

# IDENTIFICATION OF PROGNOSTIC AND DIAGNOSTIC MARKERS IN THYMIC EPITHELIAL TUMORS

PhD thesis

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# 1. Introduction

Thymic epithelial tumors (TETs) are an orphan disease with a reported incidence of 3.2/1,000,000 (de Jong, Blaauwgeers et al. 2008). Two distinct forms of TETs can be distinguished: thymomas and thymic carcinomas (TC). To date no risk factors for the development of TETs have been discovered. 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 (TC) (J.). There is a strong association between thymomas and autoimmune diseases, particularly myasthenia gravis. TETs can present as an incidental finding or due to symptoms from local compression of (thoracic) organs or systemic symptoms from TET associated paraneoplastic diseases. TETs 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.

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).

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). 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).

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. Depending on the disease distribution several surgical techniques were developed for resection: extrapleural pneumonectomy (EPP), total pleurectomy (TP) or local pleurectomy (LP).

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 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)

The most widely accepted prognostic factors for the treatment of patients with TETs are completeness of resection, Masaoka–Koga stage and WHO histological type (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).

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.

CRP is a pentraxin family member with pentameric structure (Volanakis 2001, 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).

Fibrinogen is a heterodimeric molecule consisting of two parts with different polypeptide chains: A $\alpha$ , B $\beta$ ,  $\gamma$  linked by disulphide bridges. 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).

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 interaction of neutrophils with tumor cells (Mollinedo 2019).

Lymphocytes can exert tumor-suppressive or tumor-promoting effects (Grivennikov, Greten et al. 2010).

Platelets have a role in the tumor microenvironment.

## 2. Objectives

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. 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.

- **Pathological prognostic information on TETs that can be drawn from institutional experience**

The *aim* of the initial part of this PhD thesis study was to determine the quality of care at the institutional thoracic surgery Division (a single European thoracic surgery center) 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* 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.

- **Prognostic factors for TETs in the special situation of pleural involvement: a multi-institutional 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.

- **C-reactive protein 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.

- **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.

### **3. Methods**

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

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. Patients undergoing thoracic surgery for TETs during the indicated time period at the thoracic surgery division were included in the study. A total of 79 patients were identified. Disease and treatment-specific documentation as well as follow-up information was complete for 72 patients.

The Masaoka-Koga staging system was used as the standard to document tumor stage. Pathologists employed the WHO histological classification system and recurrences were reported as recommended by ITMIG. 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.

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

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 retro-

spective data sets. Ten European and two Canadian institutions provided retrospective data from 152 patients of thymomas and TCs with pleural disease.

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 1). The calculated median follow-up time of the entire patient cohort was 52 months [95% confidence interval: 32.0–72.0].

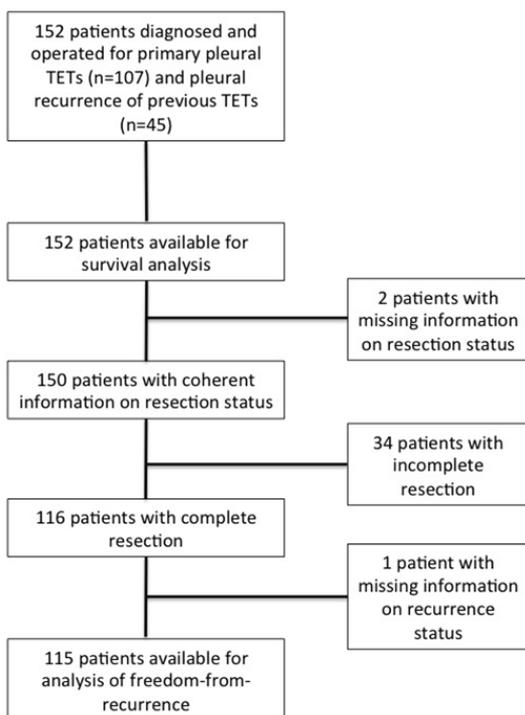


Figure 1: Flow chart illustrating the assignment of patients to different endpoints.

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%]).

- **Evaluation of CRP as a prognostic marker for TETs**

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. CRP serum concentrations were measured using a latex-enhanced immunoturbidimetric assay (Roche, Mannheim, Germany). Measurements were performed at the institutional department of laboratory medicine of the Medical University of Vienna. 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. One-hundred and twenty-eight patients with TETs were available for CRP analysis. For 68 patients pre- and postoperative CRP serum concentrations were available (recurrences: 16; no recurrence: 52 patients). CRP serum concentrations of 64 healthy volunteers matched for sex- and age served as controls. In addition we performed immunohistochemical staining on specimens of TETs with monoclonal rabbit anti-human CRP antibody (Clone Y284, Abcam, Cambridge, UK).

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

We analyzed 122 patients 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  $56.5 \pm 16.1$  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. Fifty-four percent of patients underwent multimodal therapy including surgery. Oncologic follow-up was routinely performed according to recommendations of the European Society of Thoracic Surgery (ESTS) (Ruffini, Deterbeck et al. 2014): chest CT scans every 3 to 6 months for the first three years after surgery, followed by annual CT scans.

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. 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 ( $54.6 \pm 1.4$  years) volunteers served as controls. Additionally, immunohistochemistry with monoclonal mouse anti-human CD45 (LCA; 2B11& PD7/26; Cell Marque, California, USA,) polyclonal rabbit anti-human Fibrinogen/FITC (Dako, Denmark) was performed.

## 4. Results

### Pathological predictors of outcome

- **Single center analysis: survival is dependent upon tumor stage and resection status but not histology**

This study includes 72 patients undergoing 84 surgeries (including surgery for recurrences). The mean patient age at surgery was 58.2 years. There was no perioperative mortality. The median follow-up time calculated from OS data was 47.2 months (OS and CSS is depicted in Figure 2A+B). There was decreased OS survival with more advanced disease as categorized with Masaoka-Koga stage (log rank test:  $p=0.017$ , Figure 2C). 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 2D; log-rank test:  $p<0.001$ ). WHO histological subtypes A, AB, B1, B2, B3 and TC did not display differences in survival (log rank test:  $p=0.136$ ). Survival was significantly better in patients without recurrences or progressions (log-rank test:  $p<0.001$ ; Figure 2E).

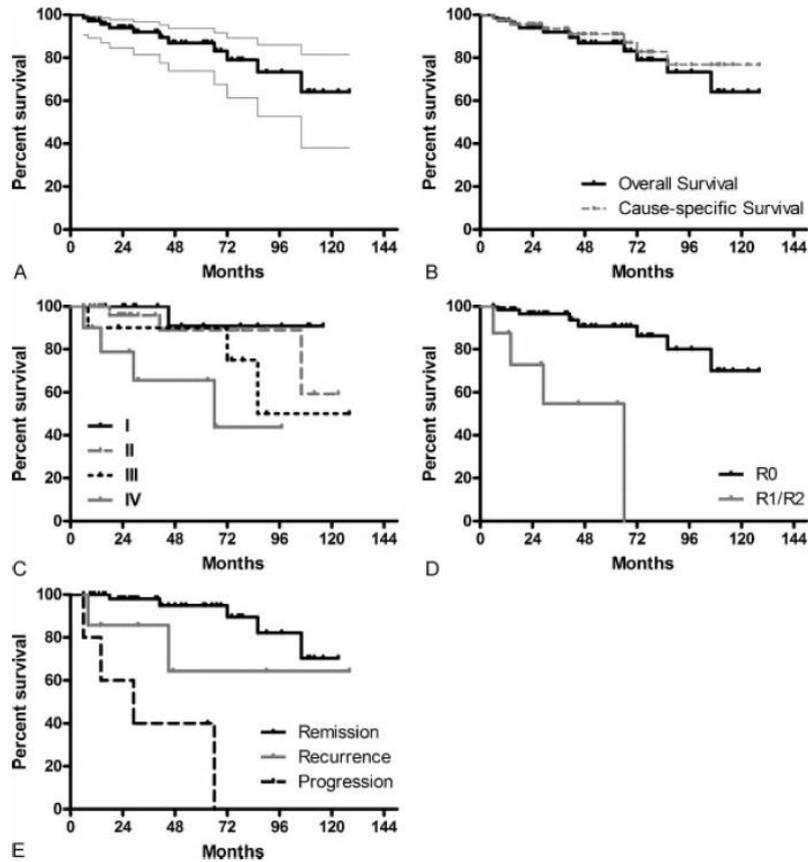


Figure 2: Overall and Cause-specific Survival in patients with TETs. OS is displayed by Kaplan–Meier curve with 95% confidence intervals (A). An overlay of OS and CSS (B), survival according to Masaoka–Koga stage (C), survival and residual tumor status (D) are shown. A comparison of survival of patients in remission or with recurrent or progressive disease is displayed (E).

Eighty-nine percent 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 3A). 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 3B).

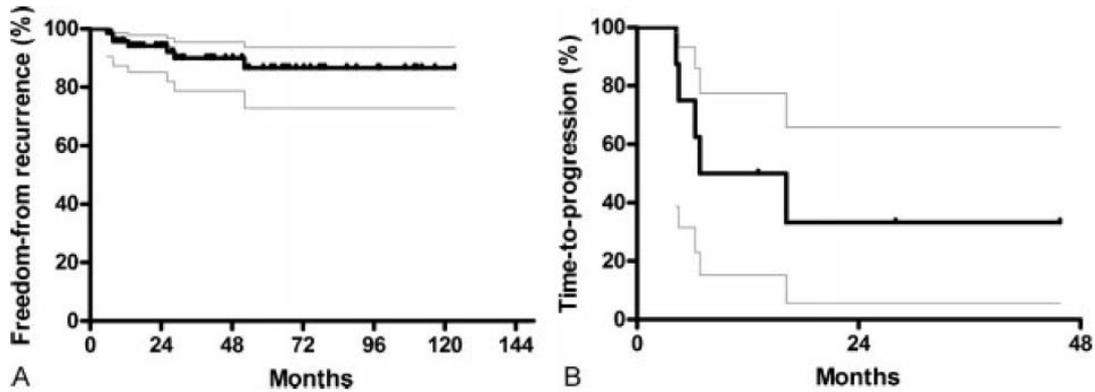


Figure 3: Outcome measures for recurrence and progression. Freedom-from recurrence (A) and time-to-progression (B) in patients undergoing thoracic surgery for TETs (including patients with multimodality treatments). Kaplan–Meier curves with 95% confidence intervals are displayed.

Stage and recurrence or progression were significantly correlated (Pearson  $\chi^2$  test:  $p=0.001$ ). 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. The frequency of surgical incisions applied was: sternotomy (45.8%), thoracotomy (23.6%), or hemiclamshell incision (13.9%). The presence or absence of MG was not a relevant factor for survival analysis (log-rank test:  $p=0.235$ ).

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

Pleural involvement resulted from thymomas in 89% and 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.

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 4 for the respective Kaplan-Meier curves.

At 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;  $p=0.040$ ]. Analysis all patients with [pseudo-]neo- and [pseudo-

adjuvant therapy combined (n=126) vs surgery alone (n=26) revealed no statistically significant differences.

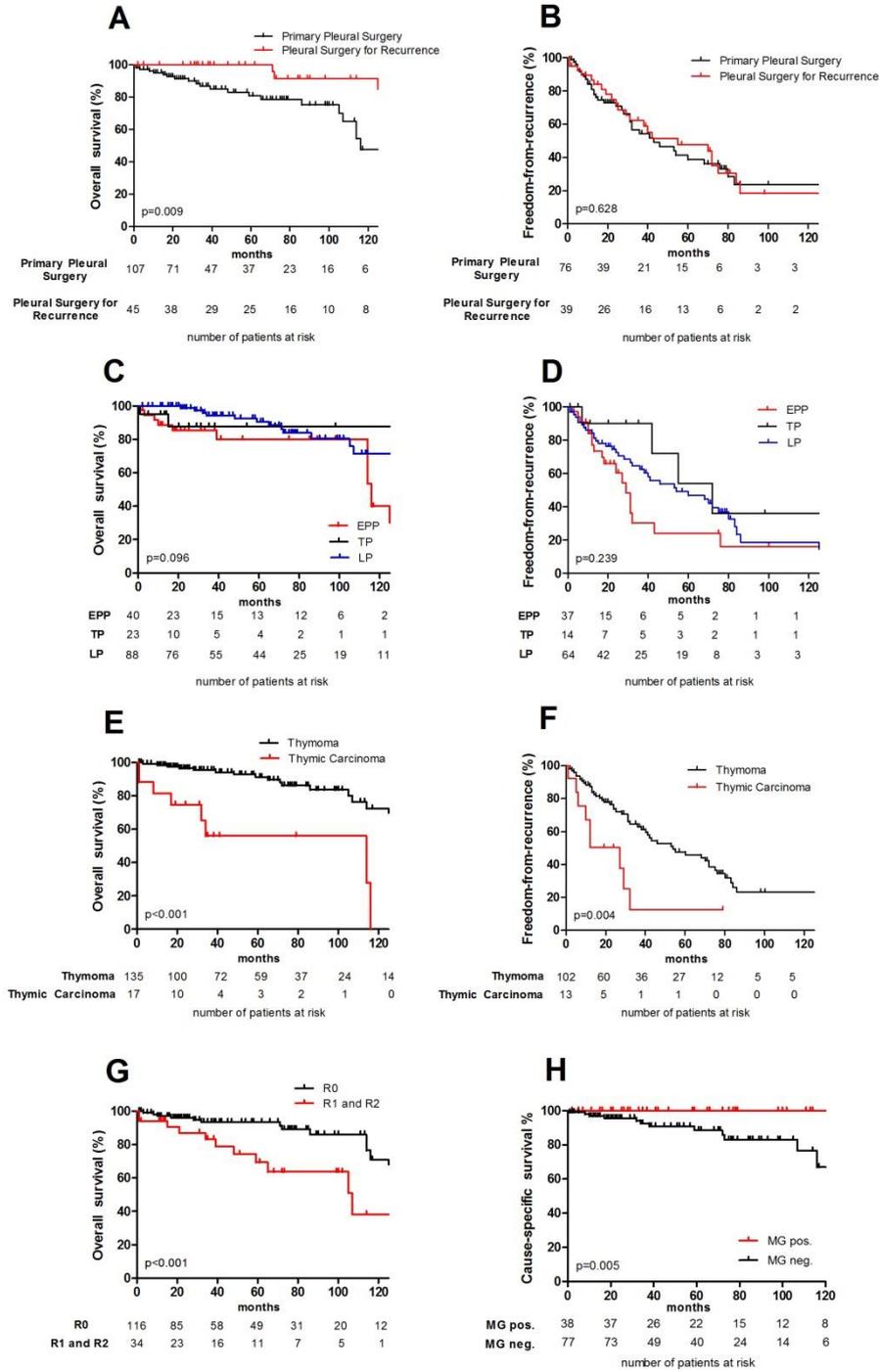


Figure 4: Survival analysis for prognostic factors. Comparison of primary pleural surgery and pleural surgery for recurrence OS (A), FFR (B), type of surgery: EPP vs TP vs LP: OS (C), FFR (D), thymoma vs TC: OS (E), FFR (F), and completeness of resection: OS (G), FFR (H).



## Serum und cellular biomarkers to predict outcome

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

Patients were dichotomized into low and high pretreatment 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. 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 5).

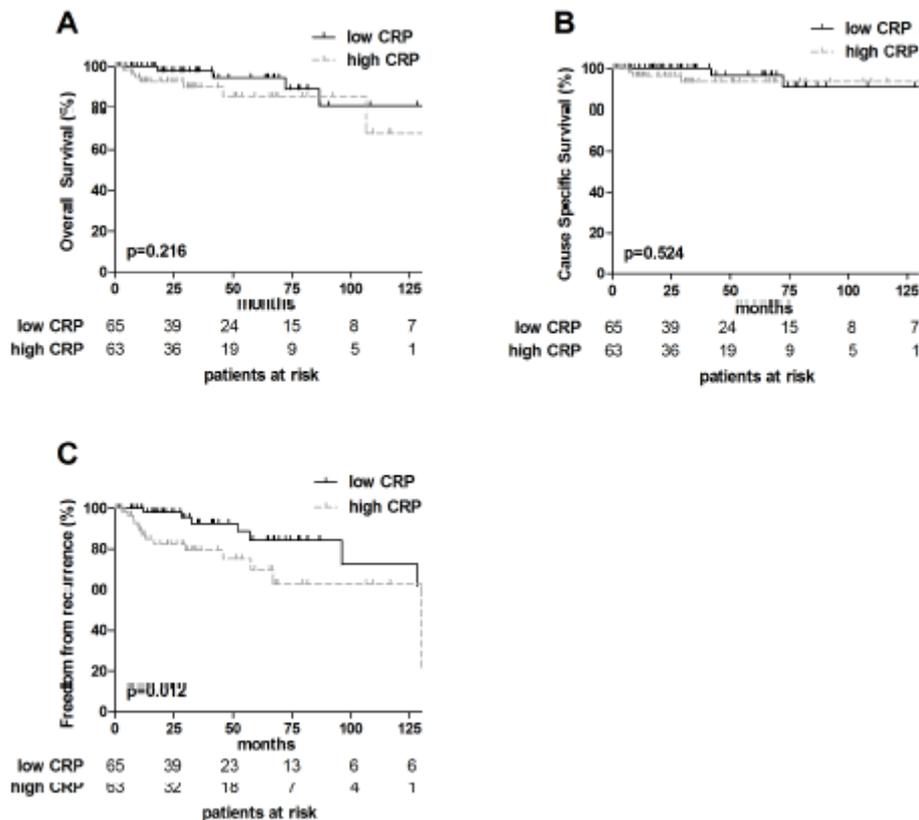


Figure 5: Prognostic impact of CRP in TETs. Overall Survival, Cause Specific Survival and Freedom From Recurrence are shown (median CRP cut off value of 0.22 mg/dL) (A–C). P-value (Log-rank test).

At univariable analysis the 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.

At multivariable analysis, the 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.

Patients with TETs showed significantly higher pretreatment CRP serum concentrations compared to sex- and age-matched controls (TETs  $1.03 \pm 0.3$  mg/dL vs. controls  $0.16 \pm 0.03$  mg/dL;  $p < 0.001$ ). Thymomas  $0.62 \pm 0.21$  mg/dL, TCs  $2.33 \pm 0.7$  mg/dL and TNETs  $0.90 \pm 0.44$  mg/dL compared to controls showed significantly different CRP serum concentrations (one-way ANOVA:  $p < 0.001$ ).

Longitudinal analysis of pre- and postoperative CRP serum concentrations was performed in a subset of 68 patients. 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  $0.57 \pm 0.21$  mg/dL preoperatively to  $0.26 \pm 0.03$  mg/dL postoperatively ( $p=0.135$ ). In case of tumor recurrence CRP serum concentrations increased significantly ( $4.72 \pm 1.61$  mg/dL;  $p=0.001$ ).

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.

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

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

High fibrinogen plasma concentrations were associated with significantly worse FFR and CSS (Figure 6A and B). High NLRs were associated with significantly worse FFR and patients with high PLR with significantly worse CSS (Figure 6C-F).

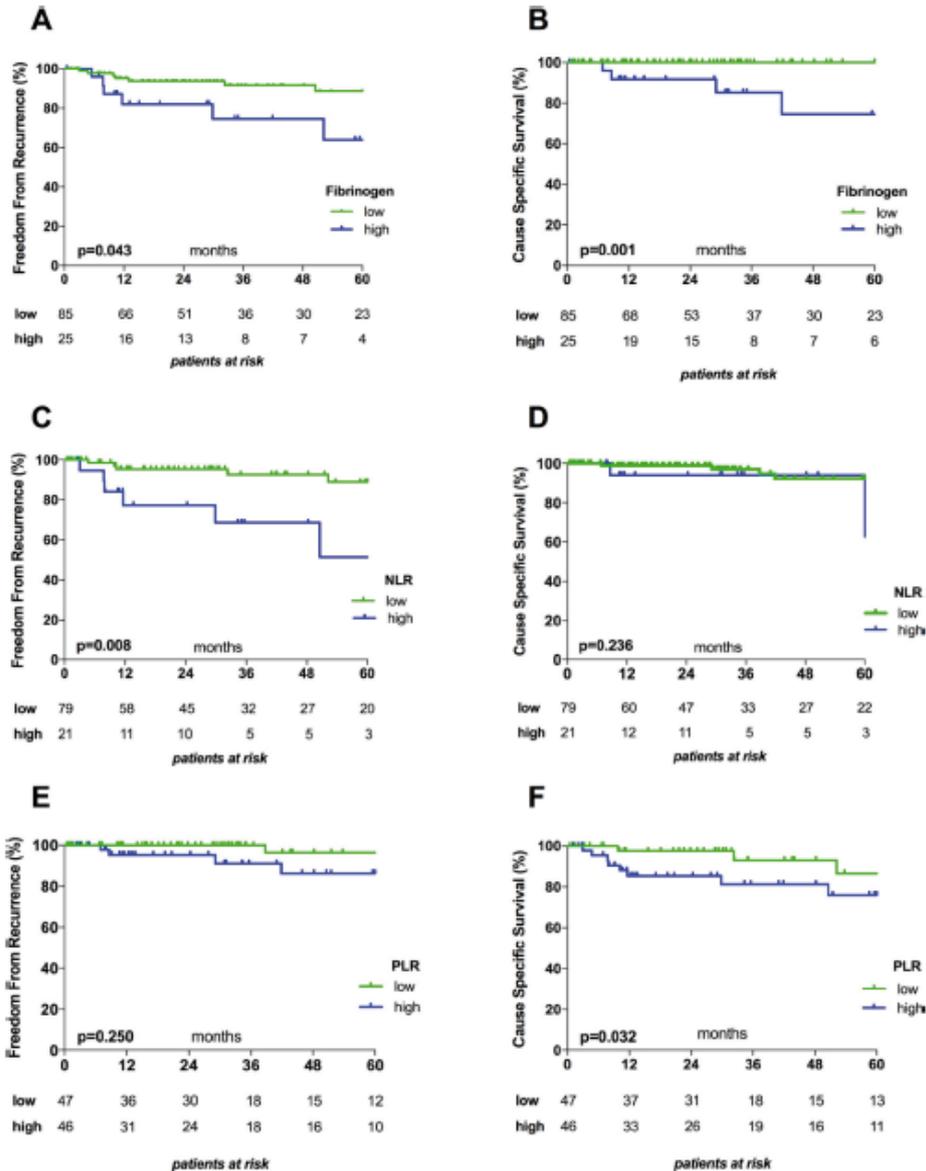


Figure 6: Kaplan–Meier survival in relation to fibrinogen plasma concentrations, NLR and PLR. Graphs show the associations between Fibrinogen and FFR (A) and CSS (B); between NLR and FFR (C) and CSS (D), and between PLR and FFR (E) and CSS (F). The cut-off values used to dichotomize patients into low and high subgroups were 452.5 mg/dL for Fibrinogen, 4.0 for NLR, and 136.5 for PLR.

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.

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). 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.

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.

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

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

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

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

with TCs ( $207.8 \pm 29.3$  vs.  $168.2 \pm 37.0$ ;  $p=0.546$ ). During oncological follow up (6-12 months) the highest PLR for thymomas ( $212.5 \pm 24.8$ ) and TCs ( $347.1 \pm 74.0$ ) was detected.

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.

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).

Plasma concentrations of fibrinogen were not significantly altered in patients with recurrence compared to those without ( $p=0.351$ ).

NLR and PLR displayed high accuracy in predicting tumor recurrences (ROC analysis): 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 ( $p = 0.043$ ;  $R^2: 0.378$ ), but not PLR ( $p = 0.078$ ;  $R^2: 0.165$ ) and Fibrinogen ( $p = 0.341$ ;  $R^2: 0.054$ ).

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.

## 5. 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. In patients with TETs of all stages completeness of resection and stage were significant pathological prognostic factors. 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 pretreatment 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 initiate prospective studies with larger number of patients to obtain higher levels of evidence for diagnosis, treatment and prognosis of patients with TETs.

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## 6.1 Publications related to the thesis

**Moser B**, Scharitzer M, Hacker S, Ankersmit HJ, Lang G, Aigner C, Taghavi S, and Klepetko W. 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

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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.

Janik S, Bekos C, Hacker P, Raunegger T, Ghanim B, Einwallner E, Klepetko W, Müllauer L, Ankersmit HJ and **Moser B**. 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.