Diagnostic and prognostic relevance of inflammatory and immune-related markers in thymic epithelial tumors

PhD thesis

Evelyn Megyesfalvi, MD

Rácz Károly Conservative Medicine Division

Doctoral College of Semmelweis University

Supervisor: Balázs Döme, MD, D.Sc.

Official reviewers: László Piros, MD, Ph.D.
Zsuzsanna Csoma, MD, Ph.D.

Head of the Complex Examination Committee:
György Losonczy MD, D.Sc.

Members of the Complex Examination Committee:
Balázs Antus, MD, D.Sc.
Gábor Horváth, MD, Ph.D.

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1. Introduction

Thymic epithelial tumors (TETs) are rare neoplasms arising from the epithelial cells of the thymus and represent the most common anterior mediastinal malignancies in adults. Long-term follow-up is particularly important in these patients due to the potential for late recurrence. Recurrences may occur many years after complete resection, especially in invasive thymomas. Surveillance strategies in TETs are primarily based on the WHO histological classification and on disease stage, yet patients with similar clinicopathological characteristics may still experience widely different clinical courses, recurrence rates, and survival outcomes. Therefore, there is an increasing need for complementary prognostic biomarkers that may support more accurate risk stratification and individualized follow-up strategies. TETs are characterized by a unique tumor immune microenvironment (TIME) because they arise within an organ that is deeply involved in immune regulation and T-cell development. In this regard, a detailed understanding of the immune landscape of thymomas and thymic carcinomas may contribute to the development of individualized follow-up strategies and offer additional insights into novel biomarker-driven immunotherapeutic approaches in TETs.

2. Objectives

Despite recent advances in the histopathological and molecular characterization of TETs, reliable biomarkers for accurate risk stratification and individualized follow-up remain limited. The WHO classification and currently used staging methods provide important prognostic information, yet the biological complexity of TETs contributes to widely different clinical outcomes and survival, even among patients with the same stage or histological subtype. Therefore, complementary prognostic markers are needed to aid patient stratification and determine the most appropriate follow-up strategies. Given the unique immunological role of the thymus, the TIME and systemic inflammatory alterations might influence treatment response, disease progression, and recurrence. Yet, the clinicopathological and prognostic relevance of immune-related markers has not been comprehensively evaluated so far in thymoma and thymic carcinoma.

Systemic inflammatory markers such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), C-reactive protein (CRP), and fibrinogen have already shown prognostic relevance in various thoracic malignancies. However, studies investigating their role in TETs yielded conflicting results, mainly because of the relatively small

number of included patients and the high degree of biological heterogeneity of these tumors. Therefore, within the framework of an international multicenter study, we first aimed to evaluate the diagnostic and prognostic relevance of blood-based inflammatory markers in a large international cohort of surgically treated TETs, with particular focus on their association with histological subtype, disease stage, and survival outcomes.

Next, taking into account the central role of the thymus in immune regulation and self-tolerance, we aimed to characterize the immune landscape of TETs at the tissue level. Accordingly, we investigated the epithelial and lymphocytic expression patterns and the clinical relevance of five immune-related markers with potential biological and therapeutic relevance (TIM3, OX40L, GTF2I, XPO1, and GITR) in surgically resected thymomas and thymic carcinomas. Besides characterizing their association with clinicopathological parameters, MG, and survival outcomes, we also evaluated whether combined immune marker expression patterns could define distinct prognostic subsets among patients with TETs.

3. Methods

Within the framework of our first study, we included histologically confirmed TET patients who underwent surgical resection at four European thoracic oncology centers: the National Koranyi Institute of Pulmonology and National Institute of Oncology, Budapest, Hungary (cohort #1, n=329); the Medical University of Vienna, Vienna, Austria (cohort #2, n=162); and the Ruhrlandklinik – University Duisburg-Essen, Essen, Germany (cohort #3, n=252). All patients underwent open or VATS thymectomy between January 1999 and December 2021. Clinical and follow-up data were collected from the available medical records and/or records from the Central Statistical Office of the given country. Laboratory parameters were recorded from the most recent preoperative blood cell count prior to surgery. Inflammatory ratios, including PLR and NLR, were calculated by dividing the absolute platelet and neutrophil counts by the lymphocyte count. Since specific comorbidities might significantly impact the absolute value of inflammatory laboratory parameters, patients with acute or chronic inflammatory diseases such as rheumatoid arthritis were excluded from the study. Given the multicenter nature of the study, certain laboratory parameters were determined by different methods and/or equipment. Thus, in order to obtain

comparable values across the participating centers, it was essential to rescale and harmonize the measured variables. For a given laboratory parameter p, with normal reference range $(N_{p,c}^{min}, N_{p,c}^{max})$ in patient cohort treated at center c, the harmonized $\bar{M}_{p,i}$ values were calculated with the following formula, given the originally measured value of $M_{p,i}$ for patient i:

$$\bar{M}_{p,i} = \frac{M_{p,i} - N_{p,i\in c}^{min}}{N_{p,i\in c}^{max} - N_{p,i\in c}^{min}} \cdot \frac{1}{n_p}\left(\sum_{c=1}^{n_p} N_{p,c}^{max} - \sum_{c=1}^{n_p} N_{p,c}^{min}\right) + \frac{1}{n_p}\sum_{c=1}^{n_p} N_{p,c}^{min},$$

where n_p is the number of treatment centers with available data for laboratory parameter p. Essentially, values were rescaled to harmonized reference ranges defined by the mean reference range of various treatment centers. For example, platelet count reference range for cohorts #1, #2, and #3 were 140-350 g/l, 150-350 g/l, and 180-380 g/l, respectively. In this case, the calculated harmonized reference range was 156.67-360 g/l.

In our second study, we investigated a representative subset of cohort #1 consisting of patients with histologically confirmed TET who underwent surgical resection (open or VATS thymectomy) at the National Koranyi Institute of Pulmonology

(Budapest, Hungary) between January 2005 and December 2020. Clinicopathological data (age at diagnosis, sex, comorbidities, paraneoplastic syndromes, and TET histological subtype) were retrospectively collected from medical records. Survival data were obtained from the National Health Insurance Office and the Central Statistical Office of Hungary. Only individuals with a sufficient amount of formalin-fixed paraffin-embedded (FFPE) tumor tissue were included. Surgically resected representative TET tissue samples were examined for the expression of five immune-related markers: TIM3, OX40L, GTF2I, XPO1, and GITR. Immunohistochemistry (IHC) staining was performed according to the recommended protocols on the Ventana BenchMark Ultra IHC/ISH System (Roche Diagnostics, Basel, Switzerland). IHC-labeled and H&E-stained slides were digitally scanned using PANNORAMIC 250 Flash III (3DHISTECH Ltd., Budapest, Hungary) and sections were evaluated with CaseViewer 2.4 (3DHISTECH Ltd., Budapest, Hungary). All H&E-stained slides were first evaluated to confirm the diagnosis of TET. Next, the IHC expression of the given marker was examined blinded to clinical data, by two independent, board-certified pathologists specializing in thoracic malignancies. If their results differed by more than 20%, a third pathologist was also

involved in the final scoring. The epithelial and lymphocytic expression of each marker was evaluated separately for the two TET components by determining the percentage of corresponding cells showing positive staining in at least 20 randomly selected areas at 20× and 40× magnification. To distinguish between epithelial and lymphocytic compartments, reference slides stained with H&E, TdT, and p63 were used and evaluated side by side with the investigated markers in accordance with routine diagnostic practice.

Statistical analyses for our first and second study were performed in R (R Foundation for Statistical Computing, Vienna, Austria) versions 4.2.1 and 4.4.1, respectively. Overall survival (OS) was defined as the elapsed time in months between surgery and the last available follow-up or death of any cause. Meanwhile, cause-specific survival (CSS) represented the interval between surgery and the last available follow-up or death from TET. The cause of death was determined by reviewing the patients' final death certificates. Individuals with unknown causes of death were not included in CSS-related measurements. Survival analyses were conducted using the Kaplan-Meier method with log-rank tests for univariate comparisons. Multivariate analysis was performed using a Cox

regression model (first study) and Fine-Gray competing risks model (second study).

Both studies were conducted in accordance with the guidelines of the Helsinki Declaration of the World Medical Association and with the approval of the national or local Ethics Committees of participating countries (approval numbers: Medical Research Council of Hungary – IV/5198-1/2021/EKU for cohort #1, The Ethic Committee of the Medical University of Vienna – EC#1053/2016 for cohort #2 and The Ethic Committee of the Medical Faculty of University Duisburg-Essen – 17-7775-BO for cohort #3). Data sharing, synchronization and pooled analysis were coordinated by the Medical University of Vienna (cooperation agreement number: 020622, DC-number: 2022-047). Due to the retrospective nature of the study, the requirement for written informed consent was waived. Tissue and data collection were approved in all institutions. After clinical information was collected, patient identifiers were removed, and subsequently, patients could not be identified either directly or indirectly.

4. Results

A total of 743 surgically treated TET patients were included in the first study, having a median age of 59 years (range: 15-86 years). All individuals had Caucasian background, and 391 patients (52.6%) were female. 19.5% of included patients had symptoms of myasthenia gravis (MG) at diagnosis. Elevated CRP levels were characteristic of increased tumor size. Accordingly, CRP was significantly higher in patients with T3 tumors (vs. T1 tumors, $p=0.035$) and the mean CRP levels were constantly rising with the tumor size. As for the WHO classification, inflammatory ratios were diagnostic for histological patterns. Namely, both NLR ($p=0.002$) and PLR ($p<0.001$) were significantly higher in thymic carcinoma than in type B thymoma (median values were 3.62 vs. 2.34 and 209.18 vs. 136.65 for NLR and PLR, respectively). Decreased lymphocyte levels were as well associated with thymic carcinoma ($p=0.037$ and $p<0.001$). Next, we assessed whether the investigated immune biomarkers are associated with the occurrence of MG among TET patients. Although the presence of MG had no statistically significant impact on the preoperative level of inflammatory markers after multiple correction testing, the average level of CRP, WBC, neutrophils, fibrinogen, NLR, and PLR was higher in MG patients (vs. those without MG). As

for the prognostic relevance of preoperative lab values, we found that high CRP (≥0.6 mg/dL), neutrophil (≥3.82 g/L), thrombocyte (≥322.38 g/L), fibrinogen (≥506.2 mg/dL), and TSH (≥ 1.72 mIU/L) levels were all suggestive of worse OS compared to those with low levels. In contrast, in our univariate models, high lymphocyte (≥ 1.98 g/L), RBC (≥4.85 g/L), Hgb (≥13.9 g/dL) and albumin (≥43 g/L) values were significantly associated with improved OS (vs. low levels). We could not gain prognostic information from the preoperative WBC and LDH levels. With regards to the inflammatory ratios, we found that high (≥3.76) NLR values were associated with impaired survival outcomes in surgically resected patients (versus low NLR; median OSs were 171 vs. 247 months, respectively, p=0.008). Importantly, TET patients with high (≥201.66) preoperative PLR levels also had significantly shorter OS (versus those with low PLR; median OSs were 171 vs. 247 months, respectively; p<0.001). In order to assess if the prognostic value of inflammatory markers was independent of other clinically relevant variables, we performed multivariate Cox-regression analyses. We found that high CRP and fibrinogen levels, along with high PLR values all remained significant negative prognosticators for OS (p=0.006, p=0.018 and p=0.002, respectively). Accordingly, hazard ratios associated with

increased (vs. low) CRP, fibrinogen and PLR were 1.77, 3.63 and 1.93, respectively. Despite its elevated hazard ratio, high NLR values did not influence the survival outcomes independently of other clinicopathological variables (p=0.113). Given that the cause of death was most likely not always TET-related in the current patient pool, we performed additional Cox regression analyses to assess the CSS. Importantly, both fibrinogen (p=0.009) and PLR (p=0.001) influenced CSS independently of other variables. In this context, the CSS-related hazard ratios of increased (vs. low) fibrinogen and PLR values were 7.09 and 2.51, respectively. CRP (p=0.150) and NLR (p=0.310) were not related to CSS in our multivariate models.

In our second study, we included 137 surgically treated TET patients, consisting of 119 thymoma (86.9%) and 18 thymic carcinoma (13.1%) cases, as classified by the WHO. Concerning immune marker expression differences between different TET compartments, we observed a statistically significant positive linear correlation between lymphocytic and epithelial expression of OX40L (p=0.036) and XPO1 (p=0.002), and a positive correlation with borderline significance for TIM3 (p=0.085). In contrast, we found no correlation between the lymphocytic and epithelial expression of GTF2I and GITR. Given that MG is an autoimmune disorder frequently observed in patients with TETs

and can significantly impact patient management and prognosis, we analyzed its association with immune marker expression. We found that, in general, both epithelial ($p=0.004$) and lymphocytic ($p=0.045$) expression of OX40L were statistically significantly higher in patients with MG compared to those without this autoimmune disorder. The median XPO1 expression was also higher in patients with MG (vs. non-MG patients), but due to the high variability in expression levels, this difference did not reach statistical significance. Across the TET subtypes, epithelial TIM3 expression was consistently moderate to high, with no significant differences overall or in pairwise comparisons between subtypes. Likewise, XPO1 and GTF2I were also highly expressed in the epithelial component, with ≥50% expression in 66 and 34 cases, respectively. In contrast, epithelial GITR expression was absent in all cases, except for one thymic carcinoma, which showed 10% positivity. With regard to lymphocytic expression of the examined markers, we found high XPO1 expression (≥50%) in 46% of cases, with no statistically significant differences between TET subtypes. Expression of other markers, including TIM3, OX40L, and GTF2I, was markedly lower in the lymphocytic component (average expression of 6%, 5%, 0%, respectively) than in the epithelial component.

To investigate whether combined immune marker expression patterns have prognostic relevance, we performed hierarchical clustering of patients and identified four distinct groups defined by the combined immune marker expression profiles. Cluster 1 was characterized by high epithelial GTF2I expression and low epithelial XPO1 expression. Cluster 2 showed low expression of both GTF2I and XPO1 in epithelial cells. Cluster 3 displayed high epithelial expression of both GTF2I and XPO1, accompanied by generally high epithelial TIM3 expression. Finally, Cluster 4 was defined by low epithelial GTF2I expression combined with high epithelial XPO1 expression. Importantly, univariate survival analysis revealed statistically significant differences in CSS across the four expression clusters ($p<0.0001$). Accordingly, patients in Clusters 1 and 3 had poorer outcomes compared with those in Clusters 2 and 4, consistent with the observation that low epithelial GTF2I expression is generally favorable, as previously suggested. Notably, Cluster 3 showed the worst survival, suggesting that the combined high epithelial expression of GTF2I, XPO1, and TIM3 confers a particularly adverse prognosis. To assess whether the prognostic value of the expression clusters was independent of other clinicopathological variables, we analyzed CSS survival by fitting a multivariate Fine-Gray competing risks model to the

data. The model was adjusted for factors including age, recurrence status, and TET subtype. We found that the expression clusters remained significant, independent predictors of CSS. Clusters 2 and 4 (both with low epithelial GTF2I expression) were associated with better CSS than Cluster 1, whereas Cluster 3 (with high epithelial GTF2I, XPO1, and TIM3 expression) had the poorest outcomes, with a hazard ratio of 6.37 (95% CI 1.27–31.96) compared with Cluster 1. Model concordance was 68.78%, indicating moderate to strong predictive power.

5. Conclusions

Our findings indicate that both systemic inflammatory alterations and tissue-level immune phenotypes contribute to the clinical heterogeneity of TETs. While the histological classification and staging systems remain the cornerstone of patient stratification in routine practice, assessment of blood- and tissue-based immune markers provides additional prognostic information that may support risk assessment. Importantly, these markers and immune phenotypes identify patient subsets with different clinical outcomes that are not fully captured by traditional clinicopathological parameters. The independent prognostic significance of PLR, fibrinogen, and distinct immune-related expression patterns further supports the concept that host immune responses and the tumor immune microenvironment play an important role in shaping disease behavior in thymoma and thymic carcinoma. In addition, the identified expression patterns of potentially targetable immune-related markers provide a framework for the development of patient selection criteria and the design of future immunotherapy trials involving TETs. To sum up, the findings of the presented studies contribute to improved patient stratification and follow-up, as well as to a more accurate risk assessment in these rare but highly heterogeneous thoracic malignancies.

6. Bibliography of the candidate's publications

(Cumulative impact factor: 42.3)

List of publications that served as a basis for the current thesis:

1. **Megyesfalvi, Evelyn**; Ghimessy, Aron ; Bauer, Jonas ; Pipek, Orsolya ; Saghi, Kevin ; Gellert, Aron ; Fillinger, Janos ; Okumus, Ozlem ; Teglas, Vivien ; Ganofszky, Erna et al. *Diagnostic and prognostic relevance of inflammatory markers in surgically treated thymic epithelial tumors: An international multicenter study.* LUNG CANCER 200 Paper: 108111 , 11 p. (2025). Impakt faktor: 4.4

2. **Megyesfalvi, Evelyn***; Teglas, Vivien* ; Ferencz, Bence ; Pipek, Orsolya ; Pozonec, Maria Dorothea ; Lippai, Zoltan ; Revi, Barnabas ; Nagy, Kristof ; Horvath, Lilla ; Ferenczy, Anita et al. *Immune-related markers define clinically relevant prognostic subgroups in thymic epithelial tumors.* LUNG CANCER 217 Paper: 109430 , 10 p. (2026). Impakt faktor: 4.4

Other publications:

3. Ferencz, Bence ; Török, Klára ; Pipek, Orsolya ; Fillinger, János ; Csende, Kristóf ; Lantos, András ; Černeková, Radoslava ; Mitták, Marcel ; Škarda, Jozef ; Delongová, Patricie ; **Megyesfalvi, Evelyn** et al. *Expression patterns of novel immunotherapy targets in intermediate- and high-grade lung neuroendocrine neoplasms.* CANCER IMMUNOLOGY IMMUNOTHERAPY 73 : 6 Paper: 114 , 15 p. (2024). Impakt faktor: 5.1

4. Török, Klára ; Ferencz, Bence ; Boettiger, Kristiina ; Pozonec, Maria Dorothea ; Pipek, Orsolya ; Bogos, Julianna ; Lantos, András ; Hegedűs, Zita ; Schelch, Karin ; Radeczky, Péter ; Bogos, Krisztina ; Teglas , Vivien ; **Megyesfalvi, Evelyn** et al. *Impact of KRAS mutation subtypes on morphological heterogeneity and immune landscape in surgically treated lung adenocarcinoma.* TRANSLATIONAL LUNG CANCER RESEARCH 14 : 6 pp. 1914-1928. 15 p (2025) Impakt faktor: 3.5

5. Csende, K ; Ferencz, B ; Boettiger, K ; Pozonec, M D ; Lantos, A ; Ferenczy, A ; Pipek, O ; Solta, A ; Ernhofer, B ; Laszlo, V ; **Megyesfalvi, E** et al. *Comparative profiling of*

surgically resected primary tumors and their lymph node metastases in small-cell lung cancer. ESMO OPEN 10 : 4 Paper: 104514 , 10 p. (2025) Impakt faktor: 8.3

6. Boettiger, Kristiina ; Kovács, Ildikó ; Horvath, Lilla ; Ernhofer, Büsra ; Schelch, Karin ; Pozonec, Maria Dorothea ; Teglas, Vivien ; Aigner, Clemens ; **Megyesfalvi, Evelyn** ; Rényi-Vámos, Ferenc et al. *Small Cell Lung Cancer Classification: Unraveling Heterogeneity to Enable Personalized Treatments* CANCER RESEARCH 86 : 5 pp. 1101-1112. , 12 p. (2026) Impakt faktor: 16.6