European Journal of Cancer 141 (2020) 193–198

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

High risk of thrombosis in patients with advanced lung cancer harboring rearrangements in *ROS1*

Nerea Muñoz-Unceta [a,*], Jon Zugazagoitia [b,z], Arancha Manzano [c], Elisabeth Jiménez-Aguilar [b], María E. Olmedo [d], Juan D. Cacho [a], Julio Oliveira [e], Manuel Dómine [f], Laura Ortega-Morán [g], Carlos Aguado [c], Ana M. Luna [h], Lourdes Fernández [i], Javier Pérez [j], Carme Font [k], Carmen Salvador [l], Jesús Corral [m], Gretel Benítez [n], Silverio Ros [o], Mercedes Biosca [p], Virginia Calvo [q], Julia Martínez [r], Manuel Sánchez-Cánovas [s], Rafael López [t], María Sereno [u], Xabier Mielgo [v], Francisco Aparisi [w], Marta Carmona [x], Rafael Carrión [y], Santiago Ponce-Aix [b,z], Marta Soares [e], Imanol Martínez-Salas [f], Marcial García-Morillo [k], Oscar Juan-Vidal [l], Ana Blasco [w], Andrés J. Muñoz [g], Luis Paz-Ares [b,z,aa,ab], Grupo de trombosis y cáncer SEOM

[a] *University Hospital Marqués de Valdecilla, IDIVAL, Santander, Spain*
[b] *University Hospital 12 de Octubre, Madrid, Spain*
[c] *San Carlos Clinical Hospital, Madrid, Spain*
[d] *University Hospital Ramón y Cajal, Madrid, Spain*
[e] *Portuguese Institute of Oncology of Porto, Portugal*
[f] *University Hospital Fundación Jiménez Díaz, IIS-FJD, Madrid, Spain*
[g] *University Hospital Gregorio Marañón, Madrid, Spain*
[h] *Hospital Ruber Internacional, Madrid, Spain*
[i] *University Hospital Virgen de la Salud, Toledo, Spain*
[j] *Hospital Virgen de los Lirios, Alcoy, Spain*
[k] *Hospital Clinic, Barcelona, Spain*
[l] *University Hospital la Fé, Valencia, Spain*
[m] *Clínica Universidad de Navarra, Madrid, Spain*
[n] *Hospital Insular de Gran Canaria, Gran Canaria, Spain*
[o] *Hospital Virgen de la Arrixaca, Murcia, Spain*
[p] *Hospital Vall d'Hebron, Barcelona, Spain*
[q] *University Hospital Puerta de Hierro, Majadahonda, Spain*
[r] *University Hospital Virgen del Rocío, Sevilla, Spain*
[s] *Hospital Morales Meseguer, Murcia, Spain*
[t] *University Hospital Clínico de Valladolid, Valladolid, Spain*

* *Corresponding author*: Medical Oncology Department, University Hospital Marqués de Valdecilla; Av. De Valdecilla 25, 39008, Santander, Cantabria, Spain.
E-mail address: nere.mun@gmail.com (N. Muñoz-Unceta).

https://doi.org/10.1016/j.ejca.2020.10.002

[u] University Hospital Infanta Sofía, San Sebastián de los Reyes, Spain
[v] Hospital Fundación de Alcorcón, Alcorcón, Spain
[w] University Hospital General de Valencia, Valencia, Spain
[x] Hospital Lucus Augusti, Lugo, Spain
[y] University Hospital del Sureste, Madrid, Spain
[z] Lung Cancer Unit H12O-CNIO, Spain
[aa] Ciberonc, Spain
[ab] University Complutense de Madrid, Spain

Received 30 June 2020; received in revised form 1 September 2020; accepted 9 October 2020

KEYWORDS
Thromboembolic event;
Advanced non–small cell lung cancer (NSCLC);
ROS1 rearrangement;
ALK translocation;
Albumin;
Recurrent thrombosis

Abstract ***Introduction:*** Based on the high incidence of thromboembolic events (TEs) observed in lung adenocarcinomas with *ALK* translocations and taking into account the biological proximity of *ROS1* and *ALK*, we conducted a retrospective analysis of patients with advanced lung carcinoma carrying rearrangements in *ROS1* from 23 centres in Spain and one centre in Portugal.
Methods: The main objective of the study was to analyse the incidence of TE in this population, looking for predictive risk factors, and its impact on overall survival.
Results: A total of 58 patients were included. The incidence of TEs throughout the disease was 46.6% (n = 27) with a median follow-up of 19 months (range: 1–78 months) and a median overall survival of 52 months in the total population and 50 months for the patients presenting TEs, with a hazards ratio of 1.12 (95% confidence interval: 0.47–2.65) p = 0.78. The majority of the events were venous (n = 24; 89%) and occurred in the ambulatory setting (n = 18; 67%). Almost half of the patients (n = 13; 48%) presented the TE in the peri-diagnostic period.
Conclusions: The high incidence of thrombosis, especially during the cancer diagnosis process, requires special attention from a clinician. Despite the limitations of such a small descriptive study, its results are in accordance with previously reported data. It would be important to design prospective studies of antithrombotic prophylaxis in this population because of their possible impact in reducing the risk of TEs.

1. Introduction

Cancer-associated thrombosis is the second cause of preventable death in cancer patients and its incidence is largely increasing [1]. Multiple factors contribute to a procoagulant context in patients with cancer, including the type of treatment, patient characteristics, and the tumour itself [2]. Lung cancer is one of the tumour types with highest incidence of thromboembolic events (TEs), accounting for 15% of venous TEs in genomically unselected patients with advanced adenocarcinoma [3,4]. Recent studies have suggested that some genomic subtypes of non–small cell lung cancer (NSCLC) might be at increased risk of developing TEs, particularly those with rearrangements in *ALK* and *ROS1* [5–10].

ROS1 rearrangements are found in up to 2% of all NSCLC. These patients are typically younger than those bearing other NSCLC subtypes and, mostly, light or never smokers. Tumour cells with this genomic aberration are highly sensitive to specific *ROS1* tyrosine kinase inhibitors, leading to durable responses and survival that exceeds 5 years in many patients [11]. Based on our previous work about TEs in lung adenocarcinomas with *ALK* translocations [5] and taking into account the biological proximity of *ROS1* and *ALK*, besides clinical and epidemiological similarities, we have conducted a retrospective analysis of patients with advanced lung carcinoma carrying a rearrangement in *ROS1* from 23 centres in Spain and one centre in Portugal.

2. Material and methods

The objective was to study the incidence, predictors, and prognostic factors associated with thromboembolic disease in this cohort of patients. Previously, a centralised institutional ethics committee approved the study's protocol at the University Hospital 12 de Octubre in Spain, and an institutional ethics committee approved it at the Portuguese Institute of Oncology of Porto.

The patients included had been diagnosed with advanced NSCLC (stage III–IV) from January 2012 to May 2018, and those with neuroendocrine histology were excluded. *ROS1* rearrangement was detected by immunohistochemistry, fluorescence *in situ* hybridization (FISH), or reverse transcription - quantitative polymerase chain reaction (RT-qPCR). Patients receiving therapeutic doses of low–molecular weight heparin or oral anticoagulants before the cancer diagnosis, and those bearing pro-thrombotic molecular alterations were excluded from this study. We defined a TE as any arterial or venous thromboembolism confirmed by radiologic imaging or ultrasound that occurred from 6 months before the diagnosis of advanced lung cancer until death or loss of follow-up.

To describe the clinical characteristics of the patients included in the study, we used frequency measures and percentages for all categorical variables. Median and range were applied for all continuous variables.

To explore the potential predictors of TEs, we performed univariate and multivariable logistic regression analysis. The latter included a maximum of three variables with a $p < 0.25$ in the univariate analysis. Continuous variables were transformed into categorical variables for the analysis, and cut-off values were chosen from previous publications (Khorana score) and from our laboratory (albumin).

Overall survival (OS) was defined as the time from lung cancer diagnosis to the date of death, whatever the cause. The follow-up of surviving patients was censored at the time of last contact.

The Kaplan–Meier method and the log-rank test were used to analyse and compare the OS functions. All p values refer to a two-sided test, and all estimates were complemented with an appropriate 95% confidence interval (CI) where applicable, with a statistical significance predefined at 0.05.

Statistical analyses were performed using SPSS 21.0 version.

3. Results

We identified and analysed a total of 58 patients with advanced NSCLC and *ROS1* rearrangement. The median age was 55 years (range 31–80), and the majority had a performance status of 0–1 (n = 48, 83%). Most of them had stage IV lung adenocarcinoma (n = 45, 78%), including 17% with brain metastasis (n = 10) and 10% with liver metastasis (n = 7). Smoking history was confirmed in more than half of the patients; 22% (n = 13) were former smokers, and 29% (n = 17) were current smokers.

After a median follow-up of 19 months (range: 1–78 months), the cumulative incidence of TEs throughout the course of the disease was of 46.6% (n = 27). The majority of the TEs were venous (n = 24, 89%) and symptomatic (n = 22, 81%), and they occurred in the ambulatory setting (n = 18, 67%). Pulmonary embolism was the most frequent TEs (n = 11, 42%), followed by deep vein thrombosis (n = 6, 23%), and nine patients presented both simultaneously (35%). Arterial thrombosis was reported in three patients: one of them in a coronary artery and another in a limb artery. Within all patients with TEs, 48% (n = 13), presented the event at the onset of advanced disease (stages III and IV) and 11% (n = 3) during the 6 months before diagnosis. Most of them had not yet started oncologic treatment (n = 16; 59%). Of note, re-thrombosis was detected in five patients (18%), while the majority of them were under appropriate anticoagulation treatment (n = 3, 60%) [Table 1].

Table 1
Characteristics of thromboembolic events.

Characteristic	Number of patients (%)
Total	27
Type of thromboembolic disease	
Venous	24 (89)
Arterial	1 (4)
Both venous and arterial	2 (7)
Clinical presentation	
Incidental	5 (19)
Symptomatic	22 (81)
Patient situation	
Ambulatory	18 (67)
Hospitalised	9 (33)
Location of thromboembolic events	
a) Venous thromboembolic events	
Pulmonary embolisms (PE)	11 (42)
Lower extremity (LE) deep vein thrombosis	4 (15)
Upper extremity (UE) deep vein thrombosis	2 (8)
Both LE and PE	6 (23)
Both UE and PE	3 (12)
b) Arterial thromboembolic events	
Peripheral	1 (33)
Coronary	1 (33)
Other	1 (33)
Time of occurrence	
During the 6 months beforediagnosis	3 (11)
At diagnosis, before starting treatment	13 (48)
Within the first 6 months of treatment	4 (15)
Beyond 6 months from start of treatment	7 (26)
Time of occurrence according to type of cancer therapy	
Treatment naïve	16 (59)
During platinum-based chemotherapy	2 (7)
During radiotherapy	2 (7)
During TKI treatment	7 (26)
Time of occurrence according to the clinical situation of the disease	
Untreated	16 (59)
Partial response	0 (0)
Stable disease	3 (11)
Progressive disease	6 (22)
Not evaluated	2 (7)
Re-thrombosis	
Yes	5 (18)
under therapeutic anticoagulation	*3 (60)
not under therapeutic anticoagulation	*2 (40)
No	20 (74)
Missing	2 (7)

TKI, tyrosine kinase inhibitor.

Table 2
Univariate and multivariate analysis for risk predictors of TEs.

Variable	Number of patients n (%)	Univariate HR (CI 95%)	p value	Multivariate HR (CI 95%)	p value
Female gender	16 (59)	1.61 (0.73–3.53)	0.234	2.94 (0.75–11.47)	0.119
BMI ≥30 kg/m^2	3 (1)	2.97 (0.85–10.42)	0.088	5.76 (0.53–62.25)	0.149
Presence of CNS metastasis	5 (19)	1.80 (0.67–4.81)	0.241	1.00 (0.17–5.74)	0.998
Albumin count <3.5 g/dl	7 (26)	3.53 (1.27–9.80)	0.015	4.22 (1.31–13.56)	0.015
Leucocyte count >11,000 cells/mm^3	5 (30)	1.00 (0.36–2.74)	0.993	–	–
Smoking history	10 (36)	0.65 (0.29–1.45)	0.302	–	–

BMI, body mass index; CI, confidence interval; CNS: central nervous system; HR, hazards ratio.

To analyse the correlation between baseline characteristics and thromboembolic disease, we conducted a Cox regression analysis [Table 2]. We found that only low albumin levels (<3.5 g/dl) were associated with a higher risk of presenting TEs, with a hazards ratio (HR) of 3.53 (CI 95%: 1.27–9.80; p = 0.015) in the univariate analysis. This association was confirmed at the multivariate analysis (which included gender, body mass index, and brain metastases), with a HR of 4.22 (CI 95%: 1.31–13.56; p = 0.015).

The median OS of patients with TEs was 50 months, which is similar to patients without TEs (52 months) (Fig. 1a). Patients diagnosed with TEs at baseline (from 6 months before diagnosis to 1 month after diagnosis) had a median OS of 46 months, which is not sstatistically significant from the remaining patients, with a HR 1.25 (CI 95%: 0.46–3.39) and p = 0.66 (Fig. 1b).

4. Discussion

The results obtained in our multicenter study confirm a high incidence of TEs in patients with advanced lung adenocarcinoma carrying rearrangements in *ROS1*, which is consistent with emerging data on patients with these characteristics [8–10]. Considering the prolonged median OS of this patient's population compared with other lung carcinomas carriers, it is not surprising to find a higher cumulative incidence of TEs. However, most of the events were diagnosed in the peri-diagnostic period, even before having started any treatment, which could indicate an inherent procoagulant status underlying this disease.

Consistent with the observation of Alexander *et al.* [10], we have failed to identify any association between the classical predictors of TEs and a higher incidence of TEs in our series, possibly because of the intrinsic risk attributable to the disease *per se*. Low serum albumin (<3.5 gr/dl) was the exception, as it was found to be predictive of TEs. This association is probably a consequence of an inflammatory status mediated by tumour cytokines, which decrease albumin hepatic synthesis and increase its degradation and transcapillary loss [12]. However, reduced protein intake due to cancer cachexia may also influence plasma albumin

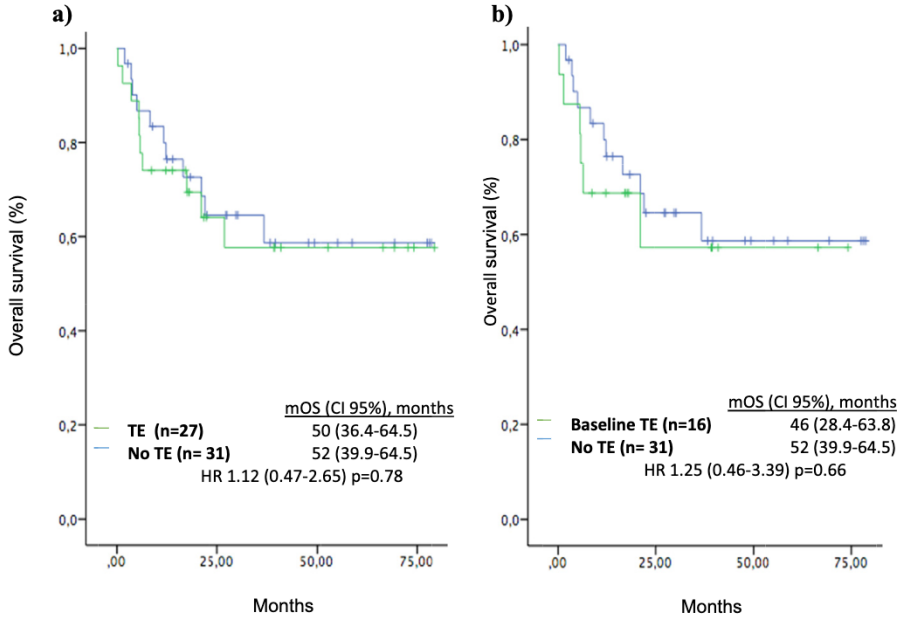

Fig. 1. **a)** Kaplan–Meier curve for overall survival for patients with and without thromboembolic disease. **b)** Kaplan–Meier curve for overall survival of patients with and without TEs at baseline (excluding patients that presented TEs after baseline).

concentration and, as a modifiable factor, nutritional status should be of special consideration at diagnosis. Body mass index was associated with a large HR, but non-significant, which agrees with the low prevalence of obese patients in this cohort (just three patients, and all had TEs). Furthermore, the high percentage of current and former smokers in our series is noteworthy, but it did not translate into a higher TE risk. Nevertheless, because of the limited size of the cohort, its retrospective nature, and the existence of missing data, these results must be taken cautiously. Unlike our *ALK*-driven NSCLC cohort [5] in which leucocytes predicted both the occurrence of thrombotic complications and reduced OS, in our series, leucocytes were not significantly higher in patients with TEs. Of note, Ng *et al.* [8] found that lymph node metastases and smoking status were associated with increased odds of TEs in multivariable analysis.

The high incidence of recurrent thrombosis under correct anticoagulation concurs with previous series [9,10] and our previous *ALK* cohort. Lee *et al.* [13] reported two patients with lung adenocarcinomas and *ROS1* rearrangement that experienced recurrent thrombosis along the course of the disease, although both of them had heterozygous factor V Leyden mutation [13]. Of relevance, this was an exclusion criterion in our cohort, when known. Some reports have proposed that adenocarcinomas rich in mucin (frequent in *ALK* and *ROS1* rearrangements) could activate platelet aggregation, which might confer a major risk of TEs [14], but this observation warrants further investigation.

Similar to previous works, we did not find a survival disadvantage for patients presenting a TE along their disease. However, it has to be considered that all of them are retrospective studies or subgroup analyses with a short time of follow-up.

5. Conclusions

To our knowledge, this is the largest series of patients with advanced lung adenocarcinoma and *ROS1* rearrangements in the Spanish and Portuguese population.

Despite the limitations of a small descriptive study, the high incidence of TEs in this population (46.6%), especially during the diagnostic process of cancer (59% before starting oncologic treatment), requires special attention from clinicians. Although our cohort was underpowered to demonstrate any significant predictive factor for TEs, the lack of them hinders the selection of patients with a higher risk of TEs. Taking into account the durable responses and high control rates with targeted therapy, when a patient recently diagnosed with advanced lung cancer and *ROS1* rearrangement experiences dyspnoea, pulmonary embolism rather than progressive disease should be considered to be a possible cause by every physician. Finally, these data warrant the design and conduct of prospective studies of thromboembolic prophylaxis in this subset of lung cancer patients given its potential to decrease morbidity and even mortality in this clinical scenario.

Role of the funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contribution statement

Nerea Muñoz-Unceta, Jon Zugazagoitia, Arancha Manzano, Luis Paz-Ares, and Andrés J. Muñoz conceived and designed the study. All authors were involved in data collection. Nerea Muñoz-Unceta did the statistical analysis using SPSS 21.0 version, with the supervision of Pedro Muñoz-Cacho. Nerea Muñoz-Unceta, Jon Zugazagoitia, and Luis Paz-Ares contributed to data interpretation and wrote the original draft of the manuscript. All authors participated in manuscript writing and editing, and approved the final version of the article.

Conflict of interest statement

Dr. Muñoz reports grants, personal fees, and non-financial support from Sanofi and Celgene; personal fees and non-financial support from Roche and Amgen; grants and personal fees from Leo Pharma; personal fees from AstraZeneca, Servier, Pfizer, Daiichi Sankyo, Bayer, Halozyme, Rovi, Merck Sharp & Dohme, and Lilly; and non-financial support from Merck Serono.

Dr. Aguado reports personal fees and non-financial support from ROCHE; personal fees from AstraZeneca, Boehringer, Sanofi, Pierre Fabre, MSD, BMS; and non-financial support from Novartis.

Dr. Oliveira reports grants from AstraZeneca and personal fees from Roche, Pierre Fabre, and Bristol-Myers Squibb.

Dr. Zugazagoitia reports personal fees from Guardant Health, NanoString, Roche, and Pfizer.

Dr. Ortega reports personal fees and non-financial support from Sanofi, Amgen, and Leo Pharma and non-financial support from Roche.

Dr. Carmona reports personal fees from Rovi and Leo Pharma.

Dr. Domine reports personal fees from AstraZeneca, BMS, Boehringer Ingelheim, MSD, Pfizer, and Roche.

Dr. Biosca reports personal fees and other from Leo Pharma, Sanofi, and Rovi.

Dr. Juan-Vidal reports personal fees from Boehringer Ingelheim, Bristol-Myers Squibb, Merck Sharp & Dohme, Roche/Genetech, AstraZeneca, Pfizer, Eli Lilly, Abbvie, and Takkeda.

Dr. Paz-Ares reports grants and personal fees from BMS and AstraZeneca and personal fees from MSD, Roche, Lilly, Merck, Novartis, Angem, Incyte, Takeda, Blueprint, and Bayer.

Dr. Ponce-Aix reports personal fees and others from MSD, AstraZeneca, BMS, Pharmamar, Roche, Boehringer, Lilly, and Merck.

Dr. Calvo reports honoraria as the speaker and consultant on advisory boards from Roche, BMS, MSD, Pfizer, Lilly, Astra-Zeneca, Boehringer, Novartis, and Takeda.

The rest of the authors declare no conflicts of interest.

References

[1] Khalil J, Bensaid B, Elkacemi H, et al. Venous thromboembolism in cancer patients: an underestimated major health problem. World J Surg Oncol 2015;13:204. https://doi.org/10.1186/s12957-015-0592-8.

[2] Khorana AA, Rao MV. Approaches to risk-stratifying cancer patients for venous thromboembolism [published correction appears in Thromb Res. 2008;123(1):187-190] Thromb Res 2007; 120(Suppl 2):S41–50. https://doi.org/10.1016/S0049-3848(07)70129-9.

[3] Kuderer NM, Culakova E, Lyman GH, Francis C, Falanga A, Khorana AA. A validated risk score for venous thromboembolism is predictive of cancer progression and mortality. Oncol 2016; 21(7):861–7. https://doi.org/10.1634/theoncologist.2015-0361.

[4] Blom JW, Osanto S, Rosendaal FR. The risk of a venous thrombotic event in lung cancer patients: higher risk for adenocarcinoma than squamous cell carcinoma. J Thromb Haemostasis 2004;2(10):1760–5. https://doi.org/10.1111/j.1538-7836.2004.00928.x.

[5] Zugazagoitia J, Biosca M, Oliveira J, et al. Incidence, predictors and prognostic significance of thromboembolic disease in patients with advanced ALK-rearranged non-small cell lung cancer. Eur Respir J 2018;51(5):1702431. https://doi.org/10.1183/13993003.02431-2017.

[6] Zer A, Moskovitz M, Hwang DM, et al. ALK-rearranged non-small-cell lung cancer is associated with a high rate of venous thromboembolism. Clin Lung Canc 2017;18(2):156–61. https://doi.org/10.1016/j.cllc.2016.10.007.

[7] Dou F, Zhang Y, Yi J, et al. Association of ALK rearrangement and risk of venous thromboembolism in patients with non-small cell lung cancer: a prospective cohort study. Thromb Res 2020; 186:36–41. https://doi.org/10.1016/j.thromres.2019.12.009.

[8] Ng TL, Smith DE, Mushtaq R, et al. ROS1 gene rearrangements are associated with an elevated risk of peridiagnosis thromboembolic events. J Thorac Oncol 2019;14(4):596–605. https://doi.org/10.1016/j.jtho.2018.12.001.

[9] Chiari R, Ricciuti B, Landi L, et al. ROS1-rearranged non-small-cell lung cancer is associated with a high rate of venous thromboembolism: analysis from a phase II, prospective, multicenter, two-arms trial (METROS). Clin Lung Canc 2020;21(1):15–20. https://doi.org/10.1016/j.cllc.2019.06.012.

[10] Alexander M, Pavlakis N, John T, et al. A multicenter study of thromboembolic events among patients diagnosed with ROS1-rearranged non-small cell lung cancer. Lung Canc 2020;142: 34–40. https://doi.org/10.1016/j.lungcan.2020.01.017.

[11] Park S, Ahn BC, Lim SW, et al. Characteristics and outcome of ROS1-positive non-small cell lung cancer patients in routine clinical practice. J Thorac Oncol 2018;13(9):1373–82. https://doi.org/10.1016/j.jtho.2018.05.026.

[12] Johnson MJ, Sproule MW, Paul J. The prevalence and associated variables of deep venous thrombosis in patients with advanced cancer. Clin Oncol 1999;11(2):105–10. https://doi.org/10.1053/clon.1999.9023.

[13] Lee A, Howell VM, Itchins M, Wheeler HR, Pavlakis N. ROS1-Rearranged non-small-cell lung cancer, factor V leiden, and recurrent venous thromboses. Clin Lung Canc 2018;19(5):457–9. https://doi.org/10.1016/j.cllc.2018.05.015.

[14] Wahrenbrock M, Borsig L, Le D, Varki N, Varki A. Selectin--mucin interactions as a probable molecular explanation for the association of Trousseau syndrome with mucinous adenocarcinomas. J Clin Invest 2003;112(6):853–62. https://doi.org/10.1172/JCI18882.