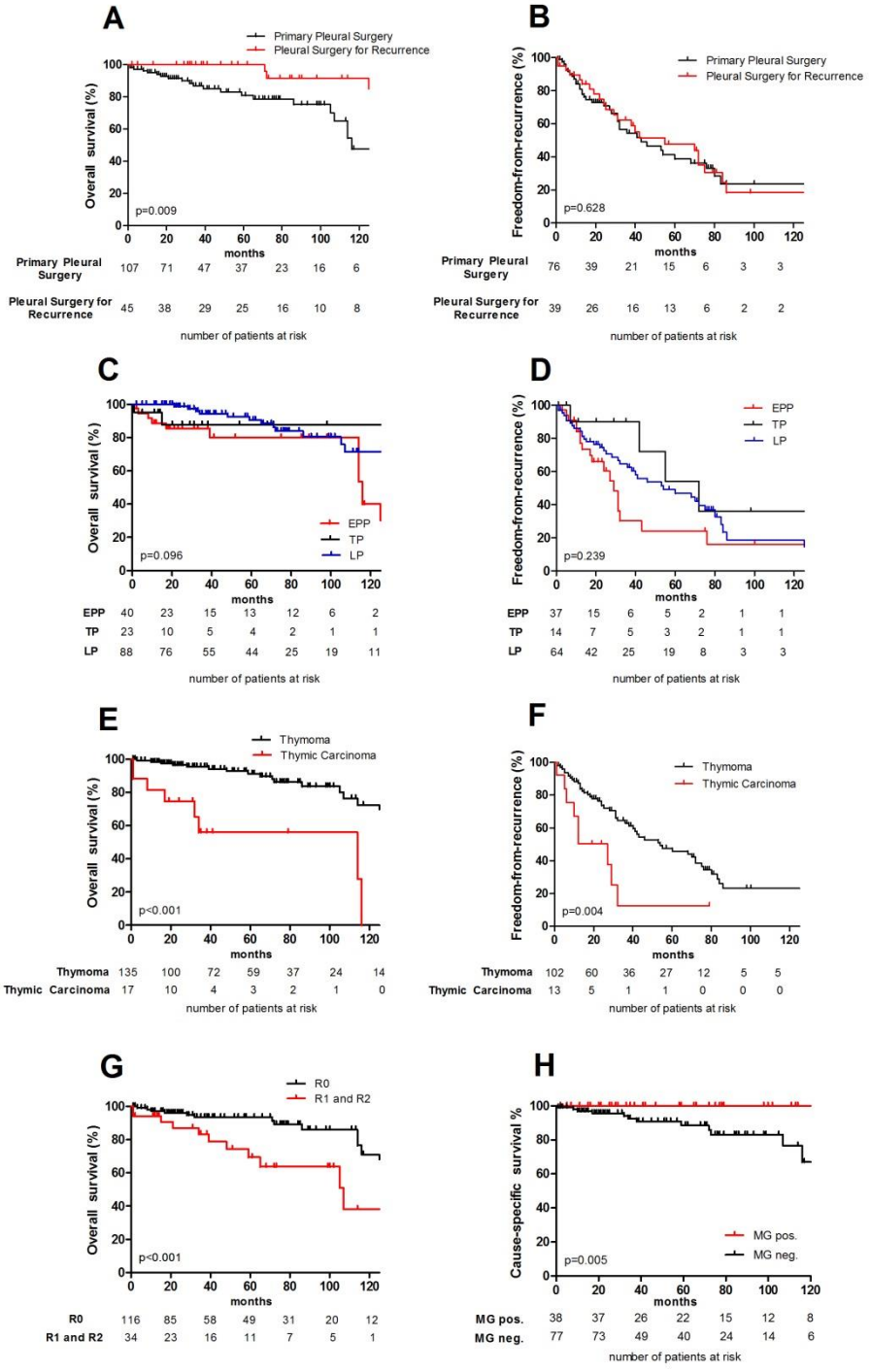

Figure 22: Survival analysis for prognostic factors. Comparison of primary pleural surgery and pleural surgery for recurrence OS (A), FFR (B), type of surgery: EPP vs TP vs LP: OS (C), FFR (D), thymoma vs TC: OS (E), FFR (F), and completeness of resection: OS (G), FFR (H).

Pericardial resection (non-EPP patients), was associated with significantly worse survival at 10 years (OS at 1, 3, 5 and 10 years for pericardial resection

yes (n=52) vs. no (n=52): 98.0% vs. 100.0% (p=0.322), 93.3% vs. 100.0% (p=0.082), 86.7% vs. 97.0% (p=0.082), and 55.1% vs. 87.2% (p=0.010), respectively.

5.2.2 The prognosis of patients with pleural disease is multifactorial: resection status, histology, primary or recurrent disease, type of pleural surgery and necessity of multimodal treatment

Univariable analysis

MG was associated with better OS [HR 0.234; p=0.019]. Incomplete resection, histology (TC vs. thymomas), primary pleural surgery vs. surgery for pleural recurrence, type of pleural surgery (EPP vs. TP/LP), preoperative and postoperative therapy were associated with worse OS (see also

Table 16). The number of resected nodules (n = 122) when dichotomized with a cut-off value of 4 (median number of resected nodules) revealed no statistically significant differences.

Multivariable analysis

TC histology in comparison with thymomas showed worse OS [HR 6.506; p=0.002], CSS [HR 13.144; p=0.001] and FFR [HR 2.442; p=0.027], respectively. Incomplete resection was a predictor of worse OS [HR 6.696; p=0.003] and male sex predicted worse FFR [HR 1.800; p=0.033]. Analysis of patients after complete resection (R0) eliminates the potential strong bias of incomplete resection on other potential predictors: There was worse OS for male sex [HR 3.176; p=0.025], TC [HR 3.988; p=0.013], primary pleural surgery compared to surgery for pleural recurrence [HR 4.132; p=0.040] (see also

DOI:10.14753/SE.2020.2356

Table 16). Analysis all patients with [pseudo-]neo- and [pseudo-]adjuvant therapy combined (n=126) vs surgery alone (n=26) revealed no statistically significant differences.

Table 16: Univariate and Multivariate Analysis of Predictors for OS, CSS and FFR.

[1]Analysis of primary pleural surgery vs. pleural surgery for recurrence (both study cohorts are operated for pleural disease of TETs for the first time). [2]Patients received (pseudo-) neoadjuvant therapy, including ChT ± RT. [3]Patients received (pseudo-) adjuvant therapy, including ChT ± RT. [4]FFR was analyzed only for patients after complete resection (R0).

	Univariate Model				**Multivariate Model**			
			95% CI				**95% CI**	
	HR	***p***	**Lo-wer**	**Upper**	**HR**	***p***	**Lo-wer**	**Upper**
Overall survival								
Sex (Male)	1.508	0.322	0.669	3.397	2.438	0.092	0.865	6.871
Age (continuous)	1.026	0.161	0.990	1.063	1.017	0.395	0.978	1.058
Myasthenia Gravis (Yes)	0.234	0.019	0.069	0.790	0.551	0.459	0.114	2.666
TC vs. Thymoma	6.315	0.000	2.652	15.040	6.506	0.002	1.956	21.646
Incomplete resection	3.916	0.001	1.727	8.882	6.696	0.003	1.944	23.060
Pleural Surgery								
Primary pleural Surgery vs. Pleural Surgery for Recur-rence[1]	4.392	0.017	1.302	14.813	1.493	0.603	0.330	6.757
Type of pleural Surgery								
EPP vs. LP+TP	2.351	0.040	1.041	5.312	2.491	0.122	0.784	7.913
Preoperative Therapy (Yes)[2]	3.086	0.013	1.271	7.463	1.495	0.427	0.554	4.032
Postoperative Therapy (Yes)[3]	2.558	0.030	1.093	5.988	1.733	0.293	0.622	4.831
Cause-specific survival								
Sex (Male)	1.740	0.331	0.569	5.325	3.206	0.128	0.715	14.372
Age (continuous)	1.041	0.108	0.991	1.093	1.039	0.197	0.980	1.101
Myasthenia Gravis (Yes)	0.022	0.110	0.000	2.387	0.000	0.952	0.000	171.39

TC vs. Thymoma	12.129	0.000	3.867	38.044	13.144	0.001	2.726	63.370
Incomplete resection	2.004	0.255	0.606	6.628	2.053	0.387	0.402	10.487
Pleural Surgery								
Primary pleural Surgery vs. Pleural Surgery for Recurrence[1]	2.198	0.239	0.593	8.130	1.213	0.836	0.194	7.576
Type of pleural Surgery								
EPP vs. LP+TP	2.301	0.155	0.729	7.265	1.063	0.934	0.252	4.488
Preoperative Therapy (Yes)[2]	2.179	0.180	0.697	6.803	1.072	0.914	0.593	3.788
Postoperative Therapy (Yes)[3]	2.066	0.203	0.675	6.329	1.020	0.976	0.288	3.613
Freedom-from-fecurrence[4]								
Sex (Male)	1.541	0.096	0.926	2.566	1.800	0.033	1.050	3.086
Age (continuous)	1.007	0.523	0.985	1.031	1.009	0.504	0.983	1.036
Myasthenia Gravis (Yes)	0.623	0.090	0.361	1.077	0.661	0.208	0.346	1.260
TC vs. Thymoma	2.748	0.006	1.330	5.676	2.442	0.027	1.106	5.388
Pleural Surgery								
Primary pleural Surgery vs. Pleural Surgery for Recurrence[1]	1.138	0.629	0.002	1.919	1.131	0.676	0.633	2.024
Type of pleural Surgery								
EPP vs. LP+TP	1.491	0.165	0.849	2.618	1.467	0.241	0.773	2.784
Preoperative Therapy (Yes)[2]	0.852	0.542	0.508	1.427	0.577	0.068	0.319	1.042
Postoperative Therapy (Yes)[3]	0.750	0.270	0.450	1.250	0.849	0.578	0.476	1.513

5.2.3 Clinical data and treatment specifics for TETs with pleural involvement

Clinical data

Initial symptoms such as cough, muscle weakness, dyspnea or pain were reported in 51.3% of patients. A preoperative biopsy was performed in 68.4%. MG was reported in 47 patients (30.9%); other autoimmune diseases in 7.2% of patients (Isaac's syndrome, paraneoplastic encephalopathy, systemic lupus erythematosus, rheumatoid arthritis, Good's syndrome, pure red cell aplasia 3, Hashimoto's thyroiditis 2, not specified 1). A previous extrathymic malignancy was present in 8.6% of patients (breast cancer 3, lymphoma 2, NSCLC 2, liposarcoma, melanoma, squamous cell carcinoma (SCC) of skin, thyroid cancer, parathyroid cancer, uterine tumor). There were 17 patients (11.2%) diagnosed with TC and 135 (88.8%) with thymoma. Pathological Masaoka–Koga Stages at their first surgery were reported as: I (3.4%), II (6.1%), III (20.5%), IVA (65.0%) and IVB (4.8%). The frequency of thymomas and TCs according to WHO histology was B2 (33.6%), B3 (23.9%), B1 (13.0%), combined B2/B3 (11.6%), TC (10.3%), AB (4.1%), B1/B2 (2.0%), A (0.7%) and combined B3/TC (0.7%).

Surgical approach, type of resection and residual tumor status

The surgical approaches for the resection of TETs with pleural involvement were: thoracotomy 61 patients (40.4%), sternotomy 65 (43.0%) clamshell 6 (4.0%), hemi-clamshell 16 (10.6%) and VATS 3 (2.0%). For primary pleural surgery (Scenario 2) EPP, TP and LP were performed in 32 cases (29.9%), 13 cases (12.1%, including 1 case of pleurectomy/decortication [P/D]) and 62 cases (57.9%), respectively. In cases of pleural surgery for recurrence (Scenario 1, first pleural surgery) EPP, TP and LP were carried out in 8 patients (18.2%) and 10 patients (22.7%, including 3 patients with P/D) and 26 patients (59.1%), respectively.

Residual tumor status following primary pleural surgery was R0 (76 patients, 71%), R1 (16 patients, 15%) and R2 (15 patients, 14%). The residual tumor status after recurrent pleural surgery was reported as: R0 (40 patients, 90.9%), R1 (1 patient, 2.3%) and R2 (2 patietns, 4.5%), respectively (missing information: 2 patients).

Treatment of the diaphragm in patients with TETs and pleural involvement

Fifty-two non-EPP patients with diagphragmatic metastasis underwent either diaphragmatic resections: 34 cases: 6 complete and 28 partial resections (missing information on resection extent in 2 cases), or 16 pleurectomies of the diaphragm without resection of the diaphragm. Patients after oncologic phrenic nerve resection (with resultant diaphragmatic paralysis underwent diaphragmatic plication (5 patients).

Multimodal therapy

Of the 152 study patients in 67 patients (62.2%) received neoadjuvant therapy and 15 patients (33.3%) pseudo-neoadjuvant therapy before surgery for recurrence. Sixty-eight patients (63.6%) underwent adjuvant treatments and 10 patients (22.2%) pseudo-adjuvant therapy after surgery for recurrence, respectively. PAC ChT (planned protocol: cisplatin 50 mg/m^2, doxorubicin 50 mg/m^2 and cyclophosphamide 500 mg/m^2) was administered as neo- and pseudo-neoadjuvant treatment in 45 patients of the 76 (59.2%) patients of patients undergoing ChT. Administration of radiotherapy was reported as neoadjuvant (8 patients), pseudo-neoadjuvant (1 patient), adjuvant (63 patients) or pseudoadjuvant (5 patients) treatment. Radiation doses ranged from 20–56 Gray (median 45Gray) for neoadjuvant therapy and 24–60 Gray (median 50 Gray) for adjuvant therapy.

Recurrence after surgery with or without chemo- and radiotherapy for pleural disease of TETs

Fifty-five months was the median time from primary TET surgery without involvement of the pleura to surgery for recurrence of disease to the pleura (first pleural surgery, Scenario 1). The median time to recurrence after the first pleural surgery was 46 months. In 59 patients (51.3%) a recurrence was diagnosed after R0 resection with or without ChT and/or RT for TETs with pleural involvement. The types of recurrence were as follows: local 15 of 59 (25.4%), regional 30 of 59 (50.8%), distant 9 of 59 (15.3%) or local and regional 5 of 59 (8.5%), respectively.

Surgical complications

There were reports on 103 patients experiencing complications (44 patients) or documented follow-up without complications (59 patients).

Table 17 lists complications allocated to type of surgery.

Table 17: Complications after pleural Surgery for TETs. EPP, Extrapleural pneumonectomy; TP, Total pleurectomy; LP, Local pleurectomy; n, number; ARDS, acute respiratory distress syndrome; MODS, multi organ dysfunction syndrome

	Type of pleural Surgery					
	EPP (n=30)		TP (n=20)		LP (n=53)	
Complications	**n**	**%**	n	**%**	**n**	**%**
Chylothorax	0	0	1	5	1	1.9
Hemothorax/postoperative bleeding	2	6.7	1	5	5	9.4
Bronchial stump fistulation	2	6.7	0	0	0	0
Prolonged air leak	0	0	1	5	3	5.7
Pulmonary Embolism	1	3.3	0	0	0	0
Wound Infection	2	6.7	0	0	1	1.9
Horner's Syndrome	0	0	1	5	1	1.9
Empyema	2	6.7	0	0	1	1.9
Pneumonia, Atelectasis, ARDS	3	10	5	15	3	5.7
Renal Failure	2	6.7	0	0	0	0
Cardiac Event	3	10	1	5	0	0
Septic Shock/MODS	1	3.3	0	0	0	0
Thrombosis of reconstructed central veins	1	3.3	0	0	0	0
Total	19	63.3	10	50	15	28.3

DOI:10.14753/SE.2020.2356

5.3 CRP serum concentrations predict poor outcome and tumor recurrence in patients with TETs

5.3.1 Survival analysis: Increased pretreatment CRP is a predictor of worse FFR

Pathological Predictors of outcome

The histopathological differentiation of thymoma and TC revealed a significant survival advantage for patients with thymomas: OS: p=0.001, CSS: p<0.001 and FFR: p=0.002, respectively. Also, patients after complete resection of their TETs (R0) had a statistically signifcant better survival outcomes than incompletely resected patients (R1+R2): OS: p=0.003, CSS: p<0.001 and FFR: p=0.039, respectively. Masaoka-Koga tumor stages III and IV were associated with significantly worse CSS: p=0.008 and FFR: p=0.002, respectively.

Ajuvant therapy and outcome

In this patient cohort OS and CSS were not statistically different with or without adjuvant therapy (p=0.424 and p=0.117; respectively). Median FFR was significantly worse in patients with adjuvant therapy (128.5 months; 95% CI 40.7–216.3 months) compared those without 160.7 months (95% CI 30.6–290.8 months; p=0.001).

Pretreatment CRP and outcome

Patients were dichotomized into low and high CRP (cut off value: median CRP serum concentration of 0.22 mg/dL). The survival outcomes OS: p=0.201 and CSS: p=0.501 were not affected by increased pretreatment CRP, respectively (See also Figure 23).

Increased pretreatment CRP was a predictor of worse FFR

Patients with TETs with high pretreatment CRP had significantly worse FFR compared to those with low CRP: FFR: 5-year: high vs. low: 68.1% vs. 84.5% and 10- year: high vs. low: 68.1% vs. 72.4% (p=0.010; see also Figure 23).

DOI:10.14753/SE.2020.2356

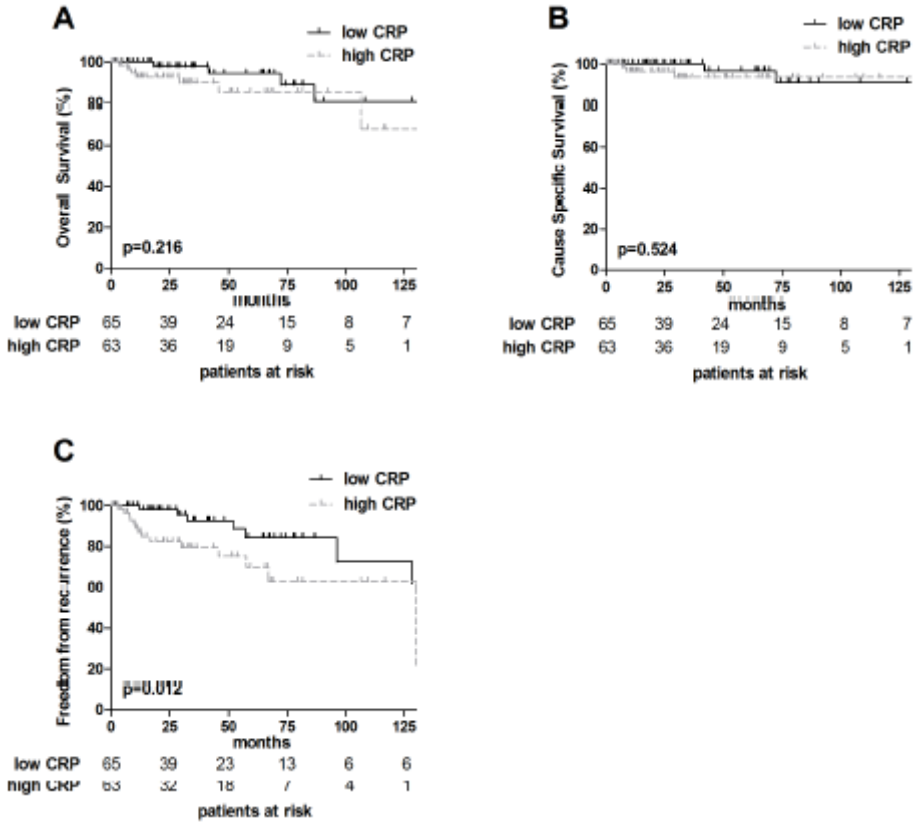

Figure 23: Prognostic impact of CRP in TETs. Overall Survival, Cause Specific Survival and Freedom From Recurrence are shown (median CRP cut off value of 0.22 mg/dL) (A–C). P-value (Log-rank test).

5.3.2 Pretreatment CRP is a prognostic factor for FFR

Univariable analysis

Presence of TC and incomplete resection were significant prognostic factors for worse OS, CSS and FFR, respectively. Advanced Masaoka-Koga tumors stage (III+IV) was also associated with shorter CSS and FFR. High pretreatment CRP serum concentrations were prognostic with regard to FFR, while OS and CSS were not affected. See Table 18 for numeric data.

Multivariable analysis

Presence of TC was statistically significant in predicting worse OS and CSS, respectively, while there was no effect on FFR. Incomplete tumor resection (R1+2) was associated with significantly worse CSS, while OS and FFR was not affected. CRP serum concentrations were not an independent prognostic marker at multivariable analysis.

Presence of paraneoplastic MG, sex or tumor size did not effect OS, CSS or FFR at univariable or multivariable analysis. Age as continuous variable significantly influenced OS, CSS and FFR, respectively. See Table 18 for numeric data.

Table 18: Univariate and multivariate Cox regression analyses. HR, hazard ratio; p, p-value; CI, confidence interval; [a]Cox-Regression; [b]median CRP (0.22mg/dL) was used for grouping patients into high and low CRP cohort.

	Univariate Model				**Multivariate Model**			
			95% CI				**95% CI**	
	HR	***p*[a]**	**Lower**	**Upper**	**HR**	***p*[a]**	**Lower**	**Upper**
Overall Survival								
Sex (Male)	1.134	0.822	0.380	3.377	0.627	0.536	0.143	2.749
Age (continuous)	1.038	0.080	0.996	1.081	1.054	0.048	1.001	1.110
MG (No)	6.361	0.077	0.820	49.340	0.358	0.360	0.040	3.234
Histology (TC vs. Thymoma)	0.183	0.003	0.059	0.568	3.561	0.130	0.687	18.457
Resection Status (R0 vs. R1-2)	0.194	0.008	0.058	0.653	2.308	0.347	0.403	13.211
Tumor Stage (I+II vs. III-IV)	0.380	0.090	0.124	1.165	2.850	0.183	0.610	13.316
CRP (low vs. high)[b]	0.477	0.240	0.139	1.639	2.601	0.188	0.627	10.799
Cause Specific Survival								
Sex (Male)	1.732	0.472	0.387	7.742	1.151	0.925	0.061	21.627
Age (continuous)	1.036	0.207	0.980	1.096	1.106	0.059	0.996	1.229
MG (No)	2.843	0.334	0.342	23.651	8.559	0.291	0.160	458.83
Histology (TC vs. Thymoma)	0.067	0.001	0.013	0.352	76.836	0.039	1.258	4694.5
Resection Status (R0 vs. R1-2)	0.071	0.001	0.016	0.319	74.810	0.041	1.191	4700.1
Tumor Stage (I+II vs. III-IV)	0.098	0.032	0.012	0.820	4.834	0.342	0.187	124.78
CRP (low vs. high)[b]	0.561	0.530	0.093	3.397	1.331	0.862	0.054	33.048
Freedom From Recurrence								
Sex (Male)	1.684	0.192	0.770	3.683	1.180	0.754	0.419	3.322
Age (continuous)	0.964	0.008	0.938	0.991	0.927	0.129	0.937	1.008
Myasthenia Gravis (No)	2.127	0.127	0.806	5.611	0.826	0.796	0.194	3.519
Histology (TC vs. Thymoma)	0.275	0.003	0.116	0.651	2.428	0.189	0.646	9.125
Resection Status (R0 vs. R1-2)	0.361	0.048	0.131	0.992	1.444	0.600	0.367	5.684
Tumor Stage (I+II vs. III-IV)	0.307	0.004	0.136	0.689	1.600	0.433	0.494	5.177
CRP (low vs. high)[b]	0.303	0.015	0.116	0.792	2.394	0.126	0.781	7.337

5.3.3 CRP serum concentrations are increased in patients with TETs

Increased CRP serum concentrations in patients with TETs

Patients with TETs showed significantly higher pretreatment CRP serum concentrations compared to sex- and age-matched controls (TETs 1.03±0.3 mg/dL vs. controls 0.16±0.03 mg/dL; $p<0.001$; Figure 24A). Thymomas 0.62±0.21 mg/dL, TCs 2.33±0.7 mg/dL and TNETs 0.90±0.44 mg/dL compared to controls showed significantly different CRP serum concentrations (one-way ANOVA: $p<0.001$; see Figure 24B for *post hoc comparisons*).

DOI:10.14753/SE.2020.2356

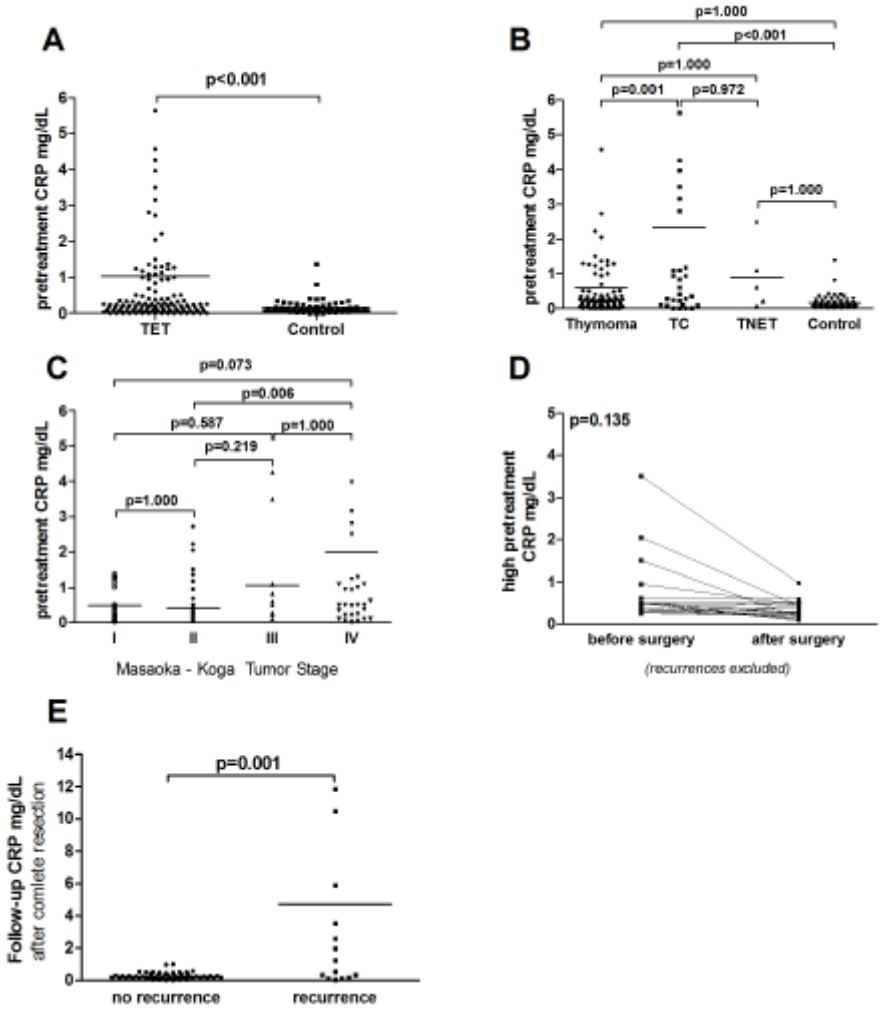

Figure 24: CRP serum concentrations in TETs. Patients with TETs (n = 128) revealed higher CRP serum concentrations compared to controls (n = 64) (A). Thymomas (n=93), TCs (n=30) and TNETs (n=5) compared to controls are shown (B). Highest CRP serum concentrations were found in metastatic TETs (Masaoka-Koga Stage IV; (C). In patients with high pretreatment CRP≥0.22 mg/dL, CRP serum concentrations decreased after complete tumor resection (n=52) (D), and increased significantly in cases of tumor recurrence (n=16) (E).

CRP serum concentrations in different WHO histological subtypes

One-way ANOVA revealed significantly different CRP serum concentrations between WHO subtypes (p=0.029), while further post hoc comparisons showed no differences. The highest CRP serum concentrations were detected in patients with TCs and the lowest in WHO type AB and B2 thymomas (see Table 19 for details).

We detected significantly higher CRP serum concentrations in patients with B3 thymomas compared to patients with A/AB/B1/B2 thymomas: 2.05±1.14 mg/dL vs. 0.32±0.049 mg/dL; p=0.009).

Table 19: Patient characteristics according to CRP serum concentrations.

*recurrences were excluded; [a]independent-samples t-test; [b]Pearson's $\chi 2$ test for independence; [c]one way ANOVA; [d]Whitney U-test; [e]paired-samples t-test.

		CRP (*mg/dL*)	
Characteristics	n	*mean(median)±SD (SEM)*	*p-value*
Age (years)			
<56	63	1.37(0.24)±3.4(0.43)	
≥56	65	0.71(0.20)±1.5(0.20)	0.165[a]
Sex			
Male	54	1.73(0.18)±3.9(0.58)	
Female	74	0.52(0.19)±0.9(0.11)	0.029[b]
Tumor Subtype			
MNT	6	0.66(0.29)±0.74(0.30)	
A	15	0.46(0.19)±0.49(0.13)	
AB	19	0.18(0.15)±0.14(0.03)	
B1	11	0.38(0.18)±0.49(0.15)	
B2	26	0.25(0.10)±0.40(0.08)	
B3	16	2.05(0.34)±4.50(1.14)	
TC	30	2.33(0.72)±4.00(0.73)	
TNET	5	0.90(0.62)±0.98(0.44)	0.029[c]
Tumor Stage			
I	27	0.57(0.20)±0.92(0.18)	
II	58	0.35(0.17)±0.56(0.07)	
III	13	2.01(0.60)±2.87(0.79)	
IV	30	2.33(0.55)±4.79(0.87)	0.003[c]
local (I-III)	98	0.63(0.20)±1.32(0.13)	
metastases (IV)	30	2.33(0.55)±4.79(0.87)	0.038[d]
non-invasive (I)	27	0.57(0.20)±0.92(0.18)	
invasive (II-IV)	101	1.15(0.22)±2.95(0.29)	0.317[a]
early stages (I-II)	85	0.42(0.19)±0.70(0.08)	
advanced stages (III-IV)	43	2.24(0.60)±4.27(0.65)	0.002[d]
Myasthenia Gravis			
Yes	34	0.91(0.13)±3.3(0.56)	
No	94	1.08(0.27)±2.4(0.25)	0.753[a]
Tumor resection			
before surgery	52	0.57(0.20)±1.51(0.21)	
after surgery *	52	0.26(0.24)±0.22(0.03)	0.135[e]
Follow up			
no recurrence	52	0.26(0.24)±0.22(0.03)	
recurrence	16	4.72(1.60)±6.44(1.61)	0.001[d]

CRP serum concentrations in different Masaoka-Koga stages

Different tumor stages displayed differing CRP serum concentrations (p=0.003; Figure 24C; Table 19). The highest CRP serum concentrations were found in patients with metastases (Stage IV disease) compared to local (stage I-III) TETs; p=0.038). For details and further comparisons see Table 19.

TET recurrences are paralleled by increased CRP serum concentrations

Longitudinal analysis was performed in a subset of 68 patients. Pre- and postoperative CRP serum concentrations were available (median follow up of 14.8 months).
In this subset of patients recurrences occurred in 16 patients (local: n=2, regional: n=4, distant: n=10), whereas 52 patients were without recurrence. In patients without tumor recurrence CRP serum concentrations decreased from 0.57±0.21 mg/dL preoperatively to 0.26±0.03 mg/dL postoperatively (p=0.135; Figure 24D).
In case of tumor recurrence CRP serum concentrations increased significantly (4.72±1.61 mg/dL; p=0.001; Table 19; Figure 24E).

Diagnostic accuracy of CRP to predict tumor recurrence

We tested the accuracy of heightened CRP serum concentrations for prediction of tumor recurrence at a CRP cut off level of 0.5 mg/dL: sensitivity 62.5% (10 out of 16), specificity 92.4% (48 out of 52), PPV)71.4% (10 out of 14) and NPV 88.9% (48 out of 54), respectively.

CRP is not expressed in TET tissue

We found no CRP expression in 27 thymoma and 6 TC specimens by immunohistochemical techniques.

DOI:10.14753/SE.2020.2356

5.4 Prognostic and diagnostic impact of fibrinogen, NLR, and PLR on TET outcome

5.4.1 Survival and freedom from recurrence are associated with fibrinogen plasma concentrations, NLR and PLR

Median follow-up time was 30.8 months. Fifteen fo the 122 patients (12.3%) suffered from a recurrence: 3 local, 4 regional, and 8 distant. High fibrinogen plasma concentrations were associated with significantly worse FFR and CSS (Figure 25A and B).

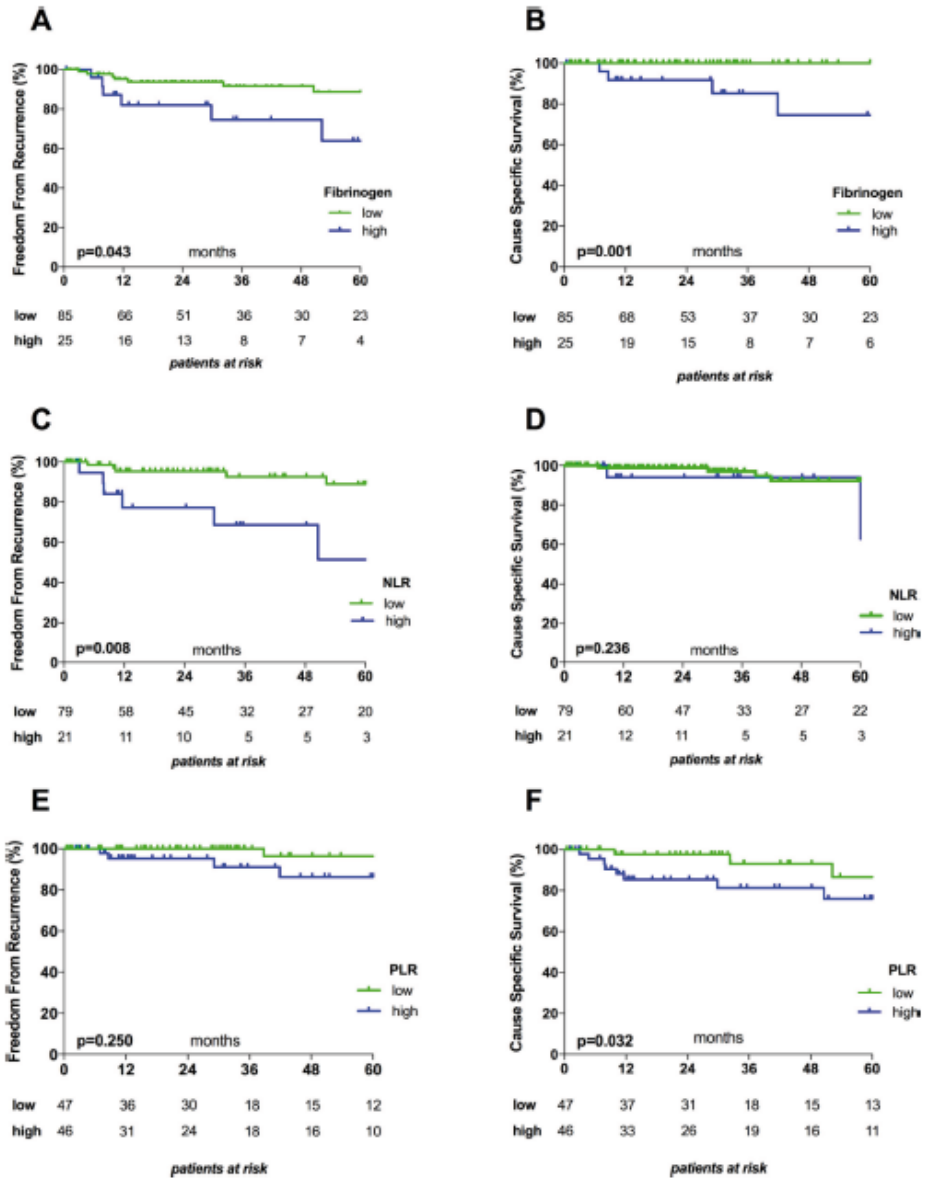

Figure 25: Kaplan–Meier survival in relation to fibrinogen plasma concentrations, NLR and PLR. Graphs show the associations between Fibrinogen and FFR (A) and CSS (B); between NLR and FFR (C) and CSS

(D) , and between PLR and FFR (E) and CSS (F). The cut-off values used to dichotomize patients into low and high subgroups were 452.5 mg/dL for Fibrinogen, 4.0 for NLR, and 136.5 for PLR.

High NLRs were associated with significantly worse FFR and patients with high PLR with significantly worse CSS (Figure 25C-F). Patients with thymomas (but not TCs) with high pretreatment fibrinogen plasma concentrations had significantly worse CSS (p=0.013). Pretreatment NLR and PLR were not associated with statistical differences in CSS or FFR in patients with thymomas or TCs.

5.4.2 Fibrinogen and NLR as predictors of survival and recurrence

FFR

TC (HR 4.93) and high pretreatment NLR (HR 3.95) were significantly associated with worse FFR (univariable analysis). In multivariable analysis TC remained a statistically significant predictor of worse FFR (HR 8.55).

CSS

TC (HR 23.3), advanced tumor stage (HR 12.35) and high fibrinogen plasma concentrations (HR 17.24) were associated with statistically significant worse CSS. See table Table 20 for details of uni- and multivariable analysis.

Table 20: Univariable and multivariable Cox regression analysis Presented data indicate the prognostic impact of tested factors. Patients were dichotomized into high and low subgroups using cut-off values of 452.5 mg/dL for Fibrinogen, 4.0 for NLR, and 136.5 for PLR.

	Univariable			**Multivariable**		
	HR	***p*-value**	**95% CI**	**HR**	***p*-value**	**95% CI**
Freedom from recurrence						
TC vs. Thymoma	4.93	0.002	1.76–13.7	8.55	0.036	1.14–62.5
Tumor Stage I–II vs. III–IV	2.60	0.066	0.94–7.14	0.68	0.673	0.12–4.02
Fibrinogen (high vs. low)	2.86	0.053	0.99–8.26	1.40	0.601	0.40–4.95
NLR (high vs. low)	3.95	0.014	1.32–11.7	3.14	0.145	0.67–14.7
PLR (high vs. low)	2.00	0.259	0.60–6.67	0.44	0.350	0.08–2.46
Cause specific survival						
TC vs. Thymoma	23.3	0.004	2.68–200.0	3.56	0.442	0.14–90.9
Tumor Stage I-II vs. III-IV	12.35	0.022	1.43–111.1	3.10	0.459	0.16–62.5
Fibrinogen (high vs. low)	17.24	0.012	1.87–166.7	9.09	0.149	0.45–166.7
NLR (high vs. low)	62.5	0.265	0.04–100.0	0.61	0.677	0.06–6.10
PLR (high vs. low)	2.83	0.257	0.47–16.5	1.07	0.963	1.00–1.60

Preoperative Fibrinogen, NLR, and PLR in patients with TETs

Patients with TETs showed significantly higher fibrinogen plasma concentrations compared to healthy volunteers. Fibrinogen was significantly higher in patients with TCs compared to patients with thymomas. There were significantly higher NLR and PLR in patients with TETs compared to controls. Mean NLR and PLR values were significantly higher in patients with TCs than in patients with thymomas. See Table 21 for details.

Thymoma subtypes according to the WHO classification

Fibrinogen plasma concentrations were lowest in patients with MNT and AB thymomas. NLR and PLR values were lowest in patients with B1 thymomas and B2 thymomas (Table 21).

Table 21: Preoperative analysis of Fibrinogen, NLR, and PLR in patients with TETs.

[a]unpaired Student's *t*-test; [b]one-way ANOVA.

		Fibrinogen			**NLR**			**PLR**	
Characteristics	n	*mean(median)±SD(SEM)*	*p-value*	n	*mean(median) ±SD(SEM)*	*p-value*	n	*mean(median)±SD(SEM)*	*p-value*
Age (years)									
<57	54	375.0(328.5)±121.3(16.5)		48	3.47(2.49)±2.79(0.40)		44	184.7(150.0)±107.2(16.2)	
≥57	58	404.3(370.5)±119.3(15.7)	0.201[a]	54	3.40(2.86)±2.42(0.33)	0.888[a]	51	175.7(142.0)±127.4(17.8)	0.715[a]
Sex									
M	46	397.6(369.5)±139.5(20.6)		40	3.69(2.92)±2.41(0.38)		37	180.3(138.7)±99.2(16.3)	
F	66	384.9(353.0)±106.3(13.1)	0.586[a]	62	3.27(2.53)±2.71(0.34)	0.419[a]	58	179.5(150.0)±129.3(17.0)	0.976[a]
WHO									
MNT	7	331.7(327.0)±90.8(34.3)		7	5.10(2.42)±3.81(1.44)		7	252.6(274.4)±142.3(53.8)	
A	14	5.2(385.5)±82.0(21.9)		14	3.32(3.04)±2.04(0.59)		12	198.8(167.8)±89.6(25.9)	
AB	16	1.5(315.5)±104.1(26.0)		16	2.93(2.97)±0.82(0.21)		14	140.7(126.3)±59.0(15.8)	
B1	10	1.9(358.5)±54.8(17.3)		10	2.19(0.18)±0.49(0.15)		10	110.7(83.8)±47.3(15.0)	
B2	22	4.7(351.5)±87.8(18.7)		22	2.70(2.47)±1.89(0.42)		18	124.5(108.6)±58.1(13.7)	
B3	15	3.5(334.0)±96.9(25.0)		15	2.71(2.53)±1.47(0.39)		13	152.4(139.5)±71.3(19.8)	
TC	28	9.4(473.0)±163.4(30.9)	0.003[b]	28	5.09(4.05)±3.84(0.82)	0.005[b]	21	268.2(230.0)±169.6(37.0)	<0.001[b]
Tumor Stage									
I	25	.5(346.0)±65.1(13.0)		25	2.84(2.44)±1.69(0.36)		20	171.3(163.6)±74.4(16.7)	
II	53	.1(352.0)±96.9(13.3)		53	3.09(2.69)±2.08(0.29)		48	145.3(129.4)±79.3(11.5)	
III	11	8.1(415.0)±126.5(38.1)		11	4.74(4.10)±3.88(1.29)		9	268.9(281.4)±145.5(48.5)	
IV	23	0.8(465.0)±169.3(35.3)	0.001[b]	23	4.45(3.22)±3.54(0.81)	0.061[b]	18	236.9(173.0)±179.7(42.4)	0.003[b]
MG									
Yes	31	351.8(330.0)± 102.3(18.4)		31	3.11(2.40)±2.75(0.53)		26	161.5(133.5)±148.4(29.1)	
No	81	404.8(368.0)± 124.5(13.8)	0.037[a]	81	3.55(2.83)±2.54(0.29)	0.455[a]	69	186.7(158.8)±104.7(12.6)	0.356[a]
Resection									
R0	101	388.7(359.0)±124.2(12.4)		101	3.51(2.65)±2.69(0.28)		86	183.5(142.2)±122.5(13.2)	
R1+2	11	403.7(406.0)±83.5(25.2)	0.696[a]	11	2.61(2.57)±0.70(.23)	0.318[a]	9	2.61(2.57)±0.70(.23)	0.384[a]
Recurrence									
Yes	14	447.8(410.5)±158.4(42.3)		13	4.70(2.61)±3.87(1.07)		12	253.7(197.3)±202.6(58.5)	
No	98	381.9(358.0)±112.9(11.4)	0.056[a]	89	3.25(2.65)±2.32(0.25)	0.209[a]	83	169.1(135.2)±97.6(10.7)	0.181[a]

Masaoka-koga tumor stage

Advanced stage tumors (stage III–IV) had significantly higher Fibrinogen plasma concentrations, NLR and PLR compared to patients with early stage tumors (stage I–II; Table 21). Fibrinogen plasma concentrations increased with tumor invasiveness: non-invasive stage I tumors 354.5±13.0 mg/dL and stage IV TETs 470.8±35.3 mg/dL (Figure 26A).

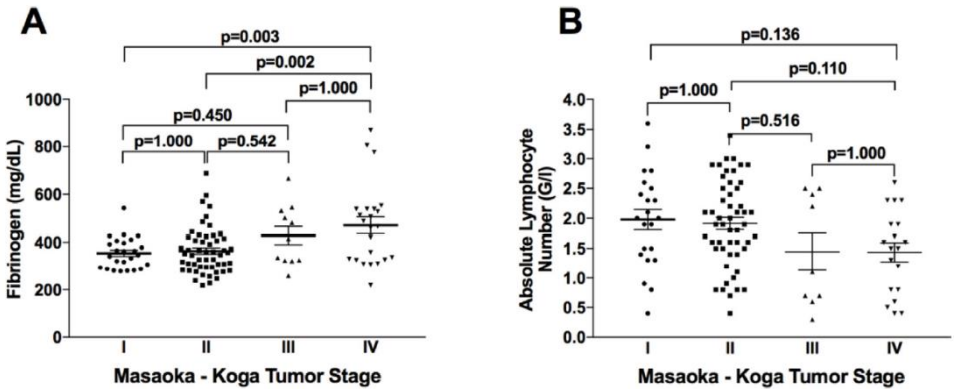

Figure 26: Fibrinogen and absolute lymphocyte numbers according to Masaoka-Koga tumor stage. Fibrinogen plasma concentrations gradually increased with invasiveness defined by stage (A). Peripheral blood absolute lymphocyte numbers gradually decreased with tumor stage (B).

Paraneoplastic MG

Twenty-six percent of 122 patients were diagnosed with MG. MG was prominent in patients with B2 thymomas (11 out of 24, 45.8%) and B3 thymomas (7 out of 17, 41.2%), respectively. Patients without MG had significantly higher preoperative fibrinogen plasma concentrations and tumor size. See Table 21 for details. There was no difference in fibrinogen plasma concentration comparing MG-negative and MG-positive thymomas (TCs excluded).

Neutrophil, lymphocyte, and platelet blood counts in patients with TETs

There was no statistically significant difference in absolute and relative neutrophil and lymphocyte counts as well as absolute platelet counts in relation to WHO histology or Masaoka-Koga stage (not shown). Absolute lymphocyte numbers in relation to TET levels of invasiveness are depicted in Figure 26B: stage I: 1.98±0.80 G/L to stage IV (metastasized) 1.43 ± 0.70 G/L (p = 0.822). We observed significantly higher absolute lymphocyte numbers in MG patients (2.09±0.7 G/L) compared to those without MG (1.69±0.1 G/L; p=0.020).

5.4.3 NLR and PLR as predictors of tumor recurrence during oncological follow-up

Further we sought to investigate the value of fibrinogen, NLR and PLR as tumor markers within oncologic follow-up. We performed analysis of longitudinal measurements of preoperative, postoperative (3-7 days after surgery) and follow-up (6-12 months after surgery) fibrinogen plasma concentration, NLR, and PLR.

Preoperative values

Patients with TETs had significantly higher mean preoperative fibrinogen plasma concentrations compared to healthy controls (390.2±11.4 mg/dL vs. 314.8±10.9 mg/dL; p<0.001), NLR (3.43±0.3 vs. 1.78±0.1; p=0.001), and PLR (179.8±12.1 vs. 133.4±7.1; p=0.001).

Early postoperative period

Fibrinogen plasma concentrations and NLR values 3-7 days after surgery were significantly elevated in thymomas compared to their respective preoperative values (fibrinogen: 445.6±14.7 mg/dL vs. 364.7±9.8 mg/dL; p<0.001; NLR: 6.22±0.5 vs. 2.99±0.2; p<0.001); similarly in TCs (fibrinogen: 541.5±34.0 mg/dL vs. 469.4±30.9 mg/dL; p=0.006; NLR: 10.5±1.4 vs. 5.1±0.8; p=0.007).

Oncological follow-up in thymoma and TC patients

Six to twelve months following thymoma resection fibrinogen plasma concentrations and NLR were significantly reduced compared to early postoperative values (fibrinogen: 356.5±25.2 mg/dL vs. 445.6±14.7 mg/dL; p=0.043; NLR: 4.37±0.5 vs. 6.22±0.5; p=0.004). In contrast, fibrinogen and NLR were not significantly reduced in TCs comparing the same time points (fibrinogen: 454.3±40.5 mg/dL vs. 541.5±34.0 mg/dL; p = 0.635; NLR: 8.7±2.0 vs. 10.5±1.4; p=0.657).

Follow-up (6-12 months) NLR values in patients with TCs were significantly higher than preoperative values (8.7±2.0 vs. 5.1±0.8; p=0.028), while Fibrinogen plasma concentrations did not significantly differ (454.3±40.5 mg/dL vs. 469.4±30.9 mg/dL; p=0.478). Follow-up fibrinogen plasma concentrations and NLR were similar to preoperative values in thymoma (fibrinogen: 356.5±25.2 mg/dL vs. 364.7±9.8 mg/dL; p=0.931; NLR: 4.37±0.5 vs. 2.99±0.2; p=0.203) (Figure 27A, B, D, E).

While postoperative PLR was significantly elevated in patients with thymomas (preop vs. postop: 141.4±9.8 vs. 156.9±10.1; p=0.014) PLR in TCs was decreased in patients with

TCs (207.8±29.3 vs. 168.2±37.0; p=0.546). During oncological follow up (6-12 months) the highest PLR for thymomas (212.5±24.8) and TCs (347.1±74.0) was detected (Figure 27G and H).

Higher NLR and PLR in patients with tumor recurrence

Patients with tumor recurrence 6–12 months after surgical resection (with and without multimodal therapy) showed significantly higher NLR (p=0.001) and PLR (p=0.031) compared to those without recurrence (Figure 27F and I).

The reasons for the NLR and PLR increases in patients with tumor recurrence was due to significantly lower absolute and relative lymphocyte numbers compared to patients without (p=0.014 and p=0.027, respectively), while platelet and neutrophil counts displayed non-significant increases (p=0.492 and p=0.154, respectively).

Serum concentrations of fibrinogen were not significantly altered in patients with recurrence compared to those without (p=0.351; Figure 27C).

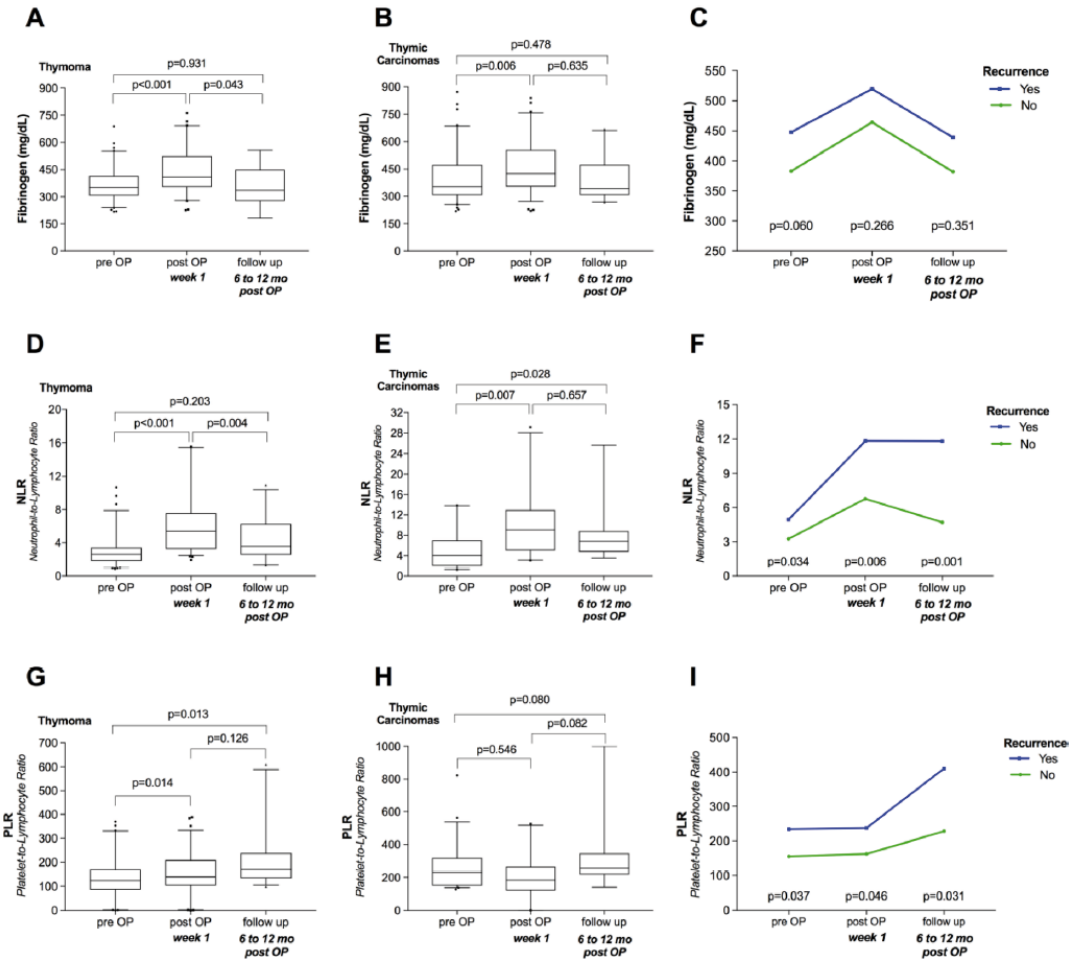

Figure 27: Fibrinogen, NLR, and PLR before and aftger surgery and during oncologic follow up. Plots illustrate how surgical tumor resection and recurrence were related to Fibrinogen, NLR, and PLR in thymomas (A, D, G) and TCs (B, E, H), as well as the longitudinal courses of Fibrinogen, NLR, and PLR according to tumor recurrence among TETs (C, F, I).

NLR and PLR as predictor of tumor recurrence during oncologic follow-up

ROC analysis of NLR and PLR for predicting tumor recurrence is shown in Figure 28. NLR and PLR displayed high accuracy in predicting tumor recurrences: area under the curve (AUC) NLR: 0.819 and PLR: 0.787.

Eighty percent sensitivity, 77% specificity, a PPV of 36.4, and a NPV of 96% were calculated employing a NLR of 6.6 as a cut-off (Youden index 0.574) to predict tumor recurrence.

One-hundred percent sensitivity, 58.1% specificity, a PPV of 27.8%, and a NPV of 100% were achieved using a PLR of 202.5 as a cut-off (Youden index 0.581).

Binary logistic regression identified NLR as a significant factor predicting tumor recurrence (p = 0.043; R2: 0.378), but not PLR (p = 0.078; R2: 0.165) and Fibrinogen (p = 0.341; R2: 0.054).

There was no additive effect in predicting tumor recurrence when using combinations of: NLR and PLR (NLR: p=0.046; PLR: p=0.847; R2: 0.379), NLR and Fibrinogen (NLR: p=0.071; Fibrinogen: p=0.913; R2: 0.369), or PLR and Fibrinogen (PLR: p=0.165; Fibrinogen: p=0.892; R2: 0.192), respectively.

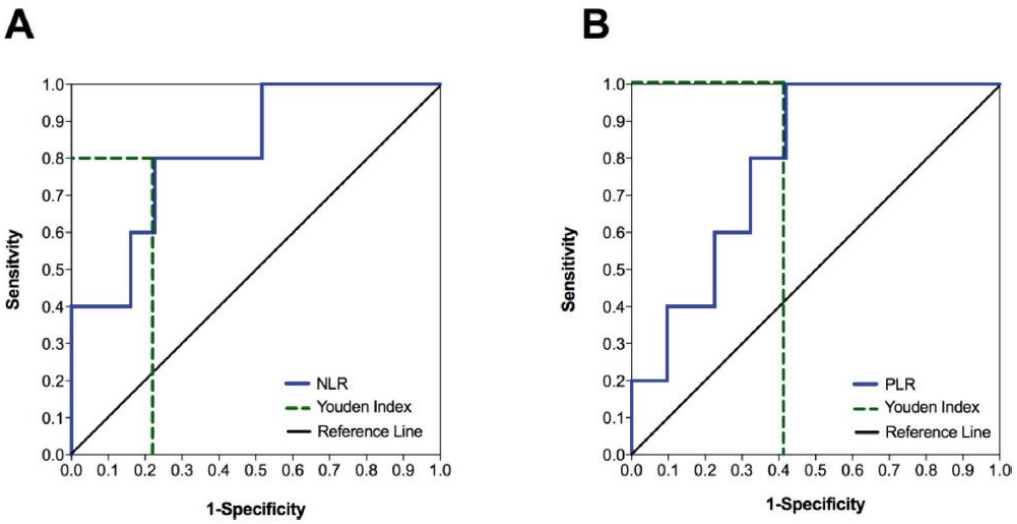

Figure 28: Accuracy of NLR and PLR in predicting tumor recurrence in patients with TETs. Receiver operating characteristic (ROC) curves for the use of NLR (A) and PLR (B) to predict tumor recurrence during oncologic follow-up showed AUC values of 0.819 (p = 0.024) and 0.787 (p = 0.042), respectively. The dotted lines indicate the highest Youden Indices for NLR (Youden Index=0.574; sensitivity=0.800; specificity=0.226; cut-off at 6.6) and PLR (Youden Index=0.581; sensitivity=1.000; specificity=0.419; cut-off at 202.5).

Fibrinogen expression in TETs

Immunohistochemistry for fibrinogen on B2 and B3 thymomas revealed fibrinogen expression in endothelial cells and within thrombotic clots, but was absent from neoplastic epithelial cells and cells of the hematopoietic lineage (see Figure 29).

DOI:10.14753/SE.2020.2356

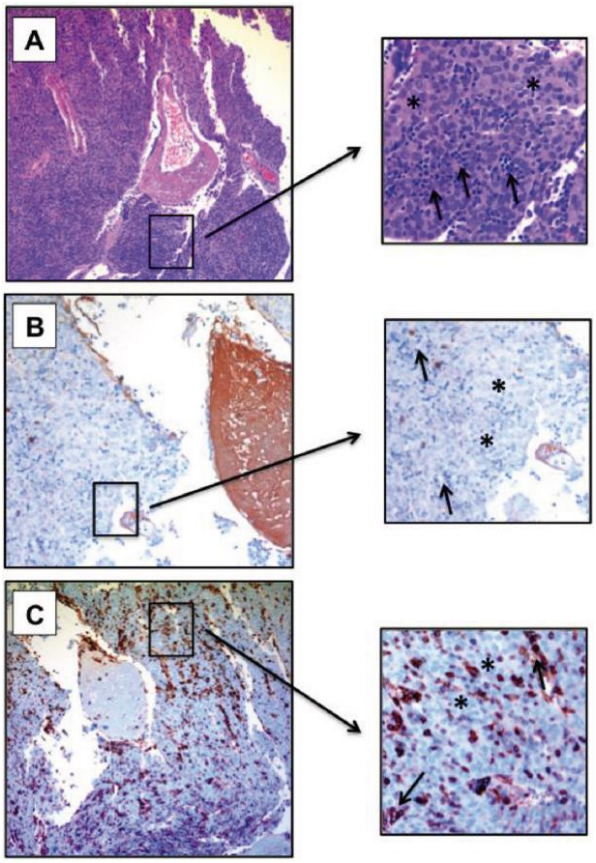

Figure 29: Fibrinogen expression in B2/B3 thymoma. Staining of a B2/B3 thymoma (B3 part) with Hematoxylin-Eosin (A). Fibrinogen (B) and CD45 (C) expression. 100× magnification. Asterisks indicate neoplastic thymic epithelial cells. Arrows indicate cells of the hematopoietic lineage. Fibrinogen expression is absent from tumor cells and lymphocytes. Lymphocytes exhibit CD45 expression.

6. Discussion

Thymomas and TCs are a rare tumor entities that display great subtype heterogeneity (Chau, Kim et al. 2010) and present in different clinical stages. Beyond that is the scattered treatment of patients with TETs that is carried out in small numbers in many different clinical departments without unified standards around the globe. The different practices for diagnosis, therapy, and follow-up of patients with TETs have resulted in poor understanding of their tumor biology and hindered the formulation of strong evidence-based treatment guidelines. As a result, current patient care has a weak evidence base. Decisions on diagnosis, therapy and follow-up are influenced by the clinical team's experience and reports from single institutions.

6.1 Pathological prognostic biomarkers for TETs

Relevance of prognositc factors at a single European thoracic surgery center

The institutional study investigated the outcome of patients with TETs treated surgically with or without multimodal therapy over the past decade and thus formed the foundation for all further studies on prognostic factors at the department. Our single center study corroborated most of the widely accepted prognostic factors for the treatment of TETs, in particular completeness of resection (R0 vs. R1+2, log-rank test $p<0.001$) and Masaoka–Koga stage log-rank test: $p=0.017$), but not WHO histological type (A, AB, B1, B2, B3, and TC, log-rank test: $p=0.136$) (Chen, Marx et al. 2002, Detterbeck and Parsons 2011, Venuta, Rendina et al. 2011). While eight (of 72) patients died from thymic neoplasms, three patients because of unrelated causes (two patients: coronary artery disease and one patient: gastric cancer) – corroborating the reporting standard of CSS in addition to OS for patients with TETs. Patients with advanced Masaoka-Koga stages not only displayed significantly worse prognosis regarding survival compared to patients in lower stages (log-rank test: $p=0.017$). This was accompanyied by a significantly higher frequency of recurrences and progressions in advanced stage tumors (Pearson $\chi 2$ test: $p=0.001$). Also, there was no significant survival difference comparing patients with MG to those without. The biologic absence of a fibrous capsule around the TET (desmoplastic reaction) as well as the disruption of loose areolar tissue surrounding it during postoperative handling and transport results in a R1 resection in the final histopathological report. This has led to an

overestimation of the number of true R1 resections in the literature. New handling, processing, and reporting guidelines will improve this misclassification (Huang, Detterbeck et al. 2011).

There are several reports indicating that preoperative biopsies may be responsible for tumor cell seeding (Nagasaka, Nakashima et al. 1993, Fujiwara, Matsumura et al. 2003, Kattach, Hasan et al. 2005, Choi, French et al. 2014). Our own data did not support a higher risk of recurrence or progression following TET biopsies. The institutional practice for performing preoperative biopsies are: (1) to identify the histological entity of the tumor, (2) in patients with suspicion of lymphoma or (3) in patients with suspected invasive TETs before the administration of neoadjuvant therapy, without traversing the pleura.

Prognositc factors for TETs with pleural involvement

The ESTS Thymic Working Group retrospective study contributes to a better understanding of the role of surgery the setting of TETs with pleural involvement. It reflects European and Canadian thoracic surgery practice patterns of institutions experienced in treating TETs with pleural and/or pericardial involvement.

Multivariable analysis revealed that complete surgical resection (R0) and thymoma histology (compared to TCs) are predictors of better OS.

It is particularly remarkable that complete surgical resection was predictive of improved OS irrespective of the surgical method (EPP, TP or LP) used. It is clear that the underlying situation and spread of disease determine the indication for a specific surgical procedure (see also 2.9.4.2). Patients requiring EPP had more advanced TETs (involving not only pleura but also the lung) than those patients in which TP or LP was sufficient to achieve R0 resections. EPP for the most advanced cases of tumor spread yielded similar OS results comparable to far less invasive procedures (TP and LP) for more limted disease.

Another key result of the study is that patients with thoracic surgery for recurrent disease to the pleura (first pleural surgery/Scenario 1) had better survival than those with surgery for primary pleural disease. A closer look at the patient cohorts reveals a bias in disease severity with resultant necessity for extended surgery in patients with primary pleural disease: (Scenario 2, primary tumor with pleural involvement): 29.9% EPPs, 15.0% TCs and 29.9% incomplete resections vs. (Scenario 1, tumor recurrence with pleural involve-

ment): 18.2% EPPs, 2.2% TCs and 6.8% incomplete resections). There is no fair comparison of the two patient cohorts. Nevertheless the data underscore the excellent outcome of surgery for recurrent disease of TETs to the pleura (Lucchi, Davini et al. 2009).

Other factors that may contribute are differences in the biology of TETs presenting with pleural involvement at first diagnosis (compared to those with first pleural involvement at recurrence) or effective follow-up after thymic surgery (tertiary prevention) succeeding in early diagnosis of recurrences after surgery (compared to those with pleural involvement without prior thymic surgery (no primary prevention).

Five-year OS of 87.2%.of the entire patient cohort in the current study compares favourably to a JART study which reported on TET patients with pleural dissemination who underwent surgical resection: 118 patients in Masaoka Stage IVA and 18 in IVB with a 5-year OS of 83.5% (Okuda, Yano et al. 2014). In studies of patients undergoing surgery for TETS with pleural dissemination with patients cohort sizes ranging from 5-21 patients 5-year OS ranging from 43.1 to 92.3% was reported (Murakawa, Karasaki et al. 2015).

In an ESTS database study on 229 TC patients including all tumor stages with 5- and 10-year OS rates of 0.61 and 0.37 and 5- and 10-year FFR rates of 0.60 and 0.43 multivariate analysis revealed incomplete resection and Masaoka–Koga stage III–IV as negative predictors of OS ($p<0.0001$ and $p=0.02$), respectively (Ruffini, Detterbeck et al. 2014). Our study revealed comparable OS for the 17 TC patients with pleural involvement: 5- and 10-year OS: 56.0% and 0% (mortality: 6 deaths by the end of 10 years), respectively.

The role adjuvant therapy in patients with pleural involvement is still unclear. In an ESTS cohort study on 2030 TET patients Masaoka–Koga stage III–IV, incomplete resection and non-thymoma histology were associated with more recurrences and worse survival while adjuvant therapy after complete resection was associated with improved survival (Ruffini, Detterbeck et al. 2014). In a JART study including 1320 patients Masaoka Stage IVA (118 patients) with ≤10 pleural nodules and macroscopic complete resection had better prognosis. The role of adjuvant therapy after R0 resection remained unclear (Kondo and Monden 2003). In another Japanese study on stage IVA patients that underwent R0 resection, no supportive data on the efficacy of ChT and/or RT were reported (Okuda, Yano et al. 2014).

The observed survival advantage of patients with MG may be just the result of more advanced disease in patients without MG: MG^+ vs MG^-: TCs 2.1% vs. 15.2%, incomplete resections 17.4% vs. 25.0% and EPPs 10.6% vs. 33.7%, respectively. Better OS of MG patients was reported in a study on 797 thymoma patients (Filosso, Evangelista et al. 2015). In patients with TAMG of all stages, MG was associated with histology and stage, but only stage had a prognostic significance on OS and DFS (Ruffini, Filosso et al. 2011). Closer follow-up of patients with MG may also be responsible for earlier diagnosis of TETs (Ruffini, Filosso et al. 2011).

Since 90% of TET patients (64 of 71) underwent extended thymectomy at the time of primary pleural surgery and only six patients had thymomectomy only with with recurrence rates of 49.0% and 50%,, no conclusions on the value of extended thymectomy compared to thymomectomy only at primary surgery for pleural disease of TETs can be drawn.

Surgery of the diaphragm in patients with TETs with diaphragmatic metastases ranges from just pleurectomy of the diaphragm to partial or complete resection of the diaphragm. The extent of resection purely depends on the metastatic involvement of diaphragmatic pleura with our without involvement of diaphragmatic musculature. Diaphragmatic metastases were present simultaneously with metastases to other pleural sites what renders the separate analysis of different extents of diaphragmatic resection or just pleurectomy impossible. Diaphragmatic surgery may also be necessary for functional reasons. After oncologic phrenic nerve resection diaphragmatic plication is indicated in order to avoid atelectasis of the lung.

Limitations of the clinical studies

The main limitations of both studies stem from their retrospective design and the long investigated study period that was necessary for gathering the large number of TET patients, particularly those with pleural involvement. During the large study period diagnostic and therapeutic algorithms may have changed due to technical advances (e.g. PET-CT).

Despite these limitations the relatively large number of patients analyzed in both studies is one of its strengths.

Prospective studies on TETs as well as TETs with pleural involvement are warranted. It is on ESTS, ITMIG or JART to prospectively collect data in large patient cohorts. We recommend close follow-up after surgery for TETs and TETs with pleural disease.

6.2 Peripheral blood derived prognostic biomarkers for TETs

High pretreatment CRP serum concentrations in our study were associated with significantly worse FFR, more aggressive tumors and advanced tumor stages. These findings are congruous with reports of increased CRP serum concentrations to be associated with worse outcome in other solid organ malignancies: breast cancer (CRP≥10 mg/dl, OS) (Villasenor, Flatt et al. 2014), gall bladder cancer (CRP≥10 mg/dl; OS) (Saqib, Pathak et al. 2018), malignant pleural mesothelioma (CRP≥1 mg/dL; OS) (Ghanim, Hoda et al. 2012), pancreatic cancer (adenocarcinoma, CRP>4.5 mg/dL; CSS) (Szkandera, Stotz et al. 2014), non-small cell lung cancer (CRP>1 mg/dL; CSS) (O'Dowd, McRae et al. 2010), hepatocellular carcinoma (CRP>0.2 mg/dL; FFR) (Nishikawa, Arimoto et al. 2013) and nasopharyngeal carcinoma (Fang, Xu et al. 2017). In studies of nasopharyngeal cancer different cut-offs were applied for different patient cohorts, all indicating worse prognosis with elevated CRP (Fang, Xu et al. 2017): high sensitivity-CRP cut-offs ranging from 1.96-3 in patients with non-metastatic (Tang, Hu et al. 2015) and primary nasopharyngeal carcinoma (Tang, Li et al. 2015) and CRP cut-offs ranging from 2.46-8 in patients with metastatic (Xia, Ye et al. 2013), newly identified (Xia, Zhang et al. 2013) and locoregional advanced nasopharyngeal carcinoma (Zeng, Wu et al. 2015). In a recent review elevated preoperative CRP serum concentrations were associated with higher mortality in adult patients with solid organ malignancies. The authors also concluded that CRP was useful to identify tumor recurrences (Shrotriya, Walsh et al. 2015).

While other groups have shown that baseline as well as postoperative CRP serum concentrations predict mortality in non-small cell lung cancer patients undergoing surgery (Pastorino, Morelli et al. 2017), our study focused on preoperative measurements. We focused out studies on preoperative CRP serum concentrations in order to avoid the influence of different surgical methods, e.g. open surgery via thoracotomy or minimally-invasive robotic or VATS surgery, on the regularly occurring rises in postoperative CRP. Different surgical approaches may impose different degrees of surgical

stress on the patients that result in quantitatively different acute phase responses and CRP values (Asteriou, Lazopoulos et al. 2016).

More evidence is accumulating the thymoma histology is of prognostic value (Ruffini, Detterbeck et al. 2014, Weis, Yao et al. 2015). In support of this are CRP serum concentrations differentiating thymoma types with better prognosis: A/AB/B1/B2 from those with poorer prognosis: B3 and TCs.

TET recurrences can be observed in 10% to 30% of patients after complete resection of the primary TET – sometimes even decades after resection (Dai, Song et al. 2015, Marulli, Margaritora et al. 2016). Surgical resection of tumor recurrence is a valid option leading to good long term OS (Sandri, Cusumano et al. 2014, Marulli, Margaritora et al. 2016). Reported 5- and 10-year OS after thoracic surgery for recurrence was 63 and 37%, respectively. Complete surgical resection was associated with a statistically significant better OS when compared to incomplete resection and non-surgical treatment (Marulli, Margaritora et al. 2016). In a multi-center study (3 centers, 81 patients) 5- and 10-year OS after TET recurrence treatment (surgery in 75% of patients) were 73.6% and 48.3%, respectively. The study included local (mediastinum: 15 cases, pleura: 44 cases) as well as distant recurrences (15 cases). Five- and 10-year OS was better with 82.4% and 65.4% following a complete re-resection (R0) (Sandri, Cusumano et al. 2014). Therefore there is a real need for biomarkers useful for tertiary prevention after TET resection in order to detect tumor recurrences early when complete resection is possible. CRP serum concentrations might serve as a useful biomarker in this regard. Thus future study of CRP serum concentrations for oncologic follow-up of patients after TET resection is warranted. CRP measurements can be readily included into follow-up routines. Due to the fact that CRP is not a specific marker for TETs or their recurrences clinicians have to evaluate other reasons for rises in circulating CRP (see also Chapter 2.11.1). Particularly infections as a source of serum CRP rises have to be ruled out before a high suspicion for recurrence can remain that prompts further clinical and radiological examinations in the search for a possible tumor recurrence. With a PPV of 71.4% and NPV of 88.9% CRP could serve as an accurate biomarker for oncologic TET follow-up. CRP has several advantages over newer but more experimental biomarkers (e.g. soluble RAGE, HMGB1(Moser, Janik et al. 2014), HSP27 and 70 (Janik, Schiefer et al. 2016)): it is cheap, its measurement is readily available in the

majority of hospitals worldwide and clinicians have already gained a vast experience with this biomarker.

CRP expression in tumor tissue has been demonstrated several solid organ malignancies, including hepatocellular carcinoma (Shin, Kim et al. 2015), esophageal squamous cell carcinoma (Nakatsu, Motoyama et al. 2012) or renal cell cancer (Jabs, Busse et al. 2005) and intrahepatic cholangiocarcinoma (Yeh, Lei et al. 2017). In the case of TETs circulating CRP might not derive from the tumors as it was not detected by immunohistochemistry in our study. The source of circulating CRP remains elusive in our study. One might speculate that increased CRP serum concentrtaions in patients with TETs stem from cancer-related inflammation: interaction of immune cells and tumor cells leading to increased CRP production by hepatocytes via cytokine stimulation.

Previously published work on HSPs (Janik, Schiefer et al. 2016) and RAGE axis molecules (Moser, Janik et al. 2014) from our group support cancer-related inflammation in TET patients. Proinflammatory HSPs and RAGE-ligands (Hofmann, Drury et al. 1999) were significantly elevated in patients with TETs and results were again pronounced in advanced tumor stages and more aggressive tumor subtypes (Moser, Janik et al. 2014, Janik, Schiefer et al. 2016).

High fibrinogen plasma concentrations were identified in several types of cancer patients under going surgical tumor resection to be a statistically significant prognostic predictor of worse outcome and/or tumor progression (selected publications): breast cancer (≥283 mg/dl; OS) (Wen, Yang et al. 2015), gall bladder cancer (>402 mg/dL; OS) (Shu, Weng et al. 2014), gastric cancer (>407 mg/dl, OS) (Lee, Lee et al. 2012), colorectal cancer (>336 mg/dl; RFS) (Yamashita, Kitayama et al. 2009), non-small cell lung cancer (>400 mg/dl; DFS) (Jiang, Li et al. 2014) and esophageal cancer (>400 mg/dl; OS and RFS) (Wakatsuki, Matsumoto et al. 2017). A meta-analysis of studies investigating plasma fibrinogen concentrations in patients with hepatocellular carcinoma in China (7 studies) and Japan (1 study) with fibrinogen cut-off values ranging from 234.5-400 mg/dl concluded found worse prognosis (different outcomes were employed: OS, DFS, RFS) and advanced tumor progression in patients with higher circulating fibrinogen (Huang, Jiang et al. 2018).

Increasing fibrinogen plasma concentrations may indicate increasing tumor invasiveness/aggressivness. Our study revealed high pretreatment Fibrinogen plasma concentrations to be statistically associated with advanced tumor stage, and worse survival outcomes (FFR and CSS). Supporting this observation are pretreatment Fibrinogen plasma concentrations increasing from stage I to IV and the highest Fibrinogen plasma concentrations in TCs; and lowest in AB thymomas and MNT. These findings are in parallel to associations of increased pretreatment Fibrinogen plasma concentrations with advanced stage and worse outcome reported for patients with uterine leiomyosarcoma (Bekos, Grimm et al. 2017), nasopharyngeal carcinoma (He, Wang et al. 2017), malignant pleura mesothelioma (Ghanim, Hoda et al. 2014) and ovarian cancer (Hefler-Frischmuth, Lafleur et al. 2015) (selected publications).

Recent research indicates different roles for fibrinogen in tumorigenesis and tumor metastasis. Endogenously synthesized fibrinogen enhances the growth of lung and prostate cancer cells through interaction with FGF-2 (Sahni, Simpson-Haidaris et al. 2008). Fibrinogen`s VE-cadherin binding domain induces endothelial barrier permeability and enhances transendothelial migration of malignant breast epithelial cells (Sahni, Arevalo et al. 2009). The number of spontaneous hematogenous and lymphatic metastasis wa significantly reduced in fibrinogen-deficient mice (Palumbo, Potter et al. 2002). Immunohistochemistry in tumor tissue of TETs revealed absent staining for fibrinogen in malignant thymic epithelial cells. The finding that fibrinogen plasma concentrations increased progressively with invasiveness together with absent expression in TET tissue let us hypothesize that increased fibrinogen plasma concentrations are the result of an acute phase response to TETs.

Data in our study on NLR in TET patients are in line with a study on 79 Chinese TC patients undergoing complete resection. For TCs preoperative NLR>4.1 was significantly associated with larger tumor size, advanced Masaoka stages and reduced DFS and OS, but was not an independent predictor of survival in TC patients after complete resection (Yuan, Gao et al. 2016). In other solid organ malignancies, such as gall bladder cancer patients undergoing surgery high preoperative NLR (NLR cut-off range: 1.94-3.74) was a significanct predictor of worse survival at univariate (Ong, Garcea et al. 2008, Wu, Shi et al. 2014, Zhang, Jiang et al. 2015, Saqib, Pathak et al. 2018) and multivariate analysis (Wu, Shi et al. 2014). Similar trends between high PLR and NLR and worse prognosis

were reported for cancers of the larynx (Hsueh, Tao et al. 2017), esophagus (Yodying, Matsuda et al. 2016), colon(Pedrazzani, Mantovani et al. 2017) and paranasal sinus (Turri-Zanoni, Salzano et al. 2017) (selected publications). In most studies the predictive power of NLR was reported to be superior to that of PLR. Also our data indicate this: NLR but not PLR was significantly associated with worse CSS.

Recently the derived NLR (dNLR, calculated as neutrophil count divided by the subtraction of white blood cell count minus neutrophil count) was introduced in studies for the simple practical reason that only the white blood cell and neutrophil counts of patients were entered into clinical trial databases (Proctor, McMillan et al. 2012). Prognostic observations similar to NLR were reported for preoperative dNLR. DNLR was an independent prognostic predictor for time-to recurrence (TTR) and OS in patients with stage II and III colon cancer (Absenger, Szkandera et al. 2013). In patients with advanced gastric cancer undergoing preoperative ChT and followed by complete (R0) resection high baseline NLR was reported to be superior to d-NLR values in predicting postoperative outcomes (RFS and OS). Post-ChT NLR and d-NLR lost their usefulness due to the inhibition of bone marrow hematopoietic function (Jin, Sun et al. 2017). While in patients with HBV-associated hepatocellular carcinoma treated by transarterial chemoembolization high NLR and dNLR predicted poor OS with similar prognostic power (Zhou, Liang et al. 2016).

Increased NLR or PLR are a result of increased neutrophils and platelets and/or decreased lymphocytes. So far numerous tumor-promoting and tumor-suppressing functions of heterogenous neutrophil populations, the different types of lymphocytes and platelets were described. The particular pathophysiological mechanisms involved in the prognostic role of NLR or PLR in various cancer is still to be elucidated. Our data revealed that absolute lymphocyte counts gradually decreased from non-invasive to metastasized TETs. The lowest lymphocyte counts were observed in patients with TCs.

In our study, patients with TAMG had significantly higher absolute lymphocyte counts compared to TET patients without MG. This is in accordance to previous reports with higher numbers of naive helper and cytotoxic T cells in blood of TAMG patients (Buckley, Douek et al. 2001, Strobel, Helmreich et al. 2002). Significantly higher numbers of naive $CD4^+$ T cells were detected in thymoma tumor tissue of TAMG patients

(and in one MG negative thymoma patient who developed MG only 2 months after surgery) compared to MG negative thymoma patients. These intratumoral findings were paralleled by significantly reduced percentages of naïve $CD4^+$ T cells in peripheral blood of thymoma patients without MG (Strobel, Helmreich et al. 2002).

We attribute the immediate increases of Fibrinogen plasma concentrations, NLR, and PLR in the early postoperative period directly to an acute phase response triggered by surgical stress (Volanakis 2001, Tennent, Brennan et al. 2007, Asteriou, Lazopoulos et al. 2016). TET patients experiencing tumor recurrences during oncological follow-up displayed 2.5-fold higher NLR and 1.8-fold higher PLR than in TET patients without recurrence. NLR and PLR were predictors of tumor recurrence with sensitivities of 80% and 100%, and NPV of 96% and 100%, respectively. Higher NLR and PLR in TET patients suffering from tumor recurrences may be a consequence of cancer-related inflammation.

CRP, fibrinogen, NLR and PLR are accurate, in most institutions readily established and likely socio-economically feasible blood-derived biomarkers that could be of further help in the early diagnosis of TETs during oncologic follow-up. The predictive power of CRP regarding FFR (p=0.037; R2: 0.147) increased in combination with fibrinogen (R2: 0.825), NLR (R2: 1.000), or PLR (R2: 0.726), respectively.

In summary, from our data we believe that the observed increases of CRP, Fibrinogen, NLR, and PLR are result from TET-related inflammation. This is in support of our findings on RAGE axis molecules and heat shock proteins involved in TET-related inflammation (Moser, Janik et al. 2014, Janik, Schiefer et al. 2016). Elevated CRP serum concentrations, fibrinogen plasma concentrations, NLR, and PLR were associated with worse outcome; and during oncologic followup, CRP, NLR and PLR were significantly increased in patients with tumor recurrence.

Recommeded follow-up incorporating peripheral blood derived biomarkers

Up to now clinical tertiary prevention programs lack adequate (bio-)markers. Currently the only recommended tools for oncologic follow-up of TET patients are CXRs and chest CT scans (Huang, Detterbeck et al. 2011). According to ESTS Thymic working group data the majority of institutions experienced with TET treatment performed chest CT scans 3- to 6-months after tumor resection for the first 3 years, followed by lifelong annual chest CT scans (Ruffini, Detterbeck et al. 2014). Similarly, ITMIG recommends a minimum of annual chest CT scans for 5 years after tumor resection followed by alternating

chest CT scans with annual CXRs until the 11th postoperative year; followed by annual CXRs alone. Additional chest CT imaging every 6 months for 3 years is suggested for resected advanced stage III or IVa thymoma, TCs, incomplete resection, or other "high-risk" tumors (Huang, Detterbeck et al. 2011).

We strongly believe that prospective tertiary prevention trials with biomarkers are warranted, particularly in patients with increased CRP serum concentrations at diagnosis of a TET primary. Controlling CRP serum concentrations prospectively at regular intervals could probably lead to omission of chest CT scans. It certainly would not completely replace surveillance by CT. We propose the following protocol emedded into current recommendations of ESTS (Ruffini, Detterbeck et al. 2014) and ITMIG (Huang, Detterbeck et al. 2011) in order to evaluate the role of CRP serum concentrations for oncologic follow-up: Chest CT scans every 6 months for the first 3 years followed by alternating chest CT scans with CXRs until the 11th postoperative year followed by annual CXRs only. At each check up CRP serum concentrations should be recorded. Further CRP serum concentrations should be further determined every 6 months. Increased CRP serum concentrations in the absence of other reasons for the CRP rise (e.g. infection, abscess) will prompt radiological imaging for detection/exclusion of a tumor recurrence. We believe that CRP measurements will increase the chance of detecting recurrences earlier particularly when compared to CXRs (radiological sensitivity is low). Earlier detection of tumor recurrence may yield improved outcomes for patients. In an analogous manner follow-up programs incorporating plasma fibrinogen, NLR and PLR can be designed.

Limitations of the studies investigating the prognostic value of CRP, Fibrinogen, NLR and PLR

The studies on circulating CRP in serum, fibrinogen in plasma and NLR and PLR of TET patients is subject to limitations that apply to retrospective and single center experiences on orphan diseases, such as an inherent bias of selection and information. Nonetheless the studies also carry strength as they are the first to ascribe prognostic and diagnostic value to these peripheral blood parameters in TET patients. Also, the relatively large number of patients having this rare disease was sufficient for the identification of the prognostic and diagnostic power of CRP, NLR, PLR, and Fibrinogen among patients with TETs.

DOI:10.14753/SE.2020.2356

The limitations can be overcome with future prospective multicenter studies to assess CRP serum and fibrinogen plasma concentrations als well as NLR and PLR in larger patient cohorts.

DOI:10.14753/SE.2020.2356

7. Conclusions

The aim of this dissertation has been to identify pathological and clinical prognostic factors for patients with TETs. The following conclusions can be drawn from the results of the five scientific projects described in this thesis.

1. We have identified the prognostic value of pathological Masaoka-Koga stage and completeness of resection for patients with TETs at a single European thoracic surgery center. Multidisciplinary treatment decisions result in good patient care and treatment outcomes.

2. From the international multicenter study we can deduct that in the rare instances of TETs with pleural involvement complete resection remains the mainstay of treatment. Thymoma histology (compared to TC) was predictive of improved survival. The surgical procedures EPP, TP and LP that were used to treat patients with different tumor distributions showed similar survival. It is important to note that the choice of the surgical procedure depends upon the extent of tumour distribution. EPP, TP and LP when performed as part of multimodal therapy seem to be efficient procedures for local control of disease yielding excellent results regarding OS, DFS, CSS and FFR.

3. The measurement of pretreatmtent CRP serum concentrations might influence decisions regarding the use of neoadjuvant therapy since it might be useful to indicate highly aggressive TETs (TCs and metastatic TETs). High pretreatment CRP serum concentrations were associated with significantly worse 5- and 10-year FFR and a negative prognostic factor regarding FFR. CRP serum concentrations might also have a role in the oncological follow-up as they decreased after complete resection and significantly increased in cases of tumor recurrences.

4. Further evidence for the role of inflammation in the biological course of TETs can be seen in that high pretreatment Fibrinogen serum concentrations were significantly associated with worse CSS and FFR, high NLR with worse FFR, and high PLR with worse CSS. We have identified high pretreatment Fibrinogen serum

concentrations, NLR, and PLR to be associated with higher Masaoka-Koga tumor stage and more aggressive tumor behavior such as seen in TCs. Next to its potential predictive and diagnostic role there might also be a value of high NLR and PLR in oncological follow-up of TETs as observed in high NLR and PLR of patients with tumor recurrence (compared to those without).

The results of the thesis contribute to research on diagnosis, treatment and prognostication of patients with TETs in that previously described pathological prognostic factors have been confirmed in the setting of a single institutional experience and in the largest multi-institutional effort of TETs with pleural involvement. The prognostic potential of inflammatory parameters used in daily clinical routine were identified as promising future diagnostic, therapeutic or predictive targets.

In the era of evidence based medicine global efforts are warranted in this orphan disease to initate prospective studies with larger number of patients to obtain higher levels of evidence for diagnosis, treatment and prognosis of patients with TETs.

DOI:10.14753/SE.2020.2356

8. Summary

Objective: Thymic epithelial tumors (TETs) are a malignant orphan disease that is characterized by the frequent occurrence of recurrences. Pretreatment prognostic information and biomarkers for oncological follow-up are not available. The aim of this thesis was to investigate the role of surgery in special situations such as pleural spread of TETs, the prognostic value of pathological characteristics and laboratory parameters of clinical routine: Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), Fibrinogen and C-reactive protein (CRP).

Methods: Standardized datasets of patients undergoing thoracic surgery for TETs of all tumor stages at the Medical University Vienna as well as patients undergoing surgical therapy for TETs with pleural disease at ten European and two Canadian thoracic surgery institutions were analyzed. For the experimental part tumor tissues and serum samples of patients with TETs, benign thymic disease and healthy volunteers were collected.

Results:

Pathological predictors of outcome In patients with TETs of all stages completeness of resection and stage were significant pathological prognostic factors. Pleural involvement resulted from thymomas in 89% and thymic carcinomas (TCs) in 11.2%. Forty extrapleural pneumonectomies (EPPs), 23 total pleurectomies (TPs), and 88 local pleurectomies (LPs) were performed (completeness of resection in 76.8%). OS for the entire patient population at 1, 3, 5 and 10 years was 96.4%, 91.0%, 87.2% and 62.7%, respectively. There was no statistically significant difference regarding FFR and OS for patients with local or advanced disease undergoing EPP, TP or LP. TCs in comparison with thymomas had a negative impact on OS, CSS and FFR. Incomplete resections predicted worse OS.

Pretreatment predictors of outcome Pretreatment CRP serum and Fibrinogen plasma concentrations, NLRs and PLRs were significantly elevated in patients with TETs, particularly TCs and advanced tumor stages. High pretreatment CRP, Fibrinogen, NLR and PLR were associated with worse outcomes.

Biomarkers for follow-up

Patients experiencing tumor recurrences had higher CRP serum concentrations, NLR and PLR.

DOI:10.14753/SE.2020.2356

Conclusions: The widely accepted pathological prognosticators stage and resection status were confirmed. Furthermore in TETs with pleural involvement complete resection remains the mainstay of treatment. EPP, TP and LP employed for different tumor distributions yield excellent local control and survival outcomes. Peripheral blood-based inflammation markers: CRP, fibrinogen, NLR and PLR warrant future propective study to test their value as possible prognostic factors influencing treatment decisions and oncologic follow-up.

DOI:10.14753/SE.2020.2356

9. Összefoglalás

Célkitűzés: A thymus epitheliális daganatai (thymic epithelial tumors - TETs) ritka malignus megbetegedések, amikre jellemző a gyakori kiújulás, a recidívák megjelenése. Jelenleg nem ismerünk megbízhatóan alkalmazható prognosztikai faktorokat és biomarkereket sem a kezelés előtt a túlélés megbecsülésére, sem az onkológiai követéshez. Ennek a disszertációnak a céljai között szerepelt a sebészi eltávolítás szerepének vizsgálata speciális esetekben, mint a TET pleurális terjedésének jelenlétében; a patológiai jellemzők, valamint a klinikai gyakorlatban használt laboratóriumi paraméterek (neutrophil-lymphocyta arány - NLR, vérlemezke -lymphocyta arány - PLR, Fibrinogén és C-reaktív protein - CRP) prognosztikai jelentőségének elemzése.

Módszerek: A Bécsi Tudományegyetemen TET miatt műtéten átesett, bármilyen stádiumú, illetve tíz európai és két kanadai centrumban pleurális terjedést mutató TET miatt operált beteg standardizált adatbázisát elemeztük. A kísérletes részben tumor szöveteket és vérmintákat gyűjtöttünk betegektől, akiknél TET alakult ki, jóindulatú thymus betegségtől szenvedő páciensektől, valamint egészséges önkéntesektől.

Eredmények:

Patológiai prediktív faktorok: A teljes sebészi reszekció és a klinikai stádium voltak a szignifikáns prognosztikai faktorok minden stádiumú TET estében. A pleurális érintettséget a vizsgált esetek 89%-ában thymomák, 11.2%-ban thymus carcinoma (TC) okozta. A daganat eltávolítása érdekében negyven extrapleurális-pleuropulmonektómia (EPP), 23 teljes pleurektómia (TP) és 88 részleges pleurektómia (LP) történt (76.8%-ban teljes tumoreltávolítást elérve). A teljes túlélés (overall survival – OS) a teljes beteg populációt tekintve 1, 3, 5 és 10 év után 96.4%, 91.0%, 87.2% és 62.7% volt. Nem volt szignifikáns különbség az OS és a kiújulás mentes túlélés (freedom from recurrence – FFR) tekintetében lokalizált és kiterjed daganatok esetén sem az EPP-n, TP-n vagy LP-n áteső betegek között. A TC diagnózisa negatívan befolyásolta a OS-t,

DOI:10.14753/SE.2020.2356

FFR-t és az betegségspecifikus túlélést (cause specific survival – CSS) is. A nem teljes sebészi eltávolítás szintén rosszabb OS-t jósolt.

A túlélés kezelés előtti jelzői: A kezelés előtti szérum CRP és plazma fibrinogén koncentráció, valamint az NLR és PLR is szignifikánsan emelkedettebb volt TET jelenlétekor, különösképpen TC és késői tumorstádium esetén. A kezelés előtt mért magas CRP, fibrinogén, NLR és PLR rosszabb túlélést jelentett.

Az onkológiai követés biomarkerei:

Azoknál a betegeknél, akiknél a daganat kiújult, magasabb szérum CRP koncentráció, NLR és PLR volt megfigyelhető.

Következtetés: A széles körben elfogadott prognosztikai faktorok, mint a stádium és reszekciós státusz ebben a betegcsoportban is igazolódtak. Továbbá, TET és plurális érintettség esetén továbbra is a sebészi eltávolítás marad a kezelés legfőbb lépése. EPP-vel, TP-vel és LP-vel különböző tumor terjedések esetén alkalmazva, kiváló lokális kontrollt és túlélést lehet elérni. A perifériás vérből vehető gyulladásos markerek: a CRP, fibrinogén, NLR és PLR lehetséges prognosztikai értékének vizsgálatához prospektív tanulmányra van szükség, mivel ez a kezelési stratégiát és onkológiai követést is befolyásolhatja.

10. Bibliography

Absenger, G., J. Szkandera, M. Pichler, M. Stotz, F. Arminger, M. Weissmueller, R. Schaberl-Moser, H. Samonigg, T. Stojakovic and A. Gerger (2013). "A derived neutrophil to lymphocyte ratio predicts clinical outcome in stage II and III colon cancer patients." Br J Cancer 109(2): 395-400.

Asghar, S., F. Parvaiz and S. Manzoor (2019). "Multifaceted role of cancer educated platelets in survival of cancer cells." Thromb Res 177: 42-50.

Asteriou, C., A. Lazopoulos, T. Rallis, A. S. Gogakos, D. Paliouras, K. Tsakiridis, A. Zissimopoulos, D. Tsavlis, K. Porpodis, W. Hohenforst-Schmidt, I. Kioumis, J. Organtzis, K. Zarogoulidis, P. Zarogoulidis and N. Barbetakis (2016). "Video-assisted thoracic surgery reduces early postoperative stress. A single-institutional prospective randomized study." Ther Clin Risk Manag 12: 59-65.

Attaran, S., M. Acharya, J. R. Anderson and P. P. Punjabi (2012). "Does surgical debulking for advanced stages of thymoma improve survival?" Interact Cardiovasc Thorac Surg 15(3): 494-497.

Awad, W. I., P. J. Symmans and J. E. Dussek (1998). "Recurrence of stage I thymoma 32 years after total excision." Ann Thorac Surg 66(6): 2106-2108.

Badiyan, S. N., M. C. Roach, M. D. Chuong, S. R. Rice, N. E. Onyeuku, J. Remick, S. Chilukuri, E. Glass, P. Mohindra and C. B. Simone, 2nd (2018). "Combining immunotherapy with radiation therapy in thoracic oncology." J Thorac Dis 10(Suppl 21): S2492-s2507.

Bekos, C., C. Grimm, T. Brodowicz, E. Petru, L. Hefler, D. Reimer, H. Koch, A. Reinthaller, S. Polterauer and M. Polterauer (2017). "Prognostic role of plasma fibrinogen in patients with uterine leiomyosarcoma - a multicenter study." Sci Rep 7(1): 14474.

Bekos, C., M. Zimmermann, L. Unger, S. Janik, P. Hacker, A. Mitterbauer, M. Koller, R. Fritz, C. Gabler, M. Kessler, S. Nickl, J. Didcock, P. Altmann, T. Haider, G. Roth, W. Klepetko, H. J. Ankersmit and B. Moser (2016). "Non-professional marathon running: RAGE axis and ST2 family changes in relation to open-window effect, inflammation and renal function." Sci Rep 6: 32315.

Berardi, R., M. De Lisa, S. Pagliaretta, A. Onofri, F. Morgese, A. Savini, Z. Ballatore, M. Caramanti, M. Santoni, P. Mazzanti and S. Cascinu (2014). "Thymic neoplasms: an update on the use of chemotherapy and new targeted therapies. A literature review." Cancer Treat Rev 40(4): 495-506.

Berman, A. T., L. Litzky, V. Livolsi, S. Singhal, J. C. Kucharczuk, J. D. Cooper, J. R. Friedberg, T. L. Evans, J. P. Stevenson, J. M. Metz, S. M. Hahn and R. Rengan (2011). "Adjuvant radiotherapy for completely resected stage 2 thymoma." Cancer 117(15): 3502-3508.

Bernard, C., H. Frih, F. Pasquet, S. Kerever, Y. Jamilloux, F. Tronc, B. Guibert, S. Isaac, M. Devouassoux, L. Chalabreysse, C. Broussolle, P. Petiot, N. Girard and P. Seve (2016). "Thymoma associated with autoimmune diseases: 85 cases and literature review." Autoimmun Rev 15(1): 82-92.

Berruti, A., P. Borasio, A. Gerbino, G. Gorzegno, T. Moschini, M. Tampellini, F. Ardissone, M. P. Brizzi, A. Dolcetti and L. Dogliotti (1999). "Primary chemotherapy with adriamycin, cisplatin, vincristine and cyclophosphamide in locally advanced thymomas: a single institution experience." Br J Cancer 81(5): 841-845.

Bilancia, R., M. Nardini and D. A. Waller (2018). "Extended pleurectomy decortication: the current role." Transl Lung Cancer Res 7(5): 556-561.

Brown, J. A., D. M. Dorfman, F. R. Ma, E. L. Sullivan, O. Munoz, C. R. Wood, E. A. Greenfield and G. J. Freeman (2003). "Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production." J Immunol 170(3): 1257-1266.

Buckley, C., D. Douek, J. Newsom-Davis, A. Vincent and N. Willcox (2001). "Mature, long-lived CD4+ and CD8+ T cells are generated by the thymoma in myasthenia gravis." Ann Neurol 50(1): 64-72.

Carter, B. W., M. F. Benveniste, R. Madan, M. C. Godoy, P. M. Groot, M. T. Truong, M. L. Rosado-de-Christenson and E. M. Marom (2017). "IASLC/ITMIG Staging System and Lymph Node Map for Thymic Epithelial Neoplasms." Radiographics 37(3): 758-776.

Castleman, B. (1955). Atlas of tumor pathology. Tumors of the thymus gland,

fascicle 19. Armed Forces Institute of Pathology, Washington, DC.

Chau, N. G., E. S. Kim and I. Wistuba (2010). "The multidisciplinary approach to thymoma: combining molecular and clinical approaches." J Thorac Oncol 5(10 Suppl 4): S313-317.

Chen, G., A. Marx, W. H. Chen, J. Yong, B. Puppe, P. Stroebel and H. K. Mueller-Hermelink (2002). "New WHO histologic classification predicts prognosis of thymic epithelial tumors: a clinicopathologic study of 200 thymoma cases from China." Cancer 95(2): 420-429.

Chen, Y. D., Q. F. Feng, H. Z. Lu, Y. S. Mao, Z. M. Zhou, G. F. Ou, M. Wang, J. Zhao, H. X. Zhang, Z. F. Xiao, D. F. Chen, J. Liang, Y. R. Zhai, L. H. Wang and J. He (2010). "Role of adjuvant radiotherapy for stage II thymoma after complete tumor resection." Int J Radiat Oncol Biol Phys 78(5): 1400-1406.

Choi, A. H., S. W. French and T. L. Van Natta (2014). "Biopsy trauma dislodges thymocytes from a thymoma to fill regional lymph node sinusoids." Exp Mol Pathol 97(1): 66-68.

Clauss, A. (1957). "[Rapid physiological coagulation method in determination of fibrinogen]." Acta Haematol 17(4): 237-246.

Coussens, L. M. and Z. Werb (2002). "Inflammation and cancer." Nature 420(6917): 860-867.

Dai, J., N. Song, Y. Yang and G. Jiang (2015). "Is it valuable and safe to perform reoperation for recurrent thymoma?" Interact Cardiovasc Thorac Surg 21(4): 526-531.

de Jong, W. K., J. L. Blaauwgeers, M. Schaapveld, W. Timens, T. J. Klinkenberg and H. J. Groen (2008). "Thymic epithelial tumours: a population-based study of the incidence, diagnostic procedures and therapy." Eur J Cancer 44(1): 123-130.

Detterbeck, F. C. (2013). "The international thymic malignancy interest group." J Natl Compr Canc Netw 11(5): 589-593.

Detterbeck, F. C., A. G. Nicholson, K. Kondo, P. Van Schil and C. Moran (2011). "The Masaoka-Koga stage classification for thymic malignancies: clarification and definition of terms." J Thorac Oncol 6(7 Suppl 3): S1710-1716.

Detterbeck, F. C. and A. M. Parsons (2011). "Management of stage I and II thymoma." Thorac Surg Clin 21(1): 59-67, vi-vii.

Detterbeck, F. C., K. Stratton, D. Giroux, H. Asamura, J. Crowley, C. Falkson, P. L. Filosso, A. A. Frazier, G. Giaccone, J. Huang, J. Kim, K. Kondo, M. Lucchi, M. Marino, E. M. Marom, A. G. Nicholson, M. Okumura, E. Ruffini and P. Van Schil (2014). "The IASLC/ITMIG Thymic Epithelial Tumors Staging Project: proposal for an evidence-based stage classification system for the forthcoming (8th) edition of the TNM classification of malignant tumors." J Thorac Oncol 9(9 Suppl 2): S65-72.

Engels, E. A. (2010). "Epidemiology of thymoma and associated malignancies." J Thorac Oncol 5(10 Suppl 4): S260-265.

Engels, E. A. and R. M. Pfeiffer (2003). "Malignant thymoma in the United States: demographic patterns in incidence and associations with subsequent malignancies." Int J Cancer 105(4): 546-551.

Enkner, F., B. Pichlhofer, A. T. Zaharie, M. Krunic, T. M. Holper, S. Janik, B. Moser, K. Schlangen, B. Neudert, K. Walter, B. Migschitz and L. Mullauer (2017). "Molecular Profiling of Thymoma and Thymic Carcinoma: Genetic Differences and Potential Novel Therapeutic Targets." Pathol Oncol Res 23(3): 551-564.

Erpenbeck, L. and M. P. Schon (2010). "Deadly allies: the fatal interplay between platelets and metastasizing cancer cells." Blood 115(17): 3427-3436.

European Commission. "Public Health: non-communicable diseases: Rare diseases." Retrieved October 2, 2018, 2018, from https://ec.europa.eu/health/non_communicable_diseases/rare_diseases_en.

Evoli, A. and E. Lancaster (2014). "Paraneoplastic disorders in thymoma patients." J Thorac Oncol 9(9 Suppl 2): S143-147.

Fabre, D., E. Fadel, S. Mussot, O. Mercier, B. Petkova, B. Besse, J. Huang and P. G. Dartevelle (2011). "Long-term outcome of pleuropneumonectomy for Masaoka stage IVa thymoma." Eur J Cardiothorac Surg 39(5): e133-138.

Fang, Y., C. Xu, P. Wu, L. H. Zhang, D. W. Li, J. H. Sun, W. F. Li and Z. S. Liao (2017). "Prognostic role of C-reactive protein in patients with nasopharyngeal carcinoma: A meta-analysis and literature review." Medicine (Baltimore) 96(45): e8463.

Filosso, P. L., A. Evangelista, E. Ruffini, E. A. Rendina, S. Margaritora, P. Novellis, O. Rena, C. Casadio, C. Andreetti, F. Guerrera, P. O. Lausi, D. Diso, A. Mussi, F. Venuta, A. Oliaro and M. Lucchi (2015). "Does myasthenia gravis influence overall survival and cumulative incidence of recurrence in thymoma patients? A Retrospective clinicopathological multicentre analysis on 797 patients." Lung Cancer 88(3): 338-343.

Filosso, P. L., C. Galassi, E. Ruffini, S. Margaritora, L. Bertolaccini, C. Casadio, M. Anile and F. Venuta (2013). "Thymoma and the increased risk of developing extrathymic

malignancies: a multicentre study." Eur J Cardiothorac Surg 44(2): 219-224; discussion 224.

Filosso, P. L., E. Ruffini, P. O. Lausi, M. Lucchi, A. Oliaro and F. Detterbeck (2014). "Historical perspectives: The evolution of the thymic epithelial tumors staging system." Lung Cancer 83(2): 126-132.

Filosso, P. L., X. Yao, U. Ahmad, Y. Zhan, J. Huang, E. Ruffini, W. Travis, M. Lucchi, A. Rimner, A. Antonicelli, F. Guerrera and F. Detterbeck (2015). "Outcome of primary neuroendocrine tumors of the thymus: a joint analysis of the International Thymic Malignancy Interest Group and the European Society of Thoracic Surgeons databases." J Thorac Cardiovasc Surg 149(1): 103-109.e102.

Friedant, A. J., E. A. Handorf, S. Su and W. J. Scott (2016). "Minimally Invasive versus Open Thymectomy for Thymic Malignancies: Systematic Review and Meta-Analysis." J Thorac Oncol 11(1): 30-38.

Fujiwara, K., A. Matsumura, H. Tanaka, K. Ohmori, S. Yamamoto and K. Iuchi (2003). "[Needle tract implantation of thymoma after transthoracic needle biopsy]." Kyobu Geka 56(6): 448-451.

Fuller, C. D., E. H. Ramahi, N. Aherne, T. Y. Eng and C. R. Thomas, Jr. (2010). "Radiotherapy for thymic neoplasms." J Thorac Oncol 5(10 Suppl 4): S327-335.

Gabay, C. and I. Kushner (1999). "Acute-phase proteins and other systemic responses to inflammation." N Engl J Med 340(6): 448-454.

Gasic, G. J., T. B. Gasic and C. C. Stewart (1968). "Antimetastatic effects associated with platelet reduction." Proc Natl Acad Sci U S A 61(1): 46-52.

Gaur, P., C. Leary and J. C. Yao (2010). "Thymic neuroendocrine tumors: a SEER database analysis of 160 patients." Ann Surg 251(6): 1117-1121.

Gbolahan, O. B., R. F. Porter, J. T. Salter, C. Yiannoutsos, M. Burns, E. G. Chiorean and P. J. Loehrer, Sr. (2018). "A Phase II Study of Pemetrexed in Patients with Recurrent Thymoma and Thymic Carcinoma." J Thorac Oncol 13(12): 1940-1948.

Ghanim, B., M. A. Hoda, T. Klikovits, M. P. Winter, A. Alimohammadi, M. Grusch, B. Dome, M. Arns, P. Schenk, M. Jakopovic, M. Samarzija, L. Brcic, M. Filipits, V. Laszlo, W. Klepetko, W. Berger and B. Hegedus (2014). "Circulating fibrinogen is a prognostic and predictive biomarker in malignant pleural mesothelioma." Br J Cancer 110(4): 984-990.

Ghanim, B., M. A. Hoda, M. P. Winter, T. Klikovits, A. Alimohammadi, B. Hegedus, B. Dome, M. Grusch, M. Arns, P. Schenk, W. Pohl, C. Zielinski, M. Filipits, W. Klepetko and W. Berger (2012). "Pretreatment serum C-reactive protein levels predict benefit from multimodality treatment including radical surgery in malignant pleural mesothelioma: a retrospective multicenter analysis." Ann Surg 256(2): 357-362.

Giaccone, G., A. Ardizzoni, A. Kirkpatrick, M. Clerico, T. Sahmoud and N. van Zandwijk (1996). "Cisplatin and etoposide combination chemotherapy for locally advanced or metastatic thymoma. A phase II study of the European Organization for Research and Treatment of Cancer Lung Cancer Cooperative Group." J Clin Oncol 14(3): 814-820.

Giaccone, G., C. Kim, J. Thompson, C. McGuire, B. Kallakury, J. J. Chahine, M. Manning, R. Mogg, W. M. Blumenschein, M. T. Tan, D. S. Subramaniam, S. V. Liu, I.

M. Kaplan and J. N. McCutcheon (2018). "Pembrolizumab in patients with thymic carcinoma: a single-arm, single-centre, phase 2 study." Lancet Oncol 19(3): 347-355.

Gilhus, N. E. and J. J. Verschuuren (2015). "Myasthenia gravis: subgroup classification and therapeutic strategies." Lancet Neurol 14(10): 1023-1036.

Girard, N., E. Ruffini, A. Marx, C. Faivre-Finn and S. Peters (2015). "Thymic epithelial tumours: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up." Ann Oncol 26 Suppl 5: v40-55.

Gong, J., S. Jin, X. Pan, G. Wang, L. Ye, H. Tao, H. Wen, Y. Liu and Q. Xie (2018). "Identification of Long Non-Coding RNAs for Predicting Prognosis Among Patients with Thymoma." Clin Lab 64(7): 1193-1198.

Grivennikov, S. I., F. R. Greten and M. Karin (2010). "Immunity, inflammation, and cancer." Cell 140(6): 883-899.

Guerrera, F., E. A. Rendina, F. Venuta, S. Margaritora, A. M. Ciccone, P. Novellis, D. Novero, M. Anile, G. Bora, O. Rena, C. Casadio, A. Mussi, A. Evangelista, E. Ruffini, M. Lucchi and P. L. Filosso (2015). "Does the World Health Organization histological classification predict outcomes after thymomectomy? Results of a multicentre study on 750 patients." Eur J Cardiothorac Surg 48(1): 48-54.

Hadoux, J., N. Girard and B. Besse (2012). "[Thymic epithelial neoplasms: updates on diagnosis, staging, biology and management in France]." Bull Cancer 99(11): 1045-1055.

Hamaji, M., S. O. Ali and B. M. Burt (2015). "A meta-analysis of induction therapy for advanced thymic epithelial tumors." Ann Thorac Surg 99(5): 1848-1856.

Hamaji, M., R. M. Shah, S. O. Ali, A. Bettenhausen, H. S. Lee and B. M. Burt (2017). "A Meta-Analysis of Postoperative Radiotherapy for Thymic Carcinoma." Ann Thorac Surg 103(5): 1668-1675.

Hayes, S. A., J. Huang, A. J. Plodkowski, J. Katzen, J. Zheng, C. S. Moskowitz and M. S. Ginsberg (2014). "Preoperative computed tomography findings predict surgical resectability of thymoma." J Thorac Oncol 9(7): 1023-1030.

He, S. S., Y. Wang, L. Yang, H. Y. Chen, S. B. Liang, L. X. Lu and Y. Chen (2017). "Plasma Fibrinogen Correlates with Metastasis and is Associated with Prognosis in Human Nasopharyngeal Carcinoma." J Cancer 8(3): 403-409.

Hefler-Frischmuth, K., J. Lafleur, L. Hefler, S. Polterauer, V. Seebacher, A. Reinthaller and C. Grimm (2015). "Plasma fibrinogen levels in patients with benign and malignant ovarian tumors." Gynecol Oncol 136(3): 567-570.

Hofmann, M. A., S. Drury, C. Fu, W. Qu, A. Taguchi, Y. Lu, C. Avila, N. Kambham, A. Bierhaus, P. Nawroth, M. F. Neurath, T. Slattery, D. Beach, J. McClary, M. Nagashima, J. Morser, D. Stern and A. M. Schmidt (1999). "RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides." Cell 97(7): 889-901.

Hsueh, C., L. Tao, M. Zhang, W. Cao, H. Gong, J. Zhou and L. Zhou (2017). "The prognostic value of preoperative neutrophils, platelets, lymphocytes, monocytes and calculated ratios in patients with laryngeal squamous cell cancer." Oncotarget 8(36): 60514-60527.

Huang, G., H. Jiang, Y. Lin, Y. Wu, W. Cai, B. Shi, Y. Luo, Z. Jian and X. Zhou (2018). "Prognostic value of plasma fibrinogen in hepatocellular carcinoma: a meta-analysis." Cancer Manag Res 10: 5027-5041.

Huang, J., F. C. Detterbeck, Z. Wang and P. J. Loehrer, Sr. (2011). "Standard outcome measures for thymic malignancies." J Thorac Oncol 6(7 Suppl 3): S1691-1697.

Huong, P. T., L. T. Nguyen, X. B. Nguyen, S. K. Lee and D. H. Bach (2019). "The Role of Platelets in the Tumor-Microenvironment and the Drug Resistance of Cancer Cells." Cancers (Basel) 11(2).

Hwang, Y., C. H. Kang, S. Park, H. J. Lee, I. K. Park, Y. T. Kim, G. D. Lee, H. R. Kim, S. H. Choi, Y. H. Kim, D. K. Kim, S. I. Park, S. Shin, J. H. Cho, H. K. Kim, Y. S. Choi, J. Kim, J. I. Zo, Y. M. Shim, C. Y. Lee, J. G. Lee, D. J. Kim, H. C. Paik and K. Y. Chung (2018). "Impact of Lymph Node Dissection on Thymic Malignancies: Multi-Institutional Propensity Score Matched Analysis." J Thorac Oncol.

Imanishi, N., Y. Nabe, M. Takenaka, A. Hirai, Y. Ichiki and F. Tanaka (2018). "Extended pleurectomy decortication for thymoma with pleural dissemination." Gen Thorac Cardiovasc Surg.

Imbimbo, M., M. Ottaviano, M. Vitali, A. Fabbri, G. Leuzzi, M. Fiore, D. Franceschini, G. Pasello, M. Perrino, M. Schiavon, G. Pruneri, A. P. Dei Tos, C. Sangalli, M. C. Garassino, R. Berardi, A. Alessi, G. Calareso, I. Petrini, M. Scorsetti, V. Scotti, L. Rosso, F. Rea, U. Pastorino, P. G. Casali, S. Ramella, U. Ricardi, L. Abate-Daga, V. Torri, A. Trama, G. Palmieri, M. Marino and P. A. Zucali (2018). "Best practices for the management of thymic epithelial tumors: A position paper by the Italian collaborative group for ThYmic MalignanciEs (TYME)." Cancer Treat Rev 71: 76-87.

International Association for the Study of Lung Cancer. (2016). "Staging Manual in Thoracic Oncology." second edition. Retrieved October 3, 2018, 2018, from https://www.iaslc.org/sites/default/files/wysiwyg-assets/8th_staging_manual_2016_hi-res.pdf.

Ishikawa, Y., H. Matsuguma, R. Nakahara, H. Suzuki, A. Ui, T. Kondo, Y. Kamiyama, S. Igarashi, K. Mori, T. Kodama and K. Yokoi (2009). "Multimodality therapy for patients with invasive thymoma disseminated into the pleural cavity: the potential role of extrapleural pneumonectomy." Ann Thorac Surg 88(3): 952-957.

J., R. " Histological typing of tumours of the thymus. Berlin: Springer-Verlag, 1999.".

Jabs, W. J., M. Busse, S. Kruger, D. Jocham, J. Steinhoff and C. Doehn (2005). "Expression of C-reactive protein by renal cell carcinomas and unaffected surrounding renal tissue." Kidney Int 68(5): 2103-2110.

Janik, S., A. I. Schiefer, C. Bekos, P. Hacker, T. Haider, J. Moser, W. Klepetko, L. Mullauer, H. J. Ankersmit and B. Moser (2016). "HSP27 and 70 expression in thymic epithelial tumors and benign thymic alterations: diagnostic, prognostic and physiologic implications." Sci Rep 6: 24267.

Jaretzki, A., 3rd, A. S. Penn, D. S. Younger, M. Wolff, M. R. Olarte, R. E. Lovelace and L. P. Rowland (1988). ""Maximal" thymectomy for myasthenia gravis. Results." J Thorac Cardiovasc Surg 95(5): 747-757.

Jiang, H. G., J. Li, S. B. Shi, P. Chen, L. P. Ge, Q. Jiang and X. P. Tang (2014). "Value of fibrinogen and D-dimer in predicting recurrence and metastasis after radical surgery for non-small cell lung cancer." Med Oncol 31(7): 22.

Jin, H., J. Sun, K. Zhu, X. Liu, Q. Zhang, Q. Shen, Y. Gao and J. Yu (2017). "The prognostic value of neutrophil-lymphocyte ratio is superior to derived neutrophil-lymphocyte ratio in advanced gastric cancer treated with preoperative chemotherapy and sequential R0 resection: a 5-year follow-up." Onco Targets Ther 10: 2655-2664.

Kattach, H., S. Hasan, C. Clelland and R. Pillai (2005). "Seeding of stage I thymoma into the chest wall 12 years after needle biopsy." Ann Thorac Surg 79(1): 323-324.

Kim, E. S., J. B. Putnam, R. Komaki, G. L. Walsh, J. Y. Ro, H. J. Shin, M. Truong, H. Moon, S. G. Swisher, F. V. Fossella, F. R. Khuri, W. K. Hong and D. M. Shin (2004). "Phase II study of a multidisciplinary approach with induction chemotherapy, followed by surgical resection, radiation therapy, and consolidation chemotherapy for unresectable malignant thymomas: final report." Lung Cancer 44(3): 369-379.

Koga, K., Y. Matsuno, M. Noguchi, K. Mukai, H. Asamura, T. Goya and Y. Shimosato (1994). "A review of 79 thymomas: modification of staging system and reappraisal of conventional division into invasive and non-invasive thymoma." Pathol Int 44(5): 359-367.

Kojima, Y., H. Ito, S. Hasegawa, T. Sasaki and K. Inui (2006). "Resected invasive thymoma with multiple endocrine neoplasia type 1." Jpn J Thorac Cardiovasc Surg 54(4): 171-173.

Kondo, K. and Y. Monden (2003). "Therapy for thymic epithelial tumors: a clinical study of 1,320 patients from Japan." Ann Thorac Surg 76(3): 878-884; discussion 884-875.

Kondo, K., P. Van Schil, F. C. Detterbeck, M. Okumura, K. Stratton, D. Giroux, H. Asamura, J. Crowley, C. Falkson, P. L. Filosso, G. Giaccone, J. Huang, J. Kim, M. Lucchi, M. Marino, E. M. Marom, A. G. Nicholson and E. Ruffini (2014). "The IASLC/ITMIG Thymic Epithelial Tumors Staging Project: proposals for the N and M components for the forthcoming (8th) edition of the TNM classification of malignant tumors." J Thorac Oncol 9(9 Suppl 2): S81-87.

Korniluk, A., O. Koper, H. Kemona and V. Dymicka-Piekarska (2017). "From inflammation to cancer." Ir J Med Sci 186(1): 57-62.

Korst, R. J., S. Fernando, A. C. Catlin, J. R. Rutledge, N. Girard, J. Huang and F. Detterbeck (2017). "Positron Emission Tomography in Thymic Tumors: Analysis Using a Prospective Research Database." Ann Thorac Surg 104(6): 1815-1820.

Lee, S. E., J. H. Lee, K. W. Ryu, B. H. Nam, S. J. Cho, J. Y. Lee, C. G. Kim, I. J. Choi, M. C. Kook, S. R. Park and Y. W. Kim (2012). "Preoperative plasma fibrinogen level is a useful predictor of adjacent organ involvement in patients with advanced gastric cancer." J Gastric Cancer 12(2): 81-87.

Lemma, G. L., J. W. Lee, S. C. Aisner, C. J. Langer, W. J. Tester, D. H. Johnson and P. J. Loehrer, Sr. (2011). "Phase II study of carboplatin and paclitaxel in advanced thymoma and thymic carcinoma." J Clin Oncol 29(15): 2060-2065.

Levinson, A. I. (2013). "Modeling the intrathymic pathogenesis of myasthenia gravis." J Neurol Sci 333(1-2): 60-67.

Liang, G., Z. Gu, Y. Li, J. Fu, Y. Shen, Y. Wei, L. Tan, P. Zhang, Y. Han, C. Chen, R. Zhang, K. Chen, H. Chen, Y. Liu, Y. Cui, Y. Wang, L. Pang, Z. Yu, X. Zhou, Y. Liu, Y. Liu and W. Fang (2016). "Comparison of the Masaoka-Koga staging and the International Association for the Study of Lung Cancer/the International Thymic Malignancies Interest Group proposal for the TNM staging systems based on the Chinese Alliance for Research in Thymomas retrospective database." J Thorac Dis 8(4): 727-737.

Lim, Y. J., E. Kim, H. J. Kim, H. G. Wu, J. Yan, Q. Liu and S. Patel (2016). "Survival Impact of Adjuvant Radiation Therapy in Masaoka Stage II to IV Thymomas: A Systematic Review and Meta-analysis." Int J Radiat Oncol Biol Phys 94(5): 1129-1136.

Liu, T. J., M. W. Lin, M. S. Hsieh, M. W. Kao, K. C. Chen, C. C. Chang, S. W. Kuo, P. M. Huang, H. H. Hsu, J. S. Chen, H. S. Lai and J. M. Lee (2014). "Video-assisted thoracoscopic surgical thymectomy to treat early thymoma: a comparison with the conventional transsternal approach." Ann Surg Oncol 21(1): 322-328.

Loehrer, P. J., Sr., M. Chen, K. Kim, S. C. Aisner, L. H. Einhorn, R. Livingston and D. Johnson (1997). "Cisplatin, doxorubicin, and cyclophosphamide plus thoracic radiation therapy for limited-stage unresectable thymoma: an intergroup trial." J Clin Oncol 15(9): 3093-3099.

Loehrer, P. J., Sr., M. Jiroutek, S. Aisner, J. Aisner, M. Green, C. R. Thomas, Jr., R. Livingston and D. H. Johnson (2001). "Combined etoposide, ifosfamide, and cisplatin in the treatment of patients with advanced thymoma and thymic carcinoma: an intergroup trial." Cancer 91(11): 2010-2015.

Loehrer, P. J., Sr., K. Kim, S. C. Aisner, R. Livingston, L. H. Einhorn, D. Johnson and R. Blum (1994). "Cisplatin plus doxorubicin plus cyclophosphamide in metastatic or recurrent thymoma: final results of an intergroup trial. The Eastern Cooperative Oncology Group, Southwest Oncology Group, and Southeastern Cancer Study Group." J Clin Oncol 12(6): 1164-1168.

Loehrer, P. J., Sr., W. Wang, D. H. Johnson, S. C. Aisner and D. S. Ettinger (2004). "Octreotide alone or with prednisone in patients with advanced thymoma and thymic carcinoma: an Eastern Cooperative Oncology Group Phase II Trial." J Clin Oncol 22(2): 293-299.

Lowe, G. D., A. Rumley and I. J. Mackie (2004). "Plasma fibrinogen." Ann Clin Biochem 41(Pt 6): 430-440.

Lucchi, M., F. Davini, R. Ricciardi, L. Duranti, L. Boldrini, G. Palmiero, F. Basolo and A. Mussi (2009). "Management of pleural recurrence after curative resection of thymoma." J Thorac Cardiovasc Surg 137(5): 1185-1189.

Luzzi, L., A. Campione, A. Gorla, G. Vassallo, A. Bianchi, A. Biggi and A. Terzi (2009). "Role of fluorine-flurodeoxyglucose positron emission tomography/computed tomography in preoperative assessment of anterior mediastinal masses." Eur J Cardiothorac Surg 36(3): 475-479.

Mackie, I. J., S. Kitchen, S. J. Machin and G. D. Lowe (2003). "Guidelines on fibrinogen assays." Br J Haematol 121(3): 396-404.

Mangi, A. A., C. D. Wright, J. S. Allan, J. C. Wain, D. M. Donahue, H. C. Grillo and D. J. Mathisen (2002). "Adjuvant radiation therapy for stage II thymoma." Ann Thorac Surg 74(4): 1033-1037.

Mann, R. B., T. C. Wu, E. M. MacMahon, Y. Ling, P. Charache and R. F. Ambinder (1992). "In situ localization of Epstein-Barr virus in thymic carcinoma." Mod Pathol 5(4): 363-366.

Manoly, I., R. N. Whistance, R. Sreekumar, S. Khawaja, J. M. Horton, A. Z. Khan, G. Casali, J. A. Thorpe, K. Amer and E. Woo (2014). "Early and mid-term outcomes of trans-sternal and video-assisted thoracoscopic surgery for thymoma." Eur J Cardiothorac Surg 45(6): e187-193.

Marom, E. M. (2010). "Imaging thymoma." J Thorac Oncol 5(10 Suppl 4): S296-303.

Marom, E. M., M. L. Rosado-de-Christenson, J. F. Bruzzi, M. Hara, J. R. Sonett and L. Ketai (2011). "Standard report terms for chest computed tomography reports of anterior mediastinal masses suspicious for thymoma." J Thorac Oncol 6(7 Suppl 3): S1717-1723.

Marulli, G., S. Margaritora, M. Lucchi, G. Cardillo, P. Granone, A. Mussi, F. Carleo, E. Perissinotto and F. Rea (2016). "Surgical treatment of recurrent thymoma: is it worthwhile?dagger." Eur J Cardiothorac Surg 49(1): 327-332.

Marx, A., J. K. Chan, J. M. Coindre, F. Detterbeck, N. Girard, N. L. Harris, E. S. Jaffe, M. O. Kurrer, E. M. Marom, A. L. Moreira, K. Mukai, A. Orazi and P. Strobel (2015). "The 2015 World Health Organization Classification of Tumors of the Thymus: Continuity and Changes." J Thorac Oncol 10(10): 1383-1395.

Marx, A., N. Willcox, M. I. Leite, W. Y. Chuang, B. Schalke, W. Nix and P. Strobel (2010). "Thymoma and paraneoplastic myasthenia gravis." Autoimmunity 43(5-6): 413-427.

Masaoka, A. and Y. Monden (1981). "Comparison of the results of transsternal simple, transcervical simple, and extended thymectomy." Ann N Y Acad Sci 377: 755-765.

Masaoka, A., Y. Monden, K. Nakahara and T. Tanioka (1981). "Follow-up study of thymomas with special reference to their clinical stages." Cancer 48(11): 2485-2492.

Masaoka, A., Y. Nagaoka and Y. Kotake (1975). "Distribution of thymic tissue at the anterior mediastinum. Current procedures in thymectomy." J Thorac Cardiovasc Surg 70(4): 747-754.

Matilla JR, A. M., Benazzo A, Schwarz S, Klepetko W, Moser B. (2018). "Initial experience with a combined sequential left-sided and subxiphoid VATS approach for resection of large anterior mediastinal tumors." Mediastinum.

Matilla, J. R., W. Klepetko and B. Moser (2017). "Thymic minimally invasive surgery: state of the art across the world-Europe." J Vis Surg 3: 70.

Merveilleux du Vignaux, C., J. M. Maury and N. Girard (2017). "Novel Agents in the Treatment of Thymic Malignancies." Curr Treat Options Oncol 18(9): 52.

Mollinedo, F. (2019). "Neutrophil Degranulation, Plasticity, and Cancer Metastasis." Trends Immunol 40(3): 228-242.

Mornex, F., M. Resbeut, P. Richaud, G. M. Jung, X. Mirabel, C. Marchal, J. L. Lagrange, P. Rambert, G. Chaplain and T. D. Nguyen (1995). "Radiotherapy and chemotherapy for

invasive thymomas: a multicentric retrospective review of 90 cases. The FNCLCC trialists. Federation Nationale des Centres de Lutte Contre le Cancer." Int J Radiat Oncol Biol Phys 32(3): 651-659.

Moser, B., C. Bekos, F. Zimprich, S. Nickl, W. Klepetko and J. Ankersmit (2012). "The receptor for advanced glycation endproducts and its ligands in patients with myasthenia gravis." Biochem Biophys Res Commun 420(1): 96-101.

Moser, B., D. D. Desai, M. P. Downie, Y. Chen, S. F. Yan, K. Herold, A. M. Schmidt and R. Clynes (2007). "Receptor for advanced glycation end products expression on T cells contributes to antigen-specific cellular expansion in vivo." J Immunol 179(12): 8051-8058.

Moser, B., S. Janik, A. I. Schiefer, L. Mullauer, C. Bekos, A. Scharrer, M. Mildner, F. Renyi-Vamos, W. Klepetko and H. J. Ankersmit (2014). "Expression of RAGE and HMGB1 in thymic epithelial tumors, thymic hyperplasia and regular thymic morphology." PLoS One 9(4): e94118.

Moser, B. and W. Klepetko (2013). Kontroversen in der chirurgischen Therapie von Thymustumoren, EMCTO 2013. JATROS – Medizinisches Fachjournal: Pulmologie & HNO 3.

Moser, B., M. Scharitzer, S. Hacker, J. Ankersmit, J. R. Matilla, G. Lang, C. Aigner, S. Taghavi and W. Klepetko (2014). "Thymomas and thymic carcinomas: prognostic factors and multimodal management." Thorac Cardiovasc Surg 62(2): 153-160.

Moser, B., A. I. Schiefer, S. Janik, A. Marx, H. Prosch, W. Pohl, B. Neudert, A. Scharrer, W. Klepetko and L. Mullauer (2015). "Adenocarcinoma of the thymus, enteric type: report of 2 cases, and proposal for a novel subtype of thymic carcinoma." Am J Surg Pathol 39(4): 541-548.

Murakawa, T., T. Karasaki, K. Kitano, K. Nagayama, J. Nitadori, M. Anraku and J. Nakajima (2015). "Invasive thymoma disseminated into the pleural cavity: mid-term results of surgical resection." Eur J Cardiothorac Surg 47(3): 567-572.

Nagasaka, T., N. Nakashima and H. Nunome (1993). "Needle tract implantation of thymoma after transthoracic needle biopsy." J Clin Pathol 46(3): 278-279.

Nakatsu, T., S. Motoyama, K. Maruyama, S. Usami, Y. Sato, M. Miura, Y. Hinai, H. Saito, Y. Minamiya, K. Murata and J. Ogawa (2012). "Tumoral CRP expression in thoracic esophageal squamous cell cancers is associated with poor outcomes." Surg Today 42(7): 652-658.

Nicholson, A. G., F. C. Detterbeck, M. Marino, J. Kim, K. Stratton, D. Giroux, H. Asamura, J. Crowley, C. Falkson, P. L. Filosso, G. Giaccone, J. Huang, K. Kondo, M. Lucchi, E. M. Marom, M. Okumura, E. Ruffini and P. Van Schil (2014). "The IASLC/ITMIG Thymic Epithelial Tumors Staging Project: proposals for the T Component for the forthcoming (8th) edition of the TNM classification of malignant tumors." J Thorac Oncol 9(9 Suppl 2): S73-80.

Nicodeme, F., S. Geffroy, M. Conti, B. Delobel, V. Soenen, N. Grardel, H. Porte, M. C. Copin, J. L. Lai and J. Andrieux (2005). "Familial occurrence of thymoma and autoimmune diseases with the constitutional translocation t(14;20)(q24.1;p12.3)." Genes Chromosomes Cancer 44(2): 154-160.

Nishikawa, H., A. Arimoto, T. Wakasa, R. Kita, T. Kimura and Y. Osaki (2013). "Pre-treatment C-reactive protein as a prognostic factor for recurrence after surgical resection of hepatocellular carcinoma." Anticancer Res 33(3): 1181-1188.

O'Dowd, C., L. A. McRae, D. C. McMillan, A. Kirk and R. Milroy (2010). "Elevated preoperative C-reactive protein predicts poor cancer specific survival in patients undergoing resection for non-small cell lung cancer." J Thorac Oncol 5(7): 988-992.

Okuda, K., M. Yano, I. Yoshino, M. Okumura, M. Higashiyama, K. Suzuki, M. Tsuchida, J. Usuda and H. Tateyama (2014). "Thymoma patients with pleural dissemination: nationwide retrospective study of 136 cases in Japan." Ann Thorac Surg 97(5): 1743-1748.

Omasa, M., H. Date, T. Sozu, T. Sato, K. Nagai, K. Yokoi, T. Okamoto, N. Ikeda, F. Tanaka and Y. Maniwa (2015). "Postoperative radiotherapy is effective for thymic carcinoma but not for thymoma in stage II and III thymic epithelial tumors: the Japanese Association for Research on the Thymus Database Study." Cancer 121(7): 1008-1016.

Ong, S. L., G. Garcea, S. C. Thomasset, C. P. Neal, D. M. Lloyd, D. P. Berry and A. R. Dennison (2008). "Ten-year experience in the management of gallbladder cancer from a single hepatobiliary and pancreatic centre with review of the literature." HPB (Oxford) 10(6): 446-458.

Ose, N., H. Maeda, M. Inoue, E. Morii, Y. Shintani, H. Matsui, H. Tada, T. Tokunaga, K. Kimura, Y. Sakamaki, Y. Takeuchi, K. Fukuhara, H. Katsura, T. Iwasaki and M. Okumura (2018). "Results of treatment for thymic neuroendocrine tumours: multicentre clinicopathological study." Interact Cardiovasc Thorac Surg 26(1): 18-24.

Otsuka, H. (2012). "The utility of FDG-PET in the diagnosis of thymic epithelial tumors." J Med Invest 59(3-4): 225-234.

Ozawa, Y., M. Hara, M. Shimohira, K. Sakurai, M. Nakagawa and Y. Shibamoto (2016). "Associations between computed tomography features of thymomas and their pathological classification." Acta Radiol 57(11): 1318-1325.

Palumbo, J. S., J. M. Potter, L. S. Kaplan, K. Talmage, D. G. Jackson and J. L. Degen (2002). "Spontaneous hematogenous and lymphatic metastasis, but not primary tumor growth or angiogenesis, is diminished in fibrinogen-deficient mice." Cancer Res 62(23): 6966-6972.

Pastorino, U., D. Morelli, G. Leuzzi, M. Gisabella, P. Suatoni, F. Taverna, E. Bertocchi, M. Boeri, G. Sozzi, A. Cantarutti and G. Corrao (2017). "Baseline and postoperative C-reactive protein levels predict mortality in operable lung cancer." Eur J Cancer 79: 90-97.

Pedrazzani, C., G. Mantovani, E. Fernandes, F. Bagante, G. Luca Salvagno, N. Surci, T. Campagnaro, A. Ruzzenente, E. Danese, G. Lippi and A. Guglielmi (2017). "Assessment of neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and platelet count as predictors of long-term outcome after R0 resection for colorectal cancer." 7(1): 1494.

Pepys, M. B. and G. M. Hirschfield (2003). "C-reactive protein: a critical update." J Clin Invest 111(12): 1805-1812.

Phillips, L. H., 2nd (2003). "The epidemiology of myasthenia gravis." Ann N Y Acad Sci 998: 407-412.

Proctor, M. J., D. C. McMillan, D. S. Morrison, C. D. Fletcher, P. G. Horgan and S. J. Clarke (2012). "A derived neutrophil to lymphocyte ratio predicts survival in patients with cancer." Br J Cancer 107(4): 695-699.

Radovich, M., C. R. Pickering, I. Felau, G. Ha, H. Zhang, H. Jo, K. A. Hoadley, P. Anur, J. Zhang, M. McLellan, R. Bowlby, T. Matthew, L. Danilova, A. M. Hegde, J. Kim, M. D. M. Leiserson, G. Sethi, C. Lu, M. Ryan, X. Su, A. D. Cherniack, G. Robertson, R. Akbani, P. Spellman, J. N. Weinstein, D. N. Hayes, B. Raphael, T. Lichtenberg, K. Leraas, J. C. Zenklusen, J. Fujimoto, C. Scapulatempo-Neto, A. L. Moreira, D. Hwang, J. Huang, M. Marino, R. Korst, G. Giaccone, Y. Gokmen-Polar, S. Badve, A. Rajan, P. Strobel, N. Girard, M. S. Tsao, A. Marx, A. S. Tsao and P. J. Loehrer (2018). "The Integrated Genomic Landscape of Thymic Epithelial Tumors." Cancer Cell 33(2): 244-258.e210.

Rena, O., E. Papalia, A. Oliaro, E. Ruffini, P. Filosso, D. Novero, G. Maggi and C. Casadio (2007). "Does adjuvant radiation therapy improve disease-free survival in completely resected Masaoka stage II thymoma?" Eur J Cardiothorac Surg 31(1): 109-113.

Ried, M., A. Marx, A. Gotz, O. Hamer, B. Schalke and H. S. Hofmann (2016). "State of the art: diagnostic tools and innovative therapies for treatment of advanced thymoma and thymic carcinoma." Eur J Cardiothorac Surg 49(6): 1545-1552.

Rosai, J. and G. D. Levine (1976). Atlas of tumor pathology. Tumors of the thymus,

Second series, fascicle 13. Armed Forces Institute of Pathology, Washington, DC.

Ruffini, E., F. Detterbeck, D. Van Raemdonck, G. Rocco, P. Thomas, W. Weder, A. Brunelli, A. Evangelista and F. Venuta (2014). "Tumours of the thymus: a cohort study of prognostic factors from the European Society of Thoracic Surgeons database." Eur J Cardiothorac Surg 46(3): 361-368.

Ruffini, E., F. Detterbeck, D. Van Raemdonck, G. Rocco, P. Thomas, W. Weder, A. Brunelli, F. Guerrera, S. Keshavjee, N. Altorki, J. Schutzner, A. Arame, L. Spaggiari, E. Lim, A. Toker and F. Venuta (2014). "Thymic carcinoma: a cohort study of patients from the European society of thoracic surgeons database." J Thorac Oncol 9(4): 541-548.

Ruffini, E., P. E. Falcoz, F. Guerrera, P. L. Filosso, P. Thomas, N. Novoa, B. Moser, D. Pellicano and S. Passani (2018). "The European Society of Thoracic Surgeons (ESTS) thymic database." J Thorac Dis 10(Suppl 29): S3516-s3520.

Ruffini, E., P. L. Filosso, F. Guerrera, P. Lausi, P. Lyberis and A. Oliaro (2018). "Optimal surgical approach to thymic malignancies: New trends challenging old dogmas." Lung Cancer 118: 161-170.

Ruffini, E., P. L. Filosso, C. Mossetti, M. C. Bruna, D. Novero, P. Lista, C. Casadio and A. Oliaro (2011). "Thymoma: inter-relationships among World Health Organization histology, Masaoka staging and myasthenia gravis and their independent prognostic significance: a single-centre experience." Eur J Cardiothorac Surg 40(1): 146-153.

Sahni, A., M. T. Arevalo, S. K. Sahni and P. J. Simpson-Haidaris (2009). "The VE-cadherin binding domain of fibrinogen induces endothelial barrier permeability and enhances transendothelial migration of malignant breast epithelial cells." Int J Cancer 125(3): 577-584.

Sahni, A., P. J. Simpson-Haidaris, S. K. Sahni, G. G. Vaday and C. W. Francis (2008). "Fibrinogen synthesized by cancer cells augments the proliferative effect of fibroblast growth factor-2 (FGF-2)." J Thromb Haemost 6(1): 176-183.

Saleh, K., N. Khalifeh-Saleh and H. R. Kourie (2018). "Where do immune checkpoint inhibitors stand in the management of thymic epithelial tumors?" Immunotherapy 10(10): 823-826.

Sandri, A., G. Cusumano, F. Lococo, M. Alifano, P. Granone, S. Margaritora, A. Cesario, A. Oliaro, P. Filosso, J. F. Regnard and E. Ruffini (2014). "Long-term results after treatment for recurrent thymoma: a multicenter analysis." J Thorac Oncol 9(12): 1796-1804.

Santoni, G., C. Amantini, M. B. Morelli, D. Tomassoni, M. Santoni, O. Marinelli, M. Nabissi, C. Cardinali, V. Paolucci, M. Torniai, S. Rinaldi, F. Morgese, G. Bernardini and R. Berardi (2018). "High CTLA-4 expression correlates with poor prognosis in thymoma patients." Oncotarget 9(24): 16665-16677.

Saqib, R., S. Pathak, N. Smart, Q. Nunes, J. Rees, M. Finch Jones and G. Poston (2018). "Prognostic significance of pre-operative inflammatory markers in resected gallbladder cancer: a systematic review." ANZ J Surg 88(6): 554-559.

Schirosi, L., N. Nannini, D. Nicoli, A. Cavazza, R. Valli, S. Buti, L. Garagnani, G. Sartori, F. Calabrese, A. Marchetti, F. Buttitta, L. Felicioni, M. Migaldi, F. Rea, F. Di Chiara, M. C. Mengoli and G. Rossi (2012). "Activating c-KIT mutations in a subset of thymic carcinoma and response to different c-KIT inhibitors." Ann Oncol 23(9): 2409-2414.

Schmitt, J. and P. J. Loehrer, Sr. (2010). "The role of chemotherapy in advanced thymoma." J Thorac Oncol 5(10 Suppl 4): S357-360.

Segreto, S., R. Fonti, M. Ottaviano, S. Pellegrino, L. Pace, V. Damiano, G. Palmieri and S. Del Vecchio (2017). "Evaluation of metabolic response with (18)F-FDG PET-CT in patients with advanced or recurrent thymic epithelial tumors." Cancer Imaging 17(1): 10.

Shibata, H., H. Nomori, K. Uno, K. Sakaguchi, R. Nakashima, K. Iyama, K. Tomiyoshi, M. Kaji, T. Goya, T. Suzuki and H. Horio (2009). "18F-fluorodeoxyglucose and 11C-acetate positron emission tomography are useful modalities for diagnosing the histologic type of thymoma." Cancer 115(11): 2531-2538.

Shin, J. H., C. J. Kim, E. J. Jeon, C. O. Sung, H. J. Shin, J. Choi and E. Yu (2015). "Overexpression of C-reactive Protein as a Poor Prognostic Marker of Resectable Hepatocellular Carcinomas." J Pathol Transl Med 49(2): 105-111.

Shrotriya, S., D. Walsh, N. Bennani-Baiti, S. Thomas and C. Lorton (2015). "C-Reactive Protein Is an Important Biomarker for Prognosis Tumor Recurrence and Treatment Response in Adult Solid Tumors: A Systematic Review." PLoS One 10(12): e0143080.

Shu, Y. J., H. Weng, R. F. Bao, X. S. Wu, Q. Ding, Y. Cao, X. A. Wang, F. Zhang, S. S. Xiang, H. F. Li, M. L. Li, J. S. Mu, W. G. Wu and Y. B. Liu (2014). "Clinical and prognostic significance of preoperative plasma hyperfibrinogenemia in gallbladder cancer patients following surgical resection: a retrospective and in vitro study." BMC Cancer 14: 566.

Sollini, M., P. A. Erba, A. Fraternali, M. Casali, M. L. Di Paolo, A. Froio, A. Frasoldati and A. Versari (2014). "PET and PET/CT with 68gallium-labeled somatostatin analogues in Non GEP-NETs Tumors." ScientificWorldJournal 2014: 194123.

Sonett, J. R. and A. Jaretzki, 3rd (2008). "Thymectomy for nonthymomatous myasthenia gravis: a critical analysis." Ann N Y Acad Sci 1132: 315-328.

Strobel, P., M. Helmreich, G. Menioudakis, S. R. Lewin, T. Rudiger, A. Bauer, V. Hoffacker, R. Gold, W. Nix, B. Schalke, O. Elert, M. Semik, H. K. Muller-Hermelink and A. Marx (2002). "Paraneoplastic myasthenia gravis correlates with generation of mature naive CD4(+) T cells in thymomas." Blood 100(1): 159-166.

Strobel, P., A. Zettl, K. Shilo, W. Y. Chuang, A. G. Nicholson, Y. Matsuno, A. Gal, R. H. Laeng, P. Engel, C. Capella, M. Marino, J. K. Chan, A. Rosenwald, W. Travis, T. J. Franks, D. Ellenberger, I. M. Schaefer and A. Marx (2014). "Tumor genetics and survival of thymic neuroendocrine neoplasms: a multi-institutional clinicopathologic study." Genes Chromosomes Cancer 53(9): 738-749.

Szkandera, J., M. Stotz, G. Absenger, T. Stojakovic, H. Samonigg, P. Kornprat, R. Schaberl-Moser, W. Alzoughbi, C. Lackner, A. L. Ress, F. S. Seggewies, A. Gerger, G. Hoefler and M. Pichler (2014). "Validation of C-reactive protein levels as a prognostic indicator for survival in a large cohort of pancreatic cancer patients." Br J Cancer 110(1): 183-188.

Tang, L. Q., D. P. Hu, Q. Y. Chen, L. Zhang, X. P. Lai, Y. He, Y. X. Xu, S. H. Wen, Y. T. Peng, W. H. Chen, S. S. Guo, L. T. Liu, C. N. Qian, X. Guo, M. S. Zeng and H. Q. Mai (2015). "Elevated high-sensitivity C-reactive protein levels predict decreased survival for nasopharyngeal carcinoma patients in the intensity-modulated radiotherapy era." PLoS One 10(4): e0122965.

Tang, L. Q., C. F. Li, Q. Y. Chen, L. Zhang, X. P. Lai, Y. He, Y. X. Xu, D. P. Hu, S. H. Wen, Y. T. Peng, W. H. Chen, H. Liu, S. S. Guo, L. T. Liu, J. Li, J. P. Zhang, L. Guo, C. Zhao, K. J. Cao, C. N. Qian, Y. X. Zeng, X. Guo, H. Q. Mai and M. S. Zeng (2015). "High-sensitivity C-reactive protein complements plasma Epstein-Barr virus deoxyribonucleic acid prognostication in nasopharyngeal carcinoma: a large-scale retrospective and prospective cohort study." Int J Radiat Oncol Biol Phys 91(2): 325-336.

Tennent, G. A., S. O. Brennan, A. J. Stangou, J. O'Grady, P. N. Hawkins and M. B. Pepys (2007). "Human plasma fibrinogen is synthesized in the liver." Blood 109(5): 1971-1974.

Titulaer, M. J., R. Soffietti, J. Dalmau, N. E. Gilhus, B. Giometto, F. Graus, W. Grisold, J. Honnorat, P. A. Sillevis Smitt, R. Tanasescu, C. A. Vedeler, R. Voltz and J. J. Verschuuren (2011). "Screening for tumours in paraneoplastic syndromes: report of an EFNS task force." Eur J Neurol 18(1): 19-e13.

Turri-Zanoni, M., G. Salzano, A. Lambertoni, M. Giovannardi, A. Karligkiotis, P. Castelnuovo and P. Battaglia (2017). "Prognostic value of pretreatment peripheral blood markers in paranasal sinus cancer: Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratio." 39(4): 730-736.

Venuta, F., E. A. Rendina, W. Klepetko and G. Rocco (2011). "Surgical management of stage III thymic tumors." Thorac Surg Clin 21(1): 85-91, vii.

Vigushin, D. M., M. B. Pepys and P. N. Hawkins (1993). "Metabolic and scintigraphic studies of radioiodinated human C-reactive protein in health and disease." J Clin Invest 91(4): 1351-1357.

Villasenor, A., S. W. Flatt, C. Marinac, L. Natarajan, J. P. Pierce and R. E. Patterson (2014). "Postdiagnosis C-reactive protein and breast cancer survivorship: findings from the WHEL study." Cancer Epidemiol Biomarkers Prev 23(1): 189-199.

Vincent, A. (2002). "Unravelling the pathogenesis of myasthenia gravis." Nat Rev Immunol 2(10): 797-804.

Volanakis, J. E. (2001). "Human C-reactive protein: expression, structure, and function." Mol Immunol 38(2-3): 189-197.

Wakatsuki, K., S. Matsumoto, K. Migita, M. Ito, T. Kunishige, H. Nakade, M. Nakatani, M. Kitano and M. Sho (2017). "Preoperative Plasma Fibrinogen is Associated with Lymph Node Metastasis and Predicts Prognosis in Resectable Esophageal Cancer." World J Surg 41(8): 2068-2077.

Wang, F., L. Pang, J. Fu, Y. Shen, Y. Wei, L. Tan, P. Zhang, Y. Han, C. Chen, R. Zhang, Y. Li, K. Chen, H. Chen, Y. Liu, Y. Cui, Y. Wang, Z. Yu, X. Zhou, Y. Liu, Y. Liu, Z. Gu and W. Fang (2016). "Postoperative survival for patients with thymoma complicating myasthenia gravis-preliminary retrospective results of the ChART database." J Thorac Dis 8(4): 711-717.

Watanabe, T., H. Shimomura, T. Mutoh, R. Saito, R. Goto, T. Yamada, H. Notsuda, Y. Matsuda, M. Noda, A. Sakurada, Y. Taki and Y. Okada (2019). "Positron emission tomography/computed tomography as a clinical diagnostic tool for anterior mediastinal tumors." Surg Today 49(2): 143-149.

Weis, C. A., X. Yao, Y. Deng, F. C. Detterbeck, M. Marino, A. G. Nicholson, J. Huang, P. Strobel, A. Antonicelli and A. Marx (2015). "The impact of thymoma histotype on prognosis in a worldwide database." J Thorac Oncol 10(2): 367-372.

Wen, J., Y. Yang, F. Ye, X. Huang, S. Li, Q. Wang and X. Xie (2015). "The preoperative plasma fibrinogen level is an independent prognostic factor for overall survival of breast cancer patients who underwent surgical treatment." Breast 24(6): 745-750.

Wolfe, G. I., H. J. Kaminski, I. B. Aban, G. Minisman, H. C. Kuo, A. Marx, P. Strobel, C. Mazia, J. Oger, J. G. Cea, J. M. Heckmann, A. Evoli, W. Nix, E. Ciafaloni, G. Antonini, R. Witoonpanich, J. O. King, S. R. Beydoun, C. H. Chalk, A. C. Barboi, A. A. Amato, A. I. Shaibani, B. Katirji, B. R. Lecky, C. Buckley, A. Vincent, E. Dias-Tosta, H. Yoshikawa, M. Waddington-Cruz, M. T. Pulley, M. H. Rivner, A. Kostera-Pruszczyk, R. M. Pascuzzi, C. E. Jackson, G. S. Garcia Ramos, J. J. Verschuuren, J. M. Massey, J. T. Kissel, L. C. Werneck, M. Benatar, R. J. Barohn, R. Tandan, T. Mozaffar, R. Conwit, J. Odenkirchen, J. R. Sonett, A. Jaretzki, 3rd, J. Newsom-Davis and G. R. Cutter (2016). "Randomized Trial of Thymectomy in Myasthenia Gravis." N Engl J Med 375(6): 511-522.

Wright, C. D. (2011). "Stage IVA thymoma: patterns of spread and surgical management." Thorac Surg Clin 21(1): 93-97, vii.

Wu, X. S., L. B. Shi, M. L. Li, Q. Ding, H. Weng, W. G. Wu, Y. Cao, R. F. Bao, Y. J. Shu, Q. C. Ding, J. S. Mu, J. Gu, P. Dong and Y. B. Liu (2014). "Evaluation of two

inflammation-based prognostic scores in patients with resectable gallbladder carcinoma." Ann Surg Oncol 21(2): 449-457.

Xia, W. X., Y. F. Ye, X. Lu, L. Wang, L. R. Ke, H. B. Zhang, M. D. Roycik, J. Yang, J. L. Shi, K. J. Cao, X. Guo and Y. Q. Xiang (2013). "The impact of baseline serum C-reactive protein and C-reactive protein kinetics on the prognosis of metastatic nasopharyngeal carcinoma patients treated with palliative chemotherapy." PLoS One 8(10): e76958.

Xia, W. X., H. B. Zhang, J. L. Shi, X. Lu, L. Wang, Y. F. Ye, K. J. Cao, C. N. Qian, X. Guo and Y. Q. Xiang (2013). "A prognostic model predicts the risk of distant metastasis and death for patients with nasopharyngeal carcinoma based on pre-treatment serum C-reactive protein and N-classification." Eur J Cancer 49(9): 2152-2160.

Yamashita, H., J. Kitayama, M. Taguri and H. Nagawa (2009). "Effect of preoperative hyperfibrinogenemia on recurrence of colorectal cancer without a systemic inflammatory response." World J Surg 33(6): 1298-1305.

Yao, J. C., M. Hassan, A. Phan, C. Dagohoy, C. Leary, J. E. Mares, E. K. Abdalla, J. B. Fleming, J. N. Vauthey, A. Rashid and D. B. Evans (2008). "One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States." J Clin Oncol 26(18): 3063-3072.

Yeh, Y. C., H. J. Lei, M. H. Chen, H. L. Ho, L. Y. Chiu, C. P. Li and Y. C. Wang (2017). "C-Reactive Protein (CRP) is a Promising Diagnostic Immunohistochemical Marker for Intrahepatic Cholangiocarcinoma and is Associated With Better Prognosis." Am J Surg Pathol 41(12): 1630-1641.

Yodying, H., A. Matsuda, M. Miyashita, S. Matsumoto, N. Sakurazawa, M. Yamada and E. Uchida (2016). "Prognostic Significance of Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio in Oncologic Outcomes of Esophageal Cancer: A Systematic Review and Meta-analysis." Ann Surg Oncol 23(2): 646-654.

Yokoyama, S. and H. Miyoshi (2018). "Thymic tumors and immune checkpoint inhibitors." J Thorac Dis 10(Suppl 13): S1509-S1515.

Yuan, Z. Y., S. G. Gao, J. W. Mu, Q. Xue, Y. S. Mao, D. L. Wang, J. Zhao, Y. S. Gao, J. F. Huang and J. He (2016). "Prognostic value of preoperative neutrophil-lymphocyte ratio is superior to platelet-lymphocyte ratio for survival in patients who underwent complete resection of thymic carcinoma." J Thorac Dis 8(7): 1487-1496.

Zeng, Y. C., R. Wu, Y. P. Xiao, F. Chi, M. Xue, Z. Y. Zhang, R. Xing, W. Z. Zhong, S. L. Wang, X. Tian, W. Chen, J. J. Chen and L. N. Wu (2015). "Serum C-reactive protein predicts poor prognosis in patients with locoregionally advanced nasopharyngeal carcinoma treated with chemoradiotherapy." Curr Oncol 22(1): 20-24.

Zhang, H., N. Lu, M. Wang, X. Gu and D. Zhang (1999). "Postoperative radiotherapy for stage I thymoma: a prospective randomized trial in 29 cases." Chin Med J (Engl) 112(2): 136-138.

Zhang, Y., C. Jiang, J. Li, J. Sun and X. Qu (2015). "Prognostic significance of preoperative neutrophil/lymphocyte ratio and platelet/lymphocyte ratio in patients with gallbladder carcinoma." Clin Transl Oncol 17(10): 810-818.

Zhou, D., J. Liang, L. I. Xu, F. He, Z. Zhou, Y. Zhang and M. Chen (2016). "Derived neutrophil to lymphocyte ratio predicts prognosis for patients with HBV-associated hepatocellular carcinoma following transarterial chemoembolization." Oncol Lett 11(5): 2987-2994.

11. Bibliography of the candidate's publications

11.1 Publications related to the thesis

Bernhard Moser, Margit Scharitzer, Stefan Hacker, Hendrik Jan Ankersmit, György Lang, Clemens Aigner, Shahrokh Taghavi, and Walter Klepetko. Thymomas and thymic carcinomas: prognostic factors and multimodal management.
Thorac Cardiovasc Surg. 2014 Mar;62(2):153-60. doi: 10.1055/s-0032-1322611. Epub 2012 Dec 6. PMID:23225512

Moser B, Fadel E, Fabre D, Keshavjee S, de Perrot M, Thomas P, Brioude G, Van Raemdonck D, Viskens S, Lang-Lazdunski L, Bille A, Weder W, Jungraithmayr W, Ruffini E, Guerrera F, Gómez de Antonio D, Liberman M, Novoa N, Scarci M, Janik S, Klepetko W.
Surgical therapy of thymic tumours with pleural involvement: an ESTS Thymic Working Group Project.
Eur J Cardiothorac Surg. 2017 Apr 25. doi:10.1093/ejcts/ezx090. [Epub ahead of print] PubMed PMID: 28449028.

Stefan Janik, Christine Bekos, Philipp Hacker, Thomas Raunegger, Bahil Ghanim, Elisa Einwallner, Walter Klepetko, Leonhard Müllauer, Hendrik J Ankersmit and **Bernhard Moser**. Elevated serum C-reactive protein levels predict poor outcome and tumor recurrence in patients with thymic epithelial tumors. A pro- and retrospective single center analysis.
Oncotarget. 2017 Apr 27. doi:10.18632/oncotarget.17478. [Epub ahead of print] PubMed PMID: 28514756.

Janik S, Raunegger T, Hacker P, Ghanim B, Einwallner E, Müllauer L, Schiefer AI, Moser J, Klepetko W, Ankersmit HJ, **Moser B.** Prognostic and diagnostic impact of fibrinogen, neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio on thymic epithelial tumors outcome.
Oncotarget. 2018 Apr 24;9(31):21861-21875. doi: 10.18632/oncotarget.25076. eCollection 2018 Apr 24. PubMed PMID: 29774108; PubMed Central PMCID: PMC5955144.

Moser B, Janik S, Schiefer AI, Müllauer L, Bekos C, Scharrer A, Mildner M, Rényi-Vámos F, Klepetko W, Ankersmit HJ. Expression of RAGE and HMGB1 in thymic epithelial tumors, thymic hyperplasia and regular thymic morphology.
PLoS One. 2014 Apr 4;9(4):e94118. doi: 10.1371/journal.pone.0094118. eCollection 2014. PMID:24705787;
Impact Factor 2013: 3.534, Journal Ranking Multidisciplinary Sciences 8/55

11.2 Publications not related to the thesis

Matilla JR, Alvoeiro M, Benazzo A, Schwarz S, Klepetko W, **Moser B**. Initial experience with a combined sequential left-sided and subxiphoid VATS approach for resection of large anterior mediastinal tumors. Mediastinum 2018. doi: 10.21037/med.2018.09.01

Moser B, Jaksch P, Taghavi S, Muraközy G, Lang G, Hager H, Krenn C, Roth G, Faybik P, Bacher A, Aigner C, Matilla JR, Hoetzenecker K, Hacker P, Lang I, Klepetko W. Lung transplantation for idiopathic pulmonary arterial hypertension on intraoperative and postoperatively prolonged extracorporeal membrane oxygenation provides optimally controlled reperfusion and excellent outcome.
Eur J Cardiothorac Surg. 2018 Jan 1;53(1):178-185. doi: 10.1093/ejcts/ezx212. PubMed PMID: 28950326; PubMed Central PMCID: PMC5848802.

Janik S, Ankersmit J, Müllauer L, Klepetko W, **Moser B.**
Improving follow up for thymic epithelial tumors.
Mediastinum 2018;2:10. Editoral doi: 10.21037/med.2018.03.01

Matilla JR, Klepetko W, **Moser B.**
Thymic minimally invasive surgery: state of the art across the world - Europe.
J Vis Surg. 2017 May 16;3:70. doi:10.21037/jovs.2017.04.01. eCollection 2017. Review. PubMed PMID: 29078633; PubMed Central PMCID: PMC5637941.

Franz Enkner, Bettina Pichlhöfer, Alexandru Teodor Zaharie, Milica Grunic, Tina Maria Holper, Stefan Janik, **Bernhard Moser**, Katrin Schlangen, Barbara Neudert, Karin Walter, Brigitte Migschitz, Leonhard Müllauer. Molecular profiling demonstrates that thymoma and thymic carcinoma are genetically different and identifies novel targets for therapy. Pathol Oncol Res. 2017 Jul;23(3):551-564. doi:10.1007/s12253-016-0144-8. Epub 2016 Nov 14. PubMed PMID: 27844328; PubMedCentral PMCID: PMC5487866.

Janik S, Schiefer AI, Bekos C, Hacker P, Haider T, Moser J, Klepetko W, Müllauer L, Ankersmit HJ, **Moser B**.
HSP27 and 70 expression in thymic epithelial tumors and benign thymic alterations: diagnostic, prognostic and physiologic implications.
Sci Rep. 2016 Apr 21;6:24267. doi: 10.1038/srep24267. PMID: 27097982; Impact Factor 2014: 5.578

Bekos C, Zimmermann M, Unger L, Janik S, Hacker P, Mitterbauer A, Koller M, Fritz R, Gäbler C, Kessler M, Nickl S, Didcock J, Altmann P, Haider T, Roth G, Klepetko W, Ankersmit HJ, **Moser B.** Non-professional marathon running: RAGE axis and ST2 family changes in relation to open-window effect, inflammation and renal function.
Sci Rep. 2016 Sep 22;6:32315. doi: 10.1038/srep32315. PubMed PMID: 27653273; Impact Factor 2014: 5.578 http://rdcu.be/pMoJ

Leuzzi G, Rocco G, Ruffini E, Sperduti I, Detterbeck F, Weder W, Venuta F, Van Raemdonck D, Thomas P, Facciolo F; ESTS Thymic Working Group. ***Collaborators*** (35): Alkattan K, Arame A, Refai M, Casadio C, Carbognani P, Cerfolio R, Donati G,

Foroulis CN, Gebitekin C, de Antonio DG, Kernstine KH, Keshavjee S, **Moser B**, Lequaglie C, Liberman M, Lim E, Nicholson AG, Lang-Lazdunski L, Mancuso M, Altorki N, Nosotti M, Novoa NM, Brioude G, Oliaro A, Filosso PL, Saita S, Scarci M, Schützner J, Terzi A, Toker A, Van Veer H, Anile M, Rendina E, Voltolini L, Zurek W.
Multimodality therapy for locally advanced thymomas: A propensity score-matched cohort study from the European Society of Thoracic Surgeons Database.
J Thorac Cardiovasc Surg. 2016 Jan;151(1):47-57.e1. doi: 10.1016/j.jtcvs.2015.08.034.

Moser B, Schiefer AI, Janik S, Marx A, Prosch H, Pohl W, Neudert B, Scharrer A, Klepetko W, Müllauer L.
Adenocarcinoma of the Thymus, Enteric Type: Report of 2 Cases, and Proposal for a Novel Subtype of Thymic Carcinoma. **Am J Surg Pathol**. 2015 Apr;39(4):541-8. doi: 10.1097/PAS.0000000000000359. Review. PMID: 25517960; Impact Factor 2013: 4.592, Journal Ranking Surgery 8/204

Bernhard Moser, Anna Megerle, Christine Bekos, Stefan Janik, Tamás Szerafin, Peter Birner, Ana-Iris Schiefer, Michael Mildner, Irene Lang, Nika Skoro-Sajer, Roela Sadushi-Kolici, Shahrokh Taghavi, Walter Klepetko and Hendrik Jan Ankersmit
Local and systemic RAGE axis changes in pulmonary hypertension: CTEPH and iPAH
PLoS One. 2014 Sep 4;9(9):e106440. doi: 10.1371/journal.pone.0106440. eCollection 2014. PMID: 25188497;
Impact Factor 2013: 3.534, Journal Ranking Multidisciplinary Sciences 8/55

Kondo K, Van Schil P, Detterbeck FC, Okumura M, Stratton K, Giroux D, Asamura H, Crowley J, Falkson C, Filosso PL, Giaccone G, Huang J, Kim J, Lucchi M, Marino M, Marom EM, Nicholson AG, Ruffini E; Staging and Prognostic Factors Committee; Members of the Advisory Boards; Participating Institutions of the Thymic Domain. Collaborators (196): Goldstraw P, Rami-Porta R, Asamura H, Ball D, MacCallum P, Beer D, Beyruti R, Bolejack V, Chansky K, Crowley J, Detterbeck F, Eberhardt WE, Edwards J, Galateau-Sallé F, Giroux D, Gleeson F, Groome P, Huang J, Kennedy C, Kim J, Kim YT, Kingsbury L, Kondo H, Krasnik M, Kubota K, Lerut A, Lyons G, Marino M, Marom EM, van Meerbeeck J, Mitchell A, Nakano T, Nicholson AG, Brompton R, Nowak A, Peake M, Rice T, Rosenzweig K, Ruffini E, Rusch V, Saijo N, Van Schil P, Sculier JP, Shemanski L, Stratton K, Suzuki K, Tachimori Y, Thomas CF Jr, Travis W, Tsao MS, Turrisi A, Vansteenkiste J, Watanabe H, Wu YL, Falkson C, Filosso PL, Giaccone G, Kondo K, Lucchi M, Okumura M, Baas P, Erasmus J, Hasegawa S, Inai K, Kernstine K, Kindler H, Krug L, Nackaerts K, Pass H, Rice D, Blackstone E, Call Caja S, Ahmad U, Detterbeck F, Girard N, Haam SJ, Bae MK, Gomez DR, Marom EM, Van Schil P, Ströbel P, Marx A, Saita S, Wakelee H, Bertolaccini L, Vallieres E, Scott W, Su S, Park B, Marks J, Khella S, Shen R, Rosenberg M, Rosenberg M, Tomulescu V, Huang J, Foroulis C, Lang-Lazdunski L, Billè A, Maessen JG, Keijzers M, van Veer H, Wright C, Marino M, Facciolo F, Palmieri G, Buonerba C, Ferguson M, Marulli G, Lucchi M, Loehrer P, Kalkat M, Rohrberg K, Daugaard G, Toker A, Erus S, Kimmich M, Brunelli A, Refai M, Nicholson A, Lim E, Park IK, Wagner J, Tieu B, Fang W, Zhang J, Yu Z, Han Y, Li Y, Chen K, Chen G, Okumura M, Fujii Y, Asamura H, Nagai K, Nakajima J, Ikeda N, Haraguchi S, Onuki T, Suzuki K, Yoshino I, Tsuchida M, Takahashi S, Yokoi K, Hanyuda M, Niwa H, Date H, Maniwa Y, Miyoshi S, Kondo K, Iwasaki A, Okamoto T, Nagayasu T, Tanaka F, Suzuki M, Yoshida K, Okuma Y, Horio H, Matsumura A, Higashiyama M,

Suehisa H, Onuki T, Sano Y, Kondo K, Al Kattan K, Cerfolio R, Gebitekin C, de Antonio D, Kernstine KH, Altorki N, Novoa N, Ruffini E, Filosso PL, Saita S, Scarci M, Voltolini L, Weder W, Zurek W, Arame A, Casadio C, Carbognani P, Donati G, Keshavjee S, Klepetko W, **Moser B**, Lequaglie C, Liberman M, Mancuso M, Nosotti M, Spaggiari L, Thomas PA, Rendina E, Venuta F, Anile M, Schützner J, Rocco G.
The IASLC/ITMIG Thymic Epithelial Tumors Staging Project: proposals for the N and M components for the forthcoming (8th) edition of the TNM classification of malignant tumors.
J Thorac Oncol. 2014 Sep;9(9 Suppl 2):S81-7. doi: 10.1097/JTO.0000000000000291.

Nicholson AG, Detterbeck FC, Marino M, Kim J, Stratton K, Giroux D, Asamura H, Crowley J, Falkson C, Filosso PL, Giaccone G, Huang J, Kondo K, Lucchi M, Marom EM, Okumura M, Ruffini E, Van Schil P; Staging and Prognostic Factors Committee; Members of the Advisory Boards; Participating Institutions of the Thymic Domain. Collaborators (196): Goldstraw P, Rami-Porta R, Asamura H, Ball D, MacCallum P, Beer D, Beyruti R, Bolejack V, Chansky K, Crowley J, Detterbeck F, Eberhardt WE, Edwards J, Galateau-Sallé F, Giroux D, Gleeson F, Groome P, Huang J, Kennedy C, Kim J, Kim YT, Kingsbury L, Kondo H, Krasnik M, Kubota K, Lerut A, Lyons G, Marino M, Marom E, van Meerbeeck J, Mitchell A, Nakano T, Nicholson AG, Brompton R, Nowak A, Peake M, Rice T, Rosenzweig K, Ruffini E, Rusch V, Saijo N, Van Schil P, Sculier JP, Shemanski L, Stratton K, Suzuki K, Tachimori Y, Thomas CF Jr, Travis W, Tsao MS, Turrisi A, Vansteenkiste J, Watanabe H, Wu YL, Falkson C, Filosso PL, Giaccone G, Kondo K, Lucchi M, Okumura M, Baas P, Erasmus J, Hasegawa S, Inai K, Kernstine K, Kindler H, Krug L, Nackaerts K, Pass H, Rice D, Blackstone E, Call Caja S, Ahmad U, Detterbeck F, Girard N, Haam SJ, Bae MK, Gomez DR, Marom E, Van Schil P, Ströbel P, Marx A, Saita S, Wakelee H, Bertolaccini L, Vallieres E, Scott W, Su S, Park B, Marks J, Khella S, Shen R, Rosenberg M, Rosenberg M, Tomulescu V, Huang J, Foroulis C, Lang-Lazdunski L, Billè A, Maessen JG, Keijzers M, van Veer H, Wright C, Marino M, Facciolo F, Palmieri G, Buonerba C, Ferguson M, Marulli G, Lucchi M, Loehrer P, Kalkat M, Rohrberg K, Daugaard G, Toker A, Erus S, Kimmich M, Brunelli A, Refai M, Nicholson A, Lim E, Park IK, Wagner J, Tieu B, Fang W, Zhang J, Yu Z, Han Y, Li Y, Chen K, Chen G, Okumura M, Fujii Y, Asamura H, Nagai K, Nakajima J, Ikeda N, Haraguchi S, Onuki T, Suzuki K, Yoshino I, Tsuchida M, Takahashi S, Yokoi K, Hanyuda M, Niwa H, Date H, Maniwa Y, Miyoshi S, Kondo K, Iwasaki A, Okamoto T, Nagayasu T, Tanaka F, Suzuki M, Yoshida K, Okuma Y, Horio H, Matsumura A, Higashiyama M, Suehisa H, Onuki T, Sano Y, Kondo K, Al Kattan K, Cerfolio R, Gebitekin C, de Antonio D, Kernstine KH, Altorki N, Novoa N, Ruffini E, Filosso PL, Saita S, Scarci M, Voltolini L, Weder W, Zurek W, Arame A, Casadio C, Carbognani P, Donati G, Keshavjee S, Klepetko W, **Moser B**, Lequaglie C, Liberman M, Mancuso M, Nosotti M, Spaggiari L, Thomas PA, Rendina E, Venuta F, Anile M, Schützner J, Rocco G.
The IASLC/ITMIG Thymic Epithelial Tumors Staging Project: proposals for the T Component for the forthcoming (8th) edition of the TNM classification of malignant tumors.
J Thorac Oncol. 2014 Sep;9(9 Suppl 2):S73-80. doi: 10.1097/JTO.0000000000000303.

Detterbeck FC, Stratton K, Giroux D, Asamura H, Crowley J, Falkson C, Filosso PL, Frazier AA, Giaccone G, Huang J, Kim J, Kondo K, Lucchi M, Marino M, Marom EM, Nicholson AG, Okumura M, Ruffini E, Van Schil P; Staging and Prognostic Factors

Committee; Members of the Advisory Boards; Participating Institutions of the Thymic Domain. Collaborators (215): Goldstraw P, Rami-Porta R, Asamura H, Ball D, MacCallum P, Beer D, Beyruti R, Bolejack V, Chansky K, Crowley J, Detterbeck F, Eberhardt WE, Edwards J, Galateau-Sallé F, Giroux D, Gleeson F, Groome P, Huang J, Kennedy C, Kim J, Kim YT, Kingsbury L, Kondo H, Krasnik M, Kubota K, Lerut A, Lyons G, Marino M, Marom EM, van Meerbeeck J, Mitchell A, Nakano T, Nicholson AG, Brompton R, Nowak A, Peake M, Rice T, Rosenzweig K, Ruffini E, Rusch V, Saijo N, Van Schil P, Sculier JP, Shemanski L, Stratton K, Suzuki K, Tachimori Y, Thomas CF Jr, Travis W, Tsao MS, Turrisi A, Vansteenkiste J, Watanabe H, Wu YL, Falkson C, Filosso PL, Giaccone G, Kondo K, Lucchi M, Okumura M, Baas P, Erasmus J, Hasegawa S, Inai K, Kernstine K, Kindler H, Krug L, Nackaerts K, Pass H, Rice D, Blackstone E, Call Caja S, Ahmad U, Detterbeck F, Girard N, Haam SJ, Bae MK, Gomez DR, Marom EM, Van Schil P, Ströbel P, Marx A, Saita S, Wakelee H, Bertolaccini L, Vallieres E, Scott W, Su S, Park B, Marks J, Khella S, Shen R, Rosenberg M, Rosenberg M, Tomulescu V, Huang J, Foroulis C, Lang-Lazdunski L, Billè A, Maessen JG, Keijzers M, van Veer H, Wright C, Marino M, Facciolo F, Palmieri G, Buonerba C, Ferguson M, Marulli G, Lucchi M, Loehrer P, Kalkat M, Rohrberg K, Daugaard G, Toker A, Erus S, Kimmich M, Brunelli A, Refai M, Nicholson A, Lim E, Park IK, Wagner J, Tieu B, Fang W, Zhang J, Yu Z, Han Y, Li Y, Chen K, Chen G, Okumura M, Fujii Y, Asamura H, Nagai K, Nakajima J, Ikeda N, Haraguchi S, Onuki T, Suzuki K, Yoshino I, Tsuchida M, Takahashi S, Yokoi K, Hanyuda M, Niwa H, Date H, Maniwa Y, Miyoshi S, Kondo K, Iwasaki A, Okamoto T, Nagayasu T, Tanaka F, Suzuki M, Yoshida K, Okuma Y, Horio H, Matsumura A, Higashiyama M, Suehisa H, Onuki T, Sano Y, Kondo K, Al Kattan K, Cerfolio R, Gebitekin C, de Antonio D, Kernstine KH, Altorki N, Novoa N, Ruffini E, Filosso PL, Saita S, Scarci M, Voltolini L, Weder W, Zurek W, Arame A, Casadio C, Carbognani P, Donati G, Keshavjee S, Klepetko W, **Moser B**, Lequaglie C, Liberman M, Mancuso M, Nosotti M, Spaggiari L, Thomas PA, Rendina E, Venuta F, Anile M, Schützner J, Rocco G, Asamura H, Crowley J, Detterbeck F, Falkson C, Filosso L, Giaccone G, Giroux D, Huang J, Kim J, Kondo K, Lucchi M, Marino M, Marom EM, Nicholson AG, Brompton R, Okumura M, Ruffini E, Van Schil P, Stratton K

The IASLC/ITMIG Thymic Epithelial Tumors Staging Project: proposal for an evidence-based stage classification system for the forthcoming (8th) edition of the TNM classification of malignant tumors.

J Thorac Oncol. 2014 Sep;9(9 Suppl 2):S65-72. doi: 10.1097/JTO.0000000000000290.

Ruffini E, Detterbeck F, Van Raemdonck D, Rocco G, Thomas P, Weder W, Brunelli A, Evangelista A, Venuta F; on behalf of the European Society of Thoracic Surgeons (ESTS) Thymic Working Group. Collaborators (35): Alkattan K, Arame A, Refai M, Casadio C, Carbognani P, Cerfolio R, Donati G, Foroulis CN, Gebitekin C, de Antonio DG, Kernstine KH, Keshavjee S, **Moser B**, Lequaglie C, Liberman M, Lim E, Nicholson AG, Lang-Lazdunski L, Mancuso M, Altorki N, Nosotti M, Novoa NM, Brioude G, Oliaro A, Filosso PL, Saita S, Scarci M, Schützner J, Terzi A, Toker A, Van Veer H, Anile M, Rendina E, Voltolini L, Zurek W.

Tumours of the thymus: a cohort study of prognostic factors from the European Society of Thoracic Surgeons database.

Eur J Cardiothorac Surg. 2014 Sep;46(3):361-8. doi: 10.1093/ejcts/ezt649. Epub 2014 Jan 30. PubMed PMID:24482389; PubMed Central PMCID: PMC4155438.

Ruffini E, Detterbeck F, Van Raemdonck D, Rocco G, Thomas P, Weder W, Brunelli A, Guerrera F, Keshavjee S, Altorki N, Schützner J, Arame A, Spaggiari L, Lim E, Toker A, Venuta F; European Society of Thoracic Surgeons Thymic Working Group. Collaborators (27): AlKattan K, Refai M, Casadio C, Carbognani P, Cerfolio R, Donati G, Gebitekin C, Gomez D, Kernstine KH, **Moser B**, Lequaglie C, Liberman M, Nicholson AG, Lang-Lazdunski L, Mancuso M, Nosotti M, Novoa NM, Brioude G, Filosso PL, Saita S, Scarci M, Terzi A, Van Veer H, Anile M, Rendina E, Voltolini L, Zurek W.
Thymic carcinoma: a cohort study of patients from the European society of thoracic surgeons database.
J Thorac Oncol. 2014 Apr;9(4):541-8. doi: 10.1097/JTO.0000000000000128. PMID:24736078

Detterbeck FC, Asamura H, Crowley J, Falkson C, Giaccone G, Giroux D, Huang J, Kim J, Kondo K, Lucchi M, Marino M, Marom EM, Nicholson A, Okumura M, Ruffini E, van Schil P, Stratton K; Staging and Prognostic Factors Committee; Members of the Advisory Boards; Participating Institutions of the Thymic Domain.
Collaborators (195): Goldstraw P, Rami-Porta R, Asamura H, Ball D, Beer D, Beyruti R, Bolejack V, Chansky K, Crowley J, Detterbeck F, Eberhardt W, Edwards J, Galateau-Sallé F, Giroux D, Gleeson F, Groome P, Huang J, Kennedy C, Kim J, Kim Y, Kingsbury L, Kondo H, Krasnik M, Kubota K, Lerut A, Lyons G, Marino M, Marom E, van Meerbeeck J, Mitchell A, Nakano T, Newman J, Nicholson A, Nowak A, Peake M, Rice T, Rosenzweig K, Ruffini E, Rusch V, van Schil P, Sculier JP, Stratton K, Suzuki K, Tachimori Y, Thomas CF Jr, Travis W, Tsao M, Turrisi A, Vansteenkiste J, Watanabe H, Wu YI, Zielinski M, Falkson C, Filosso L, Giaccone G, Kondo K, Lucchi M, Okumura M, Baas P, Erasmus J, Hasegawa S, Inai K, Kernstine K, Kindler H, Krug L, Nackaerts K, Pass H, Rice D, Blackstone E, Call Caja S, Ahmad U, Detterbeck F, Girard N, Haam SJ, Bae MK, Gomez DR, Marom E, van Schil P, Ströbel P, Marx A, Saita S, Wakelee H, Bertolaccini L, Vallieres E, Scott W, Su S, Park B, Marks J, Khella S, Shen R, Rosenberg M, Aires B, Rosenberg M, Tomulescu V, Huang J, Foroulis C, Lang-Lazdunski L, Billè A, Maessen JG, Keijzers M, van Veer H, Wright C, Marino M, Palmieri G, Buonerba C, Ferguson M, Marulli G, Lucchi M, Loehrer P, Kalkat M, Rohrberg K, Daugaard G, Toker A, Erus S, Kimmich M, Brunelli A, Refai M, Nicholson A, Lim E, Park IK, Wagner J, Tieu B, Fang W, Zhang J, Yu Z, Han Y, Li Y, Chen K, Chen G, Okumura M, Fujii Y, Asamura H, Nagai K, Nakajima J, Ikeda N, Haraguchi S, Onuki T, Suzuki K, Yoshino I, Tsuchida M, Takahashi S, Yokoi K, Hanyuda M, Niwa H, Date H, Maniwa Y, Miyoshi S, Kondo K, Iwasaki A, Okamoto T, Nagayasu T, Tanaka F, Suzuki M, Yoshida K, Okuma Y, Horio H, Matsumura A, Higashiyama M, Suehisa H, Onuki T, Sano Y, Kondo K, Kattan K, Cerfolio R, Gebitekin C, de Antonio D, Kernstine KH, Altorki N, Novoa N, Ruffini E, Filosso PL, Saita S, Scarci M, Voltolini L, Weder W, Zurek W, Arame A, Casadio C, Toracica C, Carbognani P, Donati G, Keshavjee S, Klepetko W, **Moser B**, Lequaglie C, Liberman M, Mancuso M, Nosotti M, Spaggiari L, Thomas PA, Rendina E, Venuta F, Anile M, Schützner J, Rocco G.
The IASLC/ITMIG thymic malignancies staging project: development of a stage classification for thymic malignancies.
J Thorac Oncol. 2013 Dec;8(12):1467-73. doi: 10.1097/JTO.0000000000000017. PMID:24389429

Bernhard Moser, Christine Bekos, Fritz Zimprich, Stefanie Nickl, Walter Klepetko, Jan Ankersmit.
The receptor for advanced glycation endproducts and its ligands in patients with myasthenia gravis.
Biochem Biophys Res Commun. 2012 Mar 30;420(1):96-101. PMID:22405771

Jan Ankersmit H, Nickl S, Hoeltl E, Toepker M, Lambers C, Mitterbauer A, Kortuem B, Zimmermann M, **Moser B**, Bekos C, Steinlechner B, Hofbauer H, Klepetko W, Schenk P, Dome B. Increased serum levels of HSP27 as a marker for incipient chronic obstructive pulmonary disease in young smokers.
Respiration. 2012;83(5):391-9. PMID:22469636

Ruffini E, Van Raemdonck D, Detterbeck F, Rocco G, Thomas P, Venuta F; European Society of Thoracic Surgeons Thymic Questionnaire Working Group.
Collaborators (41): Al Kattan K, Anile M, Beshay M, Boaron M, Bodner J, Camargo Jde J, Carbognani P, Cardillo G, Cassivi S, Cerfolio R, Curcio C, Eggeling S, Foroulis C, Fuentes M, Godinho MT, Gomez de Antonio D, Grodzki T, Jones DR, Kernstine K, Keshavjee S, Kubisa B, Lequaglie C, Leo F, **Moser B**, Nosotti M, Okumura M, Oliaro A, Paul MA, Refai M, Rzyman W, Saita S, Scarci M, Schutzner J, Spaggiari L, Tiffet O, Toker A, Trousse DS, Voltolini L, Yellin A, Zurek W, Weder W.
Management of thymic tumors: a survey of current practice among members of the European Society of Thoracic Surgeons.
J Thorac Oncol. 2011 Mar;6(3):614-23. PMID:21266921

Ankersmit HJ, Hoetzenecker K, Dietl W, Soleiman A, Horvat R, Wolfsberger M, Gerner C, Hacker S, Mildner M, **Moser B**, Lichtenauer M, Podesser BK.
Irradiated cultured apoptotic peripheral blood mononuclear cells regenerate infarcted myocardium.
Eur J Clin Invest. 2009 Jun;39(6):445-56. PMID: 19397690

Soleiman A, Lukschal A, Hacker S, Aumayr K, Hoetzenecker K, Lichtenauer M, **Moser B**, Untersmayr E, Horvat R, Ankersmit HJ.
Myocardial lipofuscin-laden lysosomes contain the apoptosis marker caspase-cleaved cytokeratin-18.
Eur J Clin Invest. 2008 Oct;38(10):708-12. PMID: 18837795

Chen Y, Akirav EM, Chen W, Henegariu O, **Moser B**, Desai D, Shen JM, Webster JC, Andrews RC, Mjalli AM, Rothlein R, Schmidt AM, Clynes R, Herold KC.
RAGE ligation affects T cell activation and controls T cell differentiation.
J Immunol. 2008 Sep 15;181(6):4272-8. PMID: 18768885

Bernhard Moser, Dharmesh D. Desai, Matthew Downey, Yali Chen, Kevan Herold, Ann-Marie Schmidt, and Raphael Clynes.
Receptor for advanced glycation endproducts expression on T cells contributes to antigen-specific cellular expansion in vivo.
J Immunol. 2007 Dec 15;179(12):8051-8. PMID: 18056345

Hetz H, Hoetzenecker K, Brunner M, Faybik P, Hacker S, **Moser B**, Roth G, Klinger M, Wolner E, Krenn C, Ankersmit HJ.
Caspase cleaved Cytokeratin 18 and 20S Proteasome in Liver Degeneration.
J Clin Lab Anal. 2007;21(5):277-81. PMID:17847110

Adlbrecht C, Hoetzenecker K, Posch M, Steiner S, Kopp C, Hacker S, Auer J, Horvarth R, **Moser B**, Roth G, Wolner E, Lange IM and Ankersmit HJ.
Elevated levels of interleukin-1beta-converting enzyme and caspase-cleaved cytokeratin-18 in acute myocardial infarction.
Eur J Clin Invest. 2007 May;37(5):372-80. PMID: 17461983

Moser B, Szabolcs MJ, Ankersmit HJ, Lu Y, Qu W, Weinberg A, Herold KC, and Schmidt AM.
Blockade of RAGE Suppresses Alloimmune Reactions In Vitro and Delays Allograft Rejection in Murine Heart Transplantation.
Am J Transplant. 2007 Feb; 7(2): 293-302. PMID: 17241110

Roth GA**, Moser B**, Huang SJ, Brandt JS, Huang Y, Papapanou PN, Schmidt AM, Lalla E.
Infection with a periodontal pathogen induces procoagulant effects in human aortic endothelial cells.
J Thromb Haemost. 2006 Oct;4(10):2256-61. PMID: 16856978

Szerafin T, Horvath A, **Moser B**, Hacker S, Hoetzenecker K, Steinlechner B, Brunner M, Roth G, Boltz-Nitulescu G, Peterffy A, Wolner E, Ankersmit HJ.
Apoptosis-specific activation markers in on- versus off-pump coronary artery bypass graft (CABG) patients.
Clin Lab. 2006;52(5-6):255-61. PMID: 16812952

Roth GA**, Moser B**, Roth-Walter F, Giacona MB, Harja E, Papapanou PN, Schmidt AM, Lalla E.
Infection with a periodontal pathogen increases mononuclear cell adhesion to human aortic endothelial cells.
Atherosclerosis. 2007 Feb;190(2):271-81. Epub 2006 Apr 18. PMID:16620832

Szerafin T, Brunner M, Horvath A, Szentgyorgyi L, **Moser B**, Boltz-Nitulescu G, Peterffy A, Hoetzenecker K, Steinlechner B, Wolner E, Ankersmit HJ.
Soluble ST2 protein in cardiac surgery: a possible negative feedback loop to prevent uncontrolled inflammatory reactions.
Clin Lab. 2005;51(11-12):657-63. PMID: 16329625

Roth GA, **Moser B**, Krenn C, Roth-Walter F, Hetz H, Richter S, Brunner M, Jensen-Jarolim E, Wolner E, Hoetzenecker K, Boltz-Nitulescu G, Ankersmit HJ.
Heightened levels of circulating 20S proteasome in critically ill patients.
Eur J Clin Invest. 2005 Jun; 35(6):399-403. PMID: 15948901

Konakci KZ, Bohle B, Blumer R, Hoetzenecker W, Roth G, **Moser B**, Boltz-Nitulescu G, Gorlitzer M, Klepetko W, Wolner E and Ankersmit HJ.

Alpha-Gal on bioprostheses: xenograft immune response in cardiac surgery.
Eur J Clin Invest. 2005 Jan; 35(1):17–23. PMID: 15638815

Ramskogler K, Brunner M, Hertling I, Dvorak A, Kapusta N, Krenn C, **Moser B**, Roth G, Lesch OM, Ankersmit HJ, Walter H.
CDT Values Are Not Influenced by Epithelial Cell Apoptosis in Chronic Alcoholic Patients-Preliminary Results.
Alcohol Clin Exp Res. 2004 Sep; 28(9):1396-1398. PMID: 15365311

Roth GA, Krenn C, Brunner M, **Moser B**, Ploder M, Spittler A, Pelinka L, Sautner T, Wolner E, Boltz-Nitulescu G, Ankersmit HJ.
Elevated serum levels of epithelial cell apoptosis-specific cytokeratin 18 neoepitope m30 in critically ill patients.
Shock. 2004 Sep; 22(3):218-20. PMID: 15316390

Brunner M, Krenn C, Roth G, **Moser B**, Dworschak M, Jensen-Jarolim E, Spittler A, Sautner T, Bonaros N, Wolner E, Boltz-Nitulescu G, Ankersmit HJ.
Increased levels of soluble ST2 protein and IgG1 production in patients with sepsis and trauma.
Intensive Care Med. 2004 Jul; 30(7):1468-73. Epub 2004 Feb 28. PMID: 14991091

Ankersmit HJ, Weber T, Auer J, Roth G, Brunner M, Kvas E, **Moser B**, Spreitzer S, Lassnig E, Maurer E, Hartl P, Wolner E, Boltz-Nitulescu G, Eber B.
Increased serum concentrations of soluble CD95/Fas and caspase 1/ICE in patients with acute angina.
Heart. 2004 Feb; 90(2):151-4. PMID: 14729783

Moser B*, Roth G*, Krenn C, Brunner M, Haisjackl M, Almer G, Gerlitz S, Wolner E, Boltz-Nitulescu G, Ankersmit HJ.
Susceptibility to programmed cell death in T-lymphocytes from septic patients: a mechanism for lymphopenia and Th2 predominance.
Biochem Biophys Res Commun. 2003 Sep 5; 308(4):840-6. PMID: 12927795

Moser B, Roth G, Brunner M, Lilaj T, Deicher R, Wolner E, Kovarik J, Boltz-Nitulescu G, Vychytil A, Ankersmit HJ.
Aberrant T cell activation and heightened apoptotic turnover in end-stage renal failure patients: a comparative evaluation between non-dialysis, haemodialysis, and peritoneal dialysis.
Biochem Biophys Res Commun. 2003 Aug 29; 308(3):581-5. PMID: 12914790

Ankersmit HJ, Roth GA, **Moser B**, Zuckermann A, Brunner M, Rosin C, Buchta C, Bilek E, Schmid W, Jensen-Jarolin E, Wolner E, Boltz-Nitulescu G, Volf I.
CD32 mediated platelet aggregation in Vitro by Anti-thymocyte Globulin: Implication of Therapy Induced In Vivo Thrombocytopenia.
Am J Transplant. 2003 Jun; 3(6):754-9. PMID: 12780568

Wendt TM, Tanji N, Guo J, Kislinger TR, Qu W, Lu Y, Bucciarelli LG, Rong LL, **Moser B**, Markowitz GS, Stein G, Bierhaus A, Liliensiek B, Arnold B, Nawroth PP, Stern DM, D'Agati VD, Schmidt AM.
RAGE Drives the Development of Glomerulosclerosis and Implicates Podocyte Activation in the Pathogenesis of Diabetic Nephropathy.
Am J Pathol. 2003 Apr; 162(4):1123-37. PMID: 12651605

Bucciarelli LG, Wendt T, Qu W, Lu Y, Lalla E, Rong LL, Goova MT, **Moser B**, Kislinger T, Lee DC, Kashyap Y, Stern DM, Schmidt AM.
RAGE blockade stabilizes established atherosclerosis in diabetic apolipoprotein E-null mice.
Circulation. 2002 Nov 26; 106(22):2827-35. PMID: 12451010

Hofmann MA, Drury S, Hudson BI, Gleason MR, Qu W, Lu Y, Lalla E, Chitnis S, Monteiro J, Stickland MH, Bucciarelli LG, **Moser B**, Moxley G, Itescu S, Grant PJ, Gregersen PK, Stern DM, Schmidt AM.
RAGE and arthritis: The G82S polymorphism amplifies the inflammatory response.
Genes Immun. 2002 May;3(3):123-35. PMID: 12070776

Moser B*, Ankersmit HJ*, Zuckermann A, Roth G, Taghavi S, Brunner M, Wolner E, Boltz-Nitulescu G.
Activation-induced T cell death, and aberrant T cell activation via TNFR1 and CD95-CD95 ligand pathway in stable cardiac transplant recipients.
Clin Exp Immunol. 2002 Apr;128(1):175-80. PMID: 11982606

Ankersmit HJ, Wieselthaler G, **Moser B**, Gerlitz S, Roth G, Boltz-Nitulescu G, Wolner E.
Transitory immunologic response after implantation of the DeBakey VAD continuous-axial-flow pump.
J Thorac Cardiovasc Surg. 2002 Mar; 123(3):557-61. PMID: 11882831

Ankersmit HJ, **Moser B**, Roedler S, Teufel I, Zuckermann A, Roth G, Lietz K, Back C, Gerlitz S, Wolner E, Boltz-Nitulescu G.
Death-inducing receptors and apoptotic changes in lymphocytes of patients with heart transplant vasculopathy.
Clin Exp Immunol. 2002 Jan; 127(1):183-9. PMID: 11882051

Ankersmit HJ, **Moser B**, Hoffman M, Kocher AA, Schlechta B, Boltz-Nitulescu G, Wolner E.
Aberrant T-cell activation via CD95 and apoptosis in peripheral T lymphocytes in stable heart transplant recipients.
Transplant Proc. 2001 Aug; 33(5):2860-1. PMID: 11498190

Ankersmit HJ, Deicher R, **Moser B**, Teufel I, Roth G, Gerlitz S, Itescu S, Wolner E, Boltz-Nitulescu G, Kovarik J.
Impaired T cell proliferation, increased soluble death-inducing receptors and activation-induced T cell death in patients undergoing haemodialysis.
Clin Exp Immunol. 2001 Jul; 125(1):142-8. PMID: 11472437

DOI:10.14753/SE.2020.2356

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DOI:10.14753/SE.2020.2356

14. Acknowledgements

I want to thank my parents Theresia and Josef, sister Julia and my girl friend Cecilia for their love and support.

I am grateful to Hendrik Jan Ankersmit for the ongoing scientific discussion and funding of experimental studies.

I want to thank Ana-Iris Schiefer and Leonhard Müllauer from the Clinical Institute of Pathology, Medical University of Vienna (Austria) for invaluable support and expertise on thymic pathology.

I also want to thank all my colleagues at the Division of Thoracic Surgery, Medical University Vienna, particularly Walter Klepetko (head of division), György Lang, Shahrokh Taghavi for their continued clinical and scientific support.

I am grateful to Balazs Döme to serve a my PhD supervisor and for scientific discussion.

Also I want to thank all current and former students that are writing or wrote their diploma thesis on thymic diseases under my supervision: Margit Scharitzer, Christine Bekos, Stefan Janik and Jürgen Thanner.

I want to thank Enrico Ruffini (head of the ESTS Thymic Working Group) his continued support and in particular all international contributors to the herein described project on TETs with pleural involvement.