Universitat Autònoma de Barcelona

VENOUS THROMBOEMBOLISM AFTER LUNG TRANSPLANTATION

Berta Sáez Giménez

DOCTORAL THESIS

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Author:
Berta Sáez Giménez

Directors:
Antonio Roman Broto
Carles Bravo Masgoret

Tutor:
Jaume Ferrer Sancho

Universitat Autònoma de Barcelona

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"I do know that kind fate
allowed me to find a couple of nice ideas
after many years of feverish labor."

A. Einstein

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APCR: Activated protein C resistance
APTT: Activated partial thromboplastin time
ATIII: Antithrombin III
BMI: Body mass index
BO: Bronchiolitis obliterans
CMV: Cytomegalovirus
COPD: Chronic obstructive pulmonary disease
CsA: Cyclosporine A
DVT: Deep vein thrombosis
FFP: Fresh frozen plasma
FVIII: Factor VIII activity
GT: Graft thrombosis
ICU: Intensive care unit
ILD: Interstitial lung disease
IPF: Idiopathic pulmonary fibrosis
IRR: Incidence rate ratio
LAM: Lymphangioleiomyomatosis
LT: Lung transplantation
MMF: Mycophenolate mofetil
NFH: Nonfractionated heparin
NSAIDs: Nonsteroidal anti-inflammatory drugs
OR: Odds ratio
PAH: Pulmonary arterial hypertension
PAI-1: Plasminogen activator Inhibitor -1
PC: Protein C activity
PE: Pulmonary embolism
POD: Postoperative day
PS: Free protein S
PT: prothrombin time
RR: relative risk
RT: Renal transplantation
SIR: Standardized incidence ratio
SOT: Solid organ transplantation
TF: Tissue factor
tPA: Tissue plasminogen activator
TUET: Treated upper extremity thrombosis
US: Doppler ultrasound
VAT: Vascular access thrombosis
VPS: Ventilation-perfusion scan
VTE: Venous thromboembolism
vWF: Von Willebrand factor
W: Warfarin

Venous thromboembolism is a frequent complication after solid organ transplantation and, specifically, after lung transplantation. The objectives of this study were to describe risk factors for venous thromboembolism, to assess the impact of an extended prophylaxis protocol and to describe coagulation profiles before and up to 1 year after lung transplantation.

We performed 2 studies. The first study compared a cohort (n = 138) that received 90-day extended prophylaxis with enoxaparin and a historical control cohort (n = 195) that received prophylaxis only during post-transplant hospitalization. The second study is a prospective study to describe the coagulation profiles of 48 patients before lung transplantation and at 24-72 hours, 2 weeks, 4 months and 1 year after lung transplantation.

The cumulative incidence of venous thromboembolism was 15.3% (95% CI: 11.6-19.4). Median time from transplant to the event was 40 (p25-75, 14-112) days. In this study, the risk factors associated with venous thromboembolism were male gender and interstitial lung disease. Ninety-day extended prophylaxis did not reduce the incidence of VTE.

In the second study to describe coagulation profiles up to 1 year after lung transplantation, we found that most markers of a procoagulant state normalize at 2 weeks after lung transplantation and that abnormal values of factor VIII and Von Willebrand factor persist at 1 year. Patients with venous thromboembolism at 4 months had higher values of factor VIII at 2 weeks.

Larger, multicenter studies are needed to confirm these results and to design appropriate prophylactic strategies.

Introduction

1

1.1. VENOUS THROMBOEMBOLISM IN SOLID ORGAN TRANSPLANTATION: EPIDEMIOLOGICAL DATA

The incidence of venous thromboembolism (VTE) in the general population is estimated at between 1 and 2 cases per 1000 people per year [1–3]. VTE includes deep vein thrombosis (DVT) and pulmonary embolism (PE). It is a common complication of surgery and a major cause of morbidity and mortality in various medical conditions, with an overall risk that depends on environmental and genetic factors.

Thus, the risk of VTE differs with the type of surgery performed. Approximately half of all patients undergoing orthopedic surgery without prophylaxis develop VTE [4]; when prophylaxis is implemented, the incidence decreases to around 18 per 1000 cases per year [5]. Cancer is also a risk factor for VTE, and 8% of patients experience VTE either within the first year after diagnosis or during the course of their disease [6]. Other conditions that involve an intermediate risk for VTE are shown in ***Table 1***.

Table 1: Incidence of VTE in different populations

Population studied	Incidence
General population [1–3]	0.1 - 0.2%
After orthopedic surgery [5]	
Without prophylaxis	50%
With prophylaxis	1.1 - 10.6%
Cancer [6]	1 - 8%
Abdominal surgery [7]	0.6 - 3.1%
Low-risk: *appendectomy, cholecystectomy, or lysis of adhesions*	0.6%
Intermediate-risk: *gastrointestinal tract*	1.8%
High-risk: *splenectomy*	3.1%
Thoracic surgery	
Lobectomy/pneumonectomy [8]	0.18%
Cardiac surgery [9,10]	0.56%
Renal transplantation	2 - 14%
Liver transplantation	3 - 5%
Heart transplantation	18 - 34%
Lung transplantation	8 - 29%

Less is known about the real incidence of this complication following solid organ transplantation (SOT); available data are summarized in ***Table 1***.

Renal transplantation

The incidence of VTE after renal transplantation (RT) has been reported to range between 2% and 14% [11]. In 1987, *Allen et al.* [12] published a descriptive retrospective study of 480 kidney recipients and reported an incidence of 8.3%, which peaked within the first 4 months after transplantation. In a retrospective study including 1833 kidney recipients from 1985 to 1995, *Humar et al.* [13] found a slightly lower incidence (4.2%), with a peak between the third and fifth months after transplantation.

The risk of late VTE was also assessed in a study based on the Medicare database [14]; the study population comprised 28,924 kidney recipients, and the incidence of late VTE (occurring 1.5 to 3 years after transplantation) was 1.5%. In summary, these data strongly suggest a high incidence of VTE after RT.

Liver transplantation

VTE after liver transplantation has been described as a relevant complication with an incidence of between 3% and 5% and significant morbidity and mortality both during surgery and during the early postoperative period. In their analysis of 495 liver recipients between 2004 and 2006, *Sakai et al.* [15] reported PE in 20 patients (i.e., a 4% incidence of intraoperative PE).

Ishitani et al. [16] reported a 3.7% incidence of clinically symptomatic VTE during a 6-year follow-up, with a peak during the first 2 months after transplantation. The VTE prophylactic protocol included treatment with intermittent pneumatic leg compression devices until patients were able to sit. *Salami et al.* [17] later conducted a similar retrospective study and reported an incidence of VTE of 4.6%.

Diagnostic procedures were carried out when VTE was clinically suspected, and there were no prespecified prophylaxis protocols.

Heart transplantation

Several retrospective studies on the epidemiology of VTE in heart recipients, including arterial and venous thrombotic events, reported an incidence of VTE events of between 15% and 34%. It is important to emphasize that these studies included episodes of acute myocardial infarction, stroke and occlusion of retinal vessels as thrombotic events. *Forrat et al.* 18 reviewed 285 heart recipients on low-dose aspirin (250 mg/d) and found 97 cardiac and noncardiac thromboembolic complications (34%), of which 33 (11.6%) were DVT or PE events. *Miriuka et al.* [19] analyzed the frequency of thrombotic events, which were mainly related to the coronary artery tree, but which also included DVT and PE. A total of 22 patients (26.2%) had at least 1 complication of VTE, including 13 DVT and 5 PE.

In summary, the literature shows a high incidence of thromboembolic complications after heart transplantation despite the use of antiplatelet agents as an almost standard prophylactic strategy.

Lung transplantation

VTE after lung transplantation is a common finding in clinical practice and has been reported to occur in 8% to 27% of lung recipients in retrospective studies (***Table 2***). In 1995, *Kroshus et al.* [20] reported an incidence of VTE complications of 12.1% between 10 days and 36 months after surgery. All patients underwent screening with ventilation-perfusion scanning in the first week, at 6 months, and at 1 year after transplantation. Doppler ultrasonography of the legs was performed to exclude DVT only when the condition was clinically suspected. Patients also received prophylaxis with perioperative and postoperative subcutaneous heparin until discharge, as well as pneumatic compression garments until they were able

to walk again. Patients then received antiplatelet agents. Although this study was retrospective, both the diagnostic and the predefined prophylactic strategy were thorough and strongly support the relevance of VTE in lung recipients.

Izbicki et al.[21] published similar findings in a retrospective cohort, with an incidence of PE of 8.6% between 4 and 24 months after the intervention. Similar findings on the incidence of PE were reported by *Nathan et al.* (8.3%)[22], who emphasized that all the PE events were diagnosed in the subgroup of patients with idiopathic pulmonary fibrosis. This subgroup was identified as susceptible in another study[23], but with a lower incidence (only 1.78%).

In 2004, *Burns and Iacono*[24] reported an incidence of 27% for PE in postmortem examination of lung and heart-lung recipients. This incidence was higher in patients who died during the first month after transplantation, suggesting that PE may be under-diagnosed as a complication contributing to respiratory failure in the early postoperative period.

Finally, *Yegen et al.*[25] performed a study in a cohort of 121 patients who underwent lung transplantation between 2001 and 2005. All the patients received prophylaxis with heparin, enoxaparin, or pneumatic compression devices during their post-transplant stay. Asymptomatic patients were not routinely screened for VTE. The authors diagnosed 27 cases of VTE (incidence of 22%).

In summary, available information shows that there is an unacceptable incidence of VTE after SOT. Therefore, development of preventive strategies is mandatory.

1.2. THROMBOPHILIA AS A COMPLEX DISEASE: PATHOPHYSIOLOGY

Nowadays, knowledge about the etiology of thrombosis is still based on the first description made by Virchow in the 19th century, who stated that the main causes of thrombosis were venous stasis, endothelial injury and a hypercoagulable state. Using Virchow's triad as a framework, several mechanisms have been related to the pathogenesis of thrombosis [26], as follows: stasis and low oxygen tension, activation of the endothelium, activation of innate and acquired immunity, activation of blood platelets, concentration of pro- and anticoagulant proteins and concentration and nature of microparticles.

These mechanisms interact in different risk situations for VTE, as seen in the table proposed by *Reitsma et al.* [26].

Table 2: Common and Well-Established Risk Factors And Their Presumed Point(s) of Action

Risk Factors	Presumed Points of Action
Surgery	Stasis, microparticles, innate immunity
Trauma	Stasis, vascular damage, microparticles, innate immunity
Venous catheters	Vascular damage
Prolonged bed rest	Stasis
Plaster cast	Stasis, microparticles
Long haul travel	Stasis
Malignancies	Microparticles, innate immunity, platelet numbers
Chemotherapy	Microparticles, innate immunity
Pregnancy	Stasis, coagulation factor concentrations
Puerperium	Vascular damage, coagulation factor concentrations
Oral contraceptives	Coagulation factor concentrations
Hormone replacement therapy	Coagulation factor concentrations
Obesity	Stasis, coagulation factor concentrations, platelets
Infection	Innate immunity
Inflammatory disease	Innate and acquired immunity
Smoking	Innate immunity, platelets, coagulation factor concentrations
Lupus anticoagulant	Innate and acquired immunity, platelets
Genetic factors	Coagulation factor concentrations

The interaction between environment and genes is of paramount importance in this disease and explains the consideration of thrombophilia as a complex

disease for 2 reasons: a large number of environmental and genetic factors, some of which have yet to be discovered; and a variable interaction with each other. Thus, although a patient may have a genetic thrombophilic mutation, thrombotic events alternate with asymptomatic periods, suggesting that some kind of trigger is needed and emphasizing the importance of the environment. Each of the several environmental risk factors described entails a different risk (see table, adapted from *Vilalta et al.* [27]).

Table 3: Environmental risk factors related to VTE

Environmental risk factor	Risk	Reference
Age > 75 years	2.25-12.30 **(HR)**	28
Male	1.60 **(RR)**	29
Black	1.60 **(HR)**	28
BMI >30	2.33 **(OR)**	28,30
Smoking	1.06-1.49 **(HR)**	31
Arterial hypertension	1.51 **(OR)**	30
Diabetes mellitus	1.41 **(OR)**	28,30
Varicose veins	1.40 **(HR)**	31
Any serious disease	1.70 **(OR)**	32
Heart failure	1.30-1.40 **(HR)**	31
Kidney failure	1.60-1.90 **(HR)**	31
Cancer	1.80-2.20 **(HR)**	31
COPD	1.40-1.60 **(HR)**	31
Autoimmune diseases	3.90-16.40 **(SIR)**	33,34
Infections	1.74-2.70 **(RR)**	34
Hospital admission	1.90 **(HR)**	31
Antipsychotic drugs	1.55-1.84 **(HR)**	31
NSAIDs	2.50 **(IRR)**	35
Tamoxifen	1.50 **(HR)**	31
Oral contraceptives	1.33 **(HR)**	31
Hormone replacement therapy	1.20 **(HR)**	31
Pregnancy and puerperium	4.30 **(RR)**	36
Trauma	4.80-8.60 **(OR)**	37
General surgery	9.50 **(OR)**	38
Orthopedic surgery	16.25 **(OR)**	38
Prolonged bed rest	5.60 **(OR)**	38
Plaster cast	36.5 **(OR)**	30
Long haul travel	2.80 **(RR)**	39
Winter season	1.14 **(RR)**	40
Pollution	1.47 **(OR)**	41
Coffee	0.70 **(OR)**	42
Alcohol	0.75 **(HR)**	43

BMI: body mass index; ***COPD***: chronic obstructive pulmonary disease; ***NSAIDs***: non-steroidal anti-inflammatory drugs; ***IRR***: incidence rate ratio; ***OR***: odds ratio; ***RR***: relative risk; ***SIR***; standardized incidence ratio.

Although it seems clear that a number of genetic factors have yet to be identified, the fact is that only a few genetic variations have proved to have causal effect in thrombosis, namely, decreased anticoagulant proteins (antithrombin III (ATIII), protein C (PC), protein S (PS)), dysfunctional proteins (dysfibrinogenemia), factor V G1691A mutation, factor 2 G20210A mutation and increased procoagulant factors such as factor VIII (FVIII). Others, such as antiphospholipid antibodies or elevated homocysteine, have been related to an increased risk of thrombosis, although the mechanism remains unknown. ABO blood group has also been described as a risk factor: no-O carriers (specifically A1 genotype) have an increased risk of thrombotic events owing to higher levels of Von Willebrand Factor (vWF) and FVIII [44]. Other genetic factors that are infrequent in the general population and involved in thrombotic risk include C46T mutation (gene F12), R67X (gene SERPINA10), A348S (gene SERPINC1), FV Cambridge and FV Hong Kong.

The classic concept of considering these mutations dichotomous variables has been called into question, thus increasing the complexity of this condition. The possibility that they behave as a continuous risk factor is being considered and, therefore, risk may be stratified according to protein levels. This would lead to a gradient of susceptibility to the disease, explaining the differences seen in the clinical field.

1.3. RISK FACTORS FOR VTE IN LT

1.3.1. Underlying disease

It seems plausible that the underlying disease that leads to transplantation could increase the individual susceptibility to thrombosis, and that this increased risk may persist after transplantation.

Lung recipients with idiopathic pulmonary fibrosis (IPF) as the primary disease are thought to have a higher risk of thrombosis. In a retrospective review of 72 lung recipients, *Nathan et al.* [22] reported 7 PE events among 6 patients, all of which

were in the subgroup of patients who underwent transplantation owing to IPF. *Garcia-Salcedo et al.* [23] subsequently reported 5 cases of PE in a cohort of 280 patients that were retrospectively reviewed; all 5 involved IPF patients.

The reasons are not well known: some authors [25] have suggested that age could be a confounding factor; others have focused on the study of a possible procoagulant state in patients who had IPF before transplantation. *Navaratnam et al.* [45] compared 211 incident cases of IPF with 256 age- and sex- matched controls from the general population. A prothrombotic state (defined as the presence of 1 clotting defect or marker of fibrinolytic dysfunction) was >4 times more likely in patients with IPF, and patients with a prothrombotic state also had more severe disease at presentation (based on forced vital capacity) and an increased risk of death.

Coagulation has previously been related to lung injury and fibrotic lung disease; thus, extravascular intraalveolar accumulation of fibrin is characteristic of acute lung injury and acute respiratory distress syndrome, and procoagulant alterations (such as reduced ATIII or PC and increased tissue factor levels) have been described [46,47] in this population. Similar findings have been found in pulmonary fibrosis related to systemic sclerosis and IPF [48,49]. Evidence of a causal role of coagulation abnormalities in driving fibrotic responses comes from a variety of studies in animal models, where targeting the coagulation cascade led to reductions in parameters of lung injury, inflammation and fibrosis. Nevertheless, clinical trials of anticoagulants in acute lung injury and IPF have shown conflicting results. In 2005, *Kubo et al.* [50] randomized 56 patients with IPF to prednisolone plus anticoagulant therapy or prednisolone alone; anticoagulant therapy showed a beneficial effect, with lower mortality (18% vs. 71%, $p = 0.008$). However, a subsequent double-blind, randomized, placebo-controlled trial [51] demonstrated increased mortality in the anticoagulation group, and the study was prematurely stopped. In conclusion, it seems clear that there is a biological prothrombotic state in IPF that is correlated with disease severity and

outcome, although more studies are needed to assess individual thrombotic risk and prophylactic strategies.

1.3.2. CMV infection

Cytomegalovirus (CMV) infection temporary modifies the characteristics of the endothelium to a procoagulant phenotype and increases thrombotic risk both in immunosuppressed and immunocompetent hosts [52]. Two mechanisms have been proposed. Formation of antiphospholipid antibodies in response to CMV infection has been studied in animal models, but not yet in the clinical setting. Direct infection of endothelial cells has also been suggested as a possible mechanism that leads to lysis and release of tissue factor-mediated thrombin, inhibition of anticoagulants and fibrinolysis.

In SOT, the association between CMV infection and VTE has been studied in renal, liver and lung transplantation. In 1998, a retrospective study [53] in liver transplantation found a positive association between the use of a CMV-positive donor in a seronegative recipient and the incidence of hepatic artery thrombosis in the first 30 days after transplantation. In RT, *Kazory et al.* [54] retrospectively reviewed a population of 218 renal recipients and found 77 patients with acute CMV infection, of whom 13 presented a VTE event. In 7 cases, the events (thromboembolism and CMV) were simultaneous. In this study, allograft thromboses were excluded. Globally, the incidence of VTE was 9% in patients with acute CMV infection compared with 4.2% in patients without infection. Antiphospholipid antibodies were sought in patients with concomitant CMV infection and were found in 3 cases (before transplantation in 1 patient). *Moscarelli et al.* [55] subsequently reported an even higher incidence in 769 kidney recipients; CMV infection was reported in 154 patients, 37 (38%) of whom also had an episode of VTE.

Contrasting results were found in the only study regarding LT [25], where risk factors for VTE were studied in 121 recipients. Only 1 (4%) of the 27 patients with VTE also had CMV disease, compared with an incidence of 7% in the control group.

1.3.3. Immunosuppression

Calcineurin inhibitors

Calcineurin inhibitors, and specifically **cyclosporine (CsA)**, have been related to thrombotic complications (such as thrombotic microangiopathy and thrombotic thrombocytopenic purpura) after bone marrow transplantation. Their role in these complications after SOT remains unclear, and several mechanisms have been proposed (see ***Table 4***).

Suehiro et al. [56] studied the effect of calcineurin inhibitors on serotonin-induced platelet aggregation in vitro. Using platelets from healthy donors and adding CsA and tacrolimus separately, they reported a dose-dependent enhanced serotonin-induced platelet aggregate formation with both drugs. These data conflict with those of 2 studies [57,58], suggesting that tacrolimus had an antithrombotic effect in vitro through inhibition of platelet activity and coagulation and another study [59] describing normal platelet function more than 4 months after RT in 10 patients receiving CsA.

The effect of CsA on hemostasis is also a matter in controversy. In 1985, *Vanrenterghem et al.* [60] compared increased levels of factor VIII and fibrinogen in a group of 90 patients treated with CsA and prednisolone with 90 patients receiving azathioprine and prednisolone several weeks after transplantation. All patients received dipyridamole as prophylaxis. The role of CsA in these findings is not clear, because elevated levels of vWF and FVIII before transplantation have already been described [61]. Strikingly, *Vanrenterghem et al.* also found increased levels of anticoagulant proteins such as antithrombin III and protein C.

Nevertheless, the possibility of a procoagulant effect of CsA is further supported by *in vitro* studies. *Baker et al.* [62] assessed the effect of CsA over hemostasis via hemostatometry, which provides an integrated overall assessment of hemostasis. The authors found a lower hemostasis time in patients receiving CsA than in patients

receiving azathioprine and prednisolone alone. *Garcia-Maldonado et al.* [63] compared bovine aortic endothelial cells exposed to CsA with control cells. CsA reduced thrombomodulin activity, thus downregulating the protein C anticoagulant pathway and suggesting an increased risk of thrombosis in CsA-treated patients.

Previous studies have also described an impairment in fibrinolysis [64,65]. *Ueda et al.* [64] described fibrinolytic parameters in the long term after RT (more than 3 years) in 2 groups of patients: 26 receiving CsA and low-dose prednisolone and 20 patients with azathioprine and prednisolone. They found decreased fibrinolytic activity due to decreased release of tissue plasminogen activator (tPA) or increased levels of plasminogen activator inhibitor-1 (PAI-1), which were more pronounced in CsA-treated patients. Although the effect of prednisolone is not negligible, it cannot explain the differences between the 2 groups.

The many other procoagulant mechanisms proposed include activation of monocytes to express tissue factor, endothelial dysfunction, activation of the intrinsic coagulation pathway, increased levels of thromboxane and reduced production of prostacyclin by endothelial cells [11].

CsA remains controversial from the clinical point of view. *Vanrenterghem et al.* [60] reported 17 VTE events in the CsA group and only 1 event in the azathioprine group. By contrast, in their prospective study, *Brunkwall et al.* [66] did not find an increased frequency of DVT in 97 patients receiving CsA and low-dose corticosteroids (incidence of 9.3%) compared with a similar group of 83 patients treated with azathioprine and high-dose corticosteroids (incidence of 24.1%). Larger studies [67,68] have also failed to prove an association between CsA and VTE.

Although **tacrolimus** has had lower visibility in the literature than CsA, it is also subject to controversy. Once again, studies in the clinical setting conflict with

the previously mentioned *in vitro* results and are in favor of a better coagulation profile than CsA. Thus, *Pirsch et al.* [69] described a higher incidence of DVT in tacrolimus-treated patients in an open-label, randomized, multicenter study of 412 kidney recipients treated with tacrolimus (n = 205) or CsA (n = 207) and followed for 1 year. Nevertheless, risk factors for VTE were present in all patients, and 45% of the cases in the tacrolimus group were reported by a single center.

In summary, the relative importance of all these changes is difficult to assess, and there are studies confirming and refuting the association between calcineurin inhibitors and vascular thrombosis. Larger prospective, randomized studies are needed to establish a definitive association.

Corticosteroids

The prothrombotic effect of corticosteroids has been described in the literature. In Cushing's disease, for example, corticosteroids are known to induce a hypercoagulable state, thus increasing the risk for VTE. A systematic review of literature [70] described increased factor VIII, factor IX and vWF in this disease, which tended to normalize after successful treatment.

In the field of SOT, *Sartori et al.* [71] performed a study in 28 kidney recipients long after surgery (mean 22 ± 16 months). Methylprednisolone was tapered and subsequently withdrawn. Fibrinolysis was studied at different time points before and after the change in treatment, and a venous occlusion test was performed to stimulate fibrinolysis. Fibrinolytic capacity was impaired in all patients at the first time point. Only 12 remained corticosteroid-free at 6 months. Reduced fibrinolytic capacity was seen in 83% during corticosteroid treatment and in only 16.7% after corticosteroids were withdrawn (the dose of CsA and azathioprine remained unchanged). Similarly, *Patrassi et al.* [72] studied the effect of corticosteroids in 49 heart recipients and 25 healthy controls (mean time since transplantation

29.04 ± 1.27 months). Transplant recipients were divided into 2 groups depending on whether or not they received corticosteroids as part of their immunosuppression protocol and matched for sex, age and post-transplantation time. Impairment of fibrinolytic potential due to high PAI-1 levels was seen in 69.2% of heart recipients treated with corticosteroids and in 35% of patients without corticosteroids, a difference that was statistically significant.

Therefore, it seems clear that corticosteroids have an important role in the procoagulant state seen after solid organ transplantation.

mTOR inhibitors

In preclinical studies, mTOR inhibitors have been reported to have a prothrombotic effect. The mechanisms are not well known but include regulation of tissue factor expression in endothelial cells [73], increased expression of the gene PAI-1 [74] and increased platelet aggregation [75].

The association between mTOR inhibitors and risk of VTE remains controversial in the literature. In 2002, the FDA raised the alarm about a possible association of sirolimus with hepatic artery thrombosis. This warning was based on 2 studies in liver transplantation that were not submitted for peer review; therefore, data are difficult to obtain. The only abstract available [76] is from an open-label, randomized, multicenter study where patients were assigned to 2 immunosuppression protocols: sirolimus, CsA and corticosteroids (n = 111) or tacrolimus and corticosteroids (n = 52). The incidence of hepatic artery thrombosis was higher in the sirolimus group, but the difference did not reach statistical significance. The other study is mentioned in *McKenna et al.* [77] and compares 110 recipients in the sirolimus group with 112 patients in the tacrolimus group. Again, no statistically significant differences were found for the incidence of hepatic artery thrombosis, although higher rates of death and graft loss led to the early termination of the study and prompted the FDA warning.

Several subsequent studies in liver transplant and RT have not been able to resolve the controversy. Although liver recipients set off the alarm, studies have described reduced or unchanged incidence of hepatic artery thrombosis in sirolimus-treated patients [77–80]. In RT, studies based on small samples show conflicting results regarding the incidence of VTE and its association with sirolimus [81,82]. *Baas et al.* [83] performed a single-center study describing coagulation profiles after RT and found that everolimus was an independent determinant of higher levels of vWF, prothrombin fragment 1-2, PAI-1 and thrombin-activatable fibrinolysis inhibitor, which leads to increased endothelial activation, thrombin formation and impaired fibrinolysis.

In contrast, data available for lung transplantation (2 studies) and heart transplantation (1 study) endorse the association between mTOR and risk for VTE. *Lingaraju et al.* [84] performed a retrospective study in 278 lung transplant recipients and found a higher incidence of VTE in patients using sirolimus (56.7% vs. 24.4% $p < 0.001$). Although the use of sirolimus as rescue therapy after acute or chronic rejection –and therefore in a sicker population– could act as a confounder, these results were later confirmed by *Ahya et al.* [85] in a prospective, multicenter, randomized, open-label trial. The authors compared 2 groups of patients: one receiving tacrolimus, azathioprine and corticosteroids and another receiving tacrolimus, sirolimus and corticosteroids. Even though the incidence of VTE was not the primary endpoint, a subanalysis revealed a significantly higher incidence of VTE in the sirolimus cohort (17.2% vs. 3.2% $p<0.001$). Lower limb edema was reported to be another possible cofounder that could trigger more DVT screening in the sirolimus group. The only evidence in heart transplantation [86] also supports this association, with a hazard ratio for VTE associated with sirolimus of 2.74 ($p = 0.04$) in a population of 67 patients receiving sirolimus and 134 recipients receiving other immunosuppressive treatment.

Mycophenolate Mofetil

Data on the thrombotic capacity of mycophenolate mofetil (MMF) are lacking. Some in vitro some studies report decreased aggregation of platelets in RT [58,87]. However, there are some reports of local thrombosis related to intravenous administration of MMF and 1 case report [88] of recurrent DVT in a patient treated with MMF who was heterozygous for factor V Leiden.

Table 4: Coagulation abnormalities caused by immunosuppressive drugs

Immunosupressive drug	Possible pro-coagulant mechanism	Anti-coagulant alterations
Cyclosporine A (CsA)	Serotonin-induced platelet activation [56] Enhanced hemostasis [60, 62, 61] Decreased protein C [63] Impaired fibrinolysis [62, 64]	Increased antithrombin III and protein C [60]
Tacrolimus	Serotonin-induced platelet activation [56]	Inhibition of platelet activity [57] Inhibition of coagulation [57]
Corticosteroids	Impaired fibrinolysis [71,72]	Not described
mTOR inhibitors	Increased platelet aggregation [75] Impaired fibrinolysis [74] Regulation of tissue factor expression in endothelial cells [73]	Not described
Mycophenolate mofetil	Not described	Decreased platelet aggregation [58,87]

1.4. TRANSPLANTATION AS A PROCOAGULANT STATE

Coagulation profiles after SOT have yet to be fully explored. Current data are scarce and mainly refer to kidney and liver transplantation.

The early period after RT is characterized by a procoagulant state that later tends to normalize, albeit with some procoagulant factors persistently abnormal in the long term. *Nampoory et al.* [89] performed a prospective study in patients on regular hemodialysis therapy, with a 6presented an abnormal coagulation profile consisting of PC deficiency (24.4%), PS deficiency (32.1%), ATIII deficiency (19.2%) and activated protein C resistance (20%). The incidence of vascular access thrombosis was 26.8%,

and logistic regression analysis correlated vascular access thrombosis with PS deficiency, presence of lupus anticoagulant and PC deficiency (for which a borderline correlation was observed, $p = 0.06$). Twenty of these patients subsequently received a live-related donor RT, and 16 were available for study. The patients had demonstrable pre-transplantation hypercoagulable changes: decreased PC (25%), decreased PS (68.8%), decreased ATIII (25%) and activated protein C resistance (31.3%). After a mean of 9.3 ± 4.2 months of follow up, they were reevaluated, and abnormalities related to a procoagulant state had been corrected.

Cho et al. [90] later performed a similar study in 531 Asian kidney recipients. Coagulation profiles were evaluated before transplantation and during postoperative days (POD) 7, 14 and 28. Increased D-dimer and homocysteine levels before RT persisted after transplantation up to the last follow-up in the case of D-dimer and until day 14 in the case of homocysteine. A high prevalence of lupus anticoagulant and anticardiolipin antibodies were seen before RT, although this tended to decrease after surgery. The prevalence of thrombophilic risk factors decreased from 80.9% before RT to 47% at POD 28. Moreover, at all postoperative assessments, the glomerular filtration rate of kidney recipients with no thrombophilic factors was higher than that of recipients with 1 or more thrombophilic factors. Therefore, the authors suggested that hypercoagulability after RT could be corrected by improving renal function. *Pawliki et al.* [91] also described an improvement in the procoagulant profile during the first 2 weeks after RT owing to increased activity of ATIII, PC and PS. At the same time, high levels of D-dimer were observed both at day 7 and day 14.

Focusing on the long-term coagulation profile, *Irish et al.* [92] reported persistent and increased thrombin generation (increased levels of F1+2 and D-dimer) and impaired fibrinolysis in a study performed 1 year after RT (median 5 years). These changes were exacerbated in patients with cardiovascular or metabolic diseases.

Altogether, thrombophilia seems a prevalent problem after RT, and its importance lies in the fact that it has been related to an increased risk of graft loss [93–97] and increased cardiovascular risk [92].

In liver transplantation, recent attention has focused on thromboembolic complications, and coagulation profiles have been studied in the short term after surgery. *Stahl et al.* [98] performed a study of 30 liver recipients in which they analyzed coagulation factors before and after transplantation (POD 1, 3, 5 and 10). They reported rapid normalization of PT and PTT. At POD 3, all procoagulant factors were within the normal range except FVIII and vWF, which remained persistently high throughout the study. In contrast, anticoagulant proteins had a delayed recovery, with most patients having low levels of ATIII (58%) and abnormal protein C (12.5%) at POD 10.

Other studies have focused on this persistent increase in procoagulant factors. Before liver transplantation, thrombocytopenia in cirrhotic patients is balanced by high levels of vWF, although the functionality of this marker is somehow compromised [99]. A decreased platelet count has been described up to POD 10 [100], and this is associated with persistently high levels of vWF, with rising functionality and low levels of ADAMTS 13. This results in a profound imbalance in the vWF/ADAMTS13 ratio that has been related to thrombotic risk. As for fibrinolysis, a hypofibrinolytic state has been described 5 days after liver transplantation owing to an increase in PAI-1 levels [101]. To sum up, it seems clear that a procoagulant tendency has to be taken into account after liver transplantation.

1.5. PREVENTION AND TREATMENT OF VTE AFTER SOT

There is a lack of consensus about whether or not is it advisable to use a prophylactic strategy for VTE after SOT. The greatest concern is the risk of bleeding complications as a consequence of this strategy. As a result of this duality there is wide

diversity in clinical practice as was reflected in a study by *Ripert et al.* [102]. The authors performed a telephone survey of all renal transplantation centers in France, 4 cases were assessed per center, and very different protocols were proposed in each of them. This diversity is also depicted in the different strategies evaluated in the literature, some of which were applied in the immediate postoperative period and others for longer after transplantation. ***Table 5*** summarizes the published literature.

1.5.1. Postoperative prophylaxis

Heparin is the basis of most short-term prophylactic strategies that have been evaluated and is usually administered until POD 7. Available data show an overall tendency toward lower graft thrombosis, with an increased risk of major bleeding in transplant recipients with no risk factors. *Murashima et al.* [103] performed a study selecting only patients with risk factors for VTE and reported similar results. The authors retrospectively compared 32 kidney recipients without anticoagulation with 16 patients receiving different anticoagulation protocols: 12 with nonfractionated heparin followed by warfarin, 3 with nonfractionated heparin followed by aspirin and 1 receiving nonfractionated heparin alone. They reported a lower incidence of graft thrombosis in the second cohort (18.8% vs. 6.3%), although with a relevant incidence of bleeding complications (around 31%).

Despite the similarity of the results, there is no consensus on the recommendations: some authors are in favor of using heparin prophylaxis in the early postoperative period in all transplanted patients [104], although none of the differences reported were statistically significant, whereas most agree on administering anticoagulation only to high-risk patients [103,105,106] or with frequent monitoring of coagulation [107,108] owing to a significant bleeding risk that can reach 31% [103].

Shullo et al. [109] explored a different protocol, namely, the combination of 81 mg per day of aspirin plus low-molecular-weight heparin for 10 days in 13 patients

after kidney or kidney-pancreas transplantation. Despite the absence of thrombotic complications, an alarming 69% incidence of major bleeding events led to the withdrawal of the combination.

1.5.2. Extended prophylaxis

In addition to anticoagulation protocols, extended prophylaxis has also been evaluated in antiplatelet protocols, as is the case for VTE prophylaxis in orthopedic surgery.

Three protocols explore the use of anticoagulation during the long term after SOT surgery. All 3 consider prophylaxis only for high-risk patients with different durations of treatment: 1 month [110], at least 6 months [111] and indefinite [112]. They coincide in their conclusions, namely, it might be advisable to administer anticoagulation to high-risk patients for some months after transplantation, albeit with diligent observation of coagulation, mainly during the postoperative period. The precise duration and the definition of high-risk patients have yet to be confirmed.

Antiplatelet therapy seems to be a good option, and 4 large studies [113–116] reported a decrease in graft thrombosis with minimized bleeding risk (the highest incidence was reported by *Robertson et al.* [113] and is less than 3%). In contrast, *Shay et al.* [117] could not find any statistically significant effect of aspirin 325 mg per day for 3 months compared with no prophylaxis.

The rationale for extended prophylaxis is based on the description of a high incidence of thrombotic events not only after surgery, but also in the following months. Despite this clinical observation, the only study comparing short and extended prophylaxis did not find any differences between them. *Wolf et al.* [118] compared 2 different cohorts of liver recipients; the first (n = 354) was treated with aspirin 81 mg per day indefinitely. After a change in their protocol due to a concern for a

higher bleeding risk, patients received prophylaxis with nonfractionated heparin until POD 7 (n = 175). In this study, aspirin did not have any effect on reducing the incidence of hepatic artery thrombosis and was associated with a higher rate of gastrointestinal bleeding (nonsignificant). Therefore, in conclusion, this study does not support the use of long-term prophylaxis with aspirin after liver transplantation and favors the use of short-term prophylaxis.

Thus, larger, multicenter, randomized clinical trials will be needed to identify the best prophylactic strategy, especially after lung transplantation, for which no information is available.

Table 5: Prophylactic protocols evaluated in literature

	Reference	Organ	Prophylactic strategy	Results	Complications
Strategies of Postoperative prophylaxis					
Anticoagulation	Ubhi et al. [104]	Kidney	Cohort 1 (n: 37): no anticoagulation Cohort 2 (n: 32): SCaH for 7 d or until fully mobile	No statistically different (tendency to lower GT)	No increase
	Kaneko et al. [107]	Liver	Dalteparin 2d followed by NFH adjusted by ACT (n: 128)	3% thrombotic events	15% bleeding events
	Osman et al. [105]	Kidney	Cohort 1 (n: 25): no anticoagulation Cohort 2 (n: 25). LMWH for 1 w. Cohort 3 (n: 25): NFH for 1w.	No thrombotic events	1 case of massive bleeding in cohort 2
	Uchikawa et al. [108]	Liver	Cohort 1 (n: 32): fixed LMWH dose + FFP Cohort 2 (n: 10): LMWH adjusted by ACT + FFP	No statistically different (tendency to lower GT)	1 bleeding event in cohort 1 (3.1%)
	Murashima et al. [103]	Kidney	Cohort 1(n: 32): high risk patients without anticoagulation Cohort 2: high risk patients with anticoagulation (NFH+W n:12; NDH + aspirin n: 3; NFH alone n: 1)	Lower GT rate (from 18.8% vs 6.3%)	Higher incidence of bleeding complications in cohort 2 (31.3% vs. 6.3%)
	Bakkaloglu et al. [119]	Kidney	Cohort 1 (n: 25): compressive stockings Cohort 2 (n: 25): compressive stockings + LMWH until POD 7	No thrombotic events	1 case of massive bleeding in cohort 2
Anticoagulation + Antiplatelet agents	Shullo et al. [109]	Kidney and Kidney-pancreas	Aspirin 81mg/d plus LMWH until POD 10 (n: 13)	No thrombotic events	9 cases (69%) of major bleeding complication
Strategies of Extended prophylaxis					
Anticoagulation	Alkhunaizi et al. [110]	Kidney	Cohort 1: high risk W after LMWH 1 mo. Cohort 2: low risk LMHW hospitalization	No thrombotic events	No bleeding events
	Friedman et al. [112]	Kidney	Cohort 1(n: 346): no anticoagulation Cohort 2 (n: 502): W after NFH in high risk patients	2.6- fold reduction in GT incidence	60% hemorrhagic complications the first 30 POD
	Morrissey et al. [111]	Kidney	Cohort 1: low risk (n: 227). No anticoagulation Cohort 2: high risk(n: 8); W after preoperative heparin at least 6 mo.	Lower GT rate (0.8% vs 0%)	2 cases of major bleeding (25%)
Antiplatelet agents	Robertson et al. [113]	Kidney	Cohort 1 (n: 475): no prophylaxis Cohort 2 (n: 480): aspirin 75mg/d for 1 mo.	Lower GT rate (from 5.6% vs 1.2%)	13 cases (2.7%) of major bleeding complication
	Murphy et al. [114]	Kidney	Cohort 1 (n: 121): no prophylaxis Cohort 2 (n: 105): aspirin 150mg/d for 3 mo.	Lower GT rate (from 5% vs 0%)	No major bleeding complications
	Stechman etl al. [115]	Kidney	Cohort 1 (n: 396): no prophylaxis Cohort 2 (n: 401): aspirin 75mg/d	Lower GT rate (from 5.8% vs 0.25%)	No data
	Vivarelli et al. [116]	Liver	Cohort 1 (n: 592): no prophylaxis Cohort 2 (n: 236): aspirin 100mg/d	Lower GT rate (2.2% vs 0.4%) especially in high risk patients	No bleeding events
	Shay et al. [117]	Liver	Cohort 1 (n: 304) no prophylaxis Cohort 2 (n: 135): aspirin 325mg/d for 3 mo.	No statistically significant differences in overall GT	No statistically differences
Comparison of short vs. extended prophylaxis					
Postoperative NFH vs. Indefinite aspirin	Wolf et al. [118]	Liver	Cohort 1 (n: 354): aspirin 81mg/d Cohort 2 (n: 175): NFH until POD 7	No statistically different (3.7% vs 4%)	No statistically different (18.9% vs 12.8%)

LMWH: low-molecular-weight heparin; ***ACT***: Activated Clotting time; ***FFP***: fresh frozen plasma; ***POD***: postoperative day; ***GT***: graft thrombosis; ***W***: warfarin; ***NFH***: non-fractioned heparin.

Rationale and objectives

2

VTE is a relevant complication after SOT and, specifically after LT. Thus, transplantation has been considered as a complex environmental risk factor itself, which includes the risk arising for surgery and others factors like underlying disease, CMV infection and immunosuppressive treatment. These factors interplay with other environmental and with genetic factors in a somehow unpredictable way, constituting the individual risk of an individual patient. Although thrombotic complications have been related to graft loss, this is an issue scarcely addressed in literature.

Thus, this work is composed of two studies addressing in the first place, from a clinical point of view, the relevance of this complication in our population of lung transplanted patients focusing in the risk factors and the impact of a extended prophylaxis protocol; and in the second place, form a mechanistic point of view, we describe coagulation factors profiles after lung transplantation and thrombophilia genetic profiles to assess the pathogenesis of the disease. So, the main objective of this thesis is to describe the coagulation status and the risk of VTE after LT.

2.1. SPECIFIC OBJECTIVES FOR STUDY 1

- To evaluate prophylaxis with enoxaparin up to day 90 or until full mobility is recovered after LT to prevent VTE.
- To describe the epidemiology of VTE in a series of lung transplanted patients.
- To study specific risk factors for VTE in a population of lung transplanted patients.

2.2. SPECIFIC OBJECTIVES FOR STUDY 2

- To describe coagulation profiles before and after transplantation with the hypothesis that we may find a procoagulant state.

Method

3

3.1. SPECIFIC METHODOLOGY FOR STUDY 1

Study population

We retrospectively reviewed the computerized clinical records of 333 consecutive adult patients who underwent LT in our institution between January 2009 and December 2014. We recorded prophylaxis, age, gender, body mass index, diabetes mellitus, previous thrombotic events, CMV status, underlying disease, transplant type, need for cardiopulmonary bypass, mechanical ventilation, length of stay, primary graft dysfunction, medication, and mobility. The study protocol was approved by the institutional Ethics board.

We identified patients with any thrombotic event during the first year after LT, including DVT, PE, and treated upper extremity thrombosis (TUET). Patients receiving lifetime anticoagulation therapy prior to LT were excluded. Incidental PE (untreated subsegmental perfusion defects without clinical repercussion) was not taken into account.

As part of our standard protocol, all patients underwent a ventilation-perfusion scan (VPS) following lung transplantation immediately before discharge. No subsequent screening for VTE was performed. Patients who presented with symptoms suggestive of DVT were further studied using Doppler ultrasound (US). If PE was suspected, patients underwent either VPS or computed tomography (CT) pulmonary angiography.

Between 2009 and 2012 prophylaxis with the low-molecular-weight heparin (LMWH) enoxaparin (40 mg subcutaneously) was given once daily to all patients admitted to hospital after LT (control cohort). The prophylaxis began on post-operative day 1 if there were no formal contraindications. In January 2013, concern over the high incidence of VTE led us to change our standard protocol, and patients have since been receiving prophylactic enoxaparin up to day 90 or until full mobility is recovered

(study cohort). Dosing of enoxaparin was adjusted in renal impairment and according to patient weight. We did not use any protocol to monitor enoxaparin treatment.

Data analyses

Qualitative variables are expressed as absolute numbers and percentages. Normally distributed quantitative variables are expressed as the mean and standard deviation; non-normally distributed variables are expressed as the median and interquartile range (p25-p75).

The demographic and clinical variables of patients receiving conventional prophylaxis and those receiving 90-day extended prophylaxis after LT were compared using the chi-square test for qualitative variables (or the Fisher exact test when one of the expected effects was less than 5). Normally distributed quantitative variables were compared using an unpaired t test; non-normally distributed quantitative variables were compared using the Mann-Whitney test.

Cumulative incidence function for competing risk analysis was used to determine incidence of VTE through the formulas proposed by *Gooley et al.* [120] and *Greenwood et al.* (cited in *Marubini et al.* [121]) using the STATA syntax stcompet; both for the entire group and according to type of anticoagulant prophylaxis. Death or re-transplant without previous VTE were treated as competing risks.

Cox proportional hazards regression was applied by modeling time from LT to the first event, with VTE as the outcome measure. We first conducted univariate analyses based on the Cox proportional hazards model using each of the potential predictors of VTE as independent variables and VTE as the dependent variable. Then, we performed a stepwise multivariable Cox regression with a backward elimination (p-value criterion of 0.20) fitted with all candidate variables, after adjusting for type of anticoagulant prophylaxis.

Data were analyzed using Stata software (StataCorp. 2011, release 12.1).

3.2. SPECIFIC METHODOLOGY FOR STUDY 2

We performed a longitudinal prospective study of all 106 candidates included on the waiting list for LT at our institution between June 2015 and December 2016 in order to recruit a planned sample of 50 patients. The first 48 patients who underwent transplantation were finally included. The study was approved by the Institutional Ethics Board of Hospital Vall d'Hebrón, Barcelona, Spain

Demographic and clinical data were collected. We identified patients with any thrombotic event during the first 4 months after LT, including deep vein thrombosis (DVT), pulmonary embolism (PE), and treated upper extremity thrombosis. As part of our standard protocol, all patients underwent a VPS immediately before discharge. No subsequent screening for VTE was undertaken. Doppler ultrasound was performed in patients with symptoms suggestive of DVT. If PE was suspected, patients underwent either a ventilation-perfusion scan or computed tomography angiography. All patients received prophylaxis with low-molecular-weight heparin up to day 90 or until full mobility was recovered, except for 1 patient who had chronic thrombocytopenia.

Blood samples were obtained at various time points: at baseline (when patient was on the waiting list) and at 48-72 hours, 7-14 days, and 4 months after LT. We also present data from 34 LT patients who completed 1 year of follow-up.

1. ***Plasma samples for clotting and antigenic assays:*** Blood was obtained by venipuncture in calcium citrate tubes; samples were centrifuged at room temperature and 3000 rpm for 10 minutes to obtain platelet-poor plasma. When necessary, plasma aliquots were stored frozen at –80ºC and then thawed in a water bath at 37ºC for 10 minutes before the assay.
2. ***Plasma samples for the homocysteine assay:*** Blood was obtained by venipuncture in calcium oxalate tubes; samples were centrifuged at 3500 rpm for 12 minutes at 4ºC to obtain platelet-poor plasma.

3. ***Blood samples for determination of thrombophilia polymorphisms:*** DNA was purified from EDTA blood. Testing for the Prothrombin G20210A and factor V Leiden polymorphisms was performed using a real-time PCR system (Roche Diagnostics).

Relevant methods and reagents used for blood tests and assays are listed in ***Table 6***. P-selectin and ADAMTS 13 were assessed using assays performed on a Best2000 microplate reader instrument (Biokit, Barcelona, Spain). Homocysteine was measured on a BN II Nephelometer (Siemens, Marburg, Germany). All the other variables were measured using an ACL TOP 700 LAS coagulometer (Werfen, Barcelona, Spain).

Table 6: Assays used for blood testing

Parameter	Test and Reagents
PT	HemosIL PT RecombiPlasTin 2G reagent
APTT	HemosIL SynthASil
Clauss fibrinogen	HemosIL Fib-C reagent
D-dimer	HemosIL D Dimer HS latex reagent
vWF	HemosIL Acustar VWF:Ag reagent kit
FVIII	HemosIL SynthASil and HemosIL FVIII deficient plasma
PC	HemosIL Protein C chromogenic reagent kit
PS	HemosIL Free Protein S latex reagent kit
Plasminogen activity	HemosIL Plasminogen chromogenic reagent
α2-Antiplasmin	HemosIL PI chromogenic reagent
Homocysteine	Latex HCY Siemens Reagent Kit
R protein C	HemosIL FV Leiden
P-Selectin	Invitrogen P selectin (Thermo Fisher Scientific)
ADAMTS 13 Activity	Technoclone chromogenic method

PT: prothrombin time; ***APTT***: activated partial thromboplastin time; ***vWF***: Von Willebrand factor antigen; ***FVIII***: factor VIII activity; ***PC***: protein C activity; ***PS***: free protein S; ***R protein C***: activated protein C resistance; ***ACL TOP***: AcuStar and HemosIL IL are all products manufactured by Instrumentation Laboratory Co. *(Bedford, MA, USA).*

Table 7 shows the assays performed at each time point.

Table 7: Hematologic tests and assays protocol

Baseline	48-72 h post-LT	7-14 d post-LT	4 mo post-LT	1 yr post-LT
Thrombophilia panel Fibrinogen D-dimer vWF Plasminogen α2-Antiplasmin P-Selectin ADAMTS 13	PT, APTT Fibrinogen D-dimer vWF FVIII PC PS Plasminogen α2-Antiplasmin	PT, APTT Fibrinogen D-dimer vWF FVIII PC Protein S Plasminogen α2-Antiplasmin P-Selectin ADAMTS 13	PT, APTT Fibrinogen D-dimer vWF FVIII PC Protein S Plasminogen α2-Antiplasmin	PT, APTT Fibrinogen D-dimer vWF FVIII PC PS Plasminogen α2-Antiplasmin P-Selectin ADAMTS 13

Fibrinogen: Clauss fibrinogen; ***vWF***: Von Willebrand factor antigen; ***plasminogen***: plasminogen activity; ***PT***: prothrombin time; ***APTT***: activated partial thromboplastin time; ***FVIII***: factor VIII activity; ***PC***: protein C activity; ***PS***: free protein S; ***R protein C***: activated protein C resistance; ***ADAMTS13***: ADAMTS13 activity

Data analysis

Results are expressed as absolute numbers and their corresponding percentages for qualitative variables, as the mean and standard deviation for quantitative variables with a normal distribution, and as the median and 25th to 75th percentiles for quantitative variables with a non-normal distribution.

Post-LT coagulation parameters were compared with baseline (pre-transplant) using the Wilcoxon matched-pairs signed-ranks test for non-normally distributed quantitative variables. Coagulation parameters were compared according to VTE status using the Mann-Whitney test for non-normally distributed quantitative variables. Analyses were corrected for multiple comparisons using the Bonferroni method. Spearman's rank correlation coefficient was used to analyze the relationship between numerical variables. Receiver operating characteristic (ROC) curves were constructed to assess the ability of some coagulation parameters to predict VTE events occurring during follow-up.

Data were analyzed using Stata 12.1 (StataCorp, College Station, TX, USA).

Results

4

4.1. STUDY 1: PROPHYLAXIS WITH ENOXAPARIN FOR PREVENTION OF VENOUS THROMBOEMBOLISM AFTER LUNG TRANSPLANTATION

4.1.1. Clinical characteristics and epidemiology of VTE

Table 8: Characteristics of LT recipients according to anticoagulant prophylaxis

	All n = 333	Control cohort n = 195	Study cohort n = 138	p
Pretransplant variables				
Age, mean (SD)	52.0 (11.4)	50.2 (11.8)	54.5 (10.5)	**< 0.001**
Sex: Male, n (%)	201 (60.4)	119 (61.0)	82 (59.4)	0.768
BMI, n (%) [n = 327]				
<20 kg/m², n (%)	33 (10.1)	23 (12.1)	10 (7.3)	0.199
20-24.9 kg/m², n (%)	110 (33.6)	58 (30.5)	52 (38.0)	
≥25 kg/m², n (%)	184 (56.3)	109 (57.4)	75 (54.7)	
Diagnosis, n (%)				
Interstitial lung diseases (ILD)	143 (42.9)	74 (38.0)	69 (50.0)	**0.029**
COPD, bronchiectasis, or BO	123 (36.9)	73 (37.4)	50 (36.2)	0.823
Cystic fibrosis	25 (7.5)	16 (8.2)	9 (6.5)	0.566
Pulmonary arterial hypertension	17 (5.1)	13 (6.7)	4 (2.9)	0.138
LAM	5 (1.5)	3 (1.5)	2 (1.5)	0.989
Other	20 (6.0)	16 (8.2)	4 (2.9)	0.989
Pretransplant diabetes mellitus, n (%)	52 (15.6)	22 (11.3)	30 (21.7)	**0.010**
Pretransplant VTE, n (%)	10 (3.0)	8 (4.1)	2 (1.5)	0.205
Pretransplant CMV serology (positive), n (%)	282 (84.7)	161 (82.6)	121 (87.7)	0.201
Peritransplant variables				
***Type of lung transplant,* n (%)**				
Bilateral	201 (60.4)	116 (59.5)	89 (64.6)	0.699
Single	132 (39.6)	79 (40.5)	53 (38.4)	
Extracorporeal circulation, n (%)	75 (22.5)	49 (25.1)	26 (18.8)	0.176
Reintervention, n (%)	32 (9.6)	15 (7.7)	17 (12.3)	0.158
Surgical complications, n (%)	38 (11.4)	23 (11.8)	15 (10.9)	0.794
Hemodynamic instability, n (%)	213 (64.0)	135 (69.2)	78 (56.5)	**0.017**
Days on mechanical ventilation, median (p25-p75)	13 (2-37)	13 (2-42)	12 (2-36)	0.571
Days of hospitalization, median (p25-p75)	37 (25-60)	38 (25-65)	36 (24-56)	0.228
Primary graft dysfunction, n (%)	114 (34.3)	71 (36.4)	43 (31.4)	0.343
Post-transplant variables				
Treatment with mTOR, n (%)	7 (2.1)	3 (1.5)	4 (2.9)	0.454
Reduced mobility 3 months post-transplant, n (%) [n = 258] ^	41 (15.9)	19 (12.7)	22 (20.4)	0.169
CMV disease, n (%)	20 (6.0)	14 (7.2)	6 (4.4)	0.284
Incidence of thromboembolic events				
Total thromboembolic events, n (%)	50 (15.0)	31 (15.9)	19 (13.7)	0.592
Before discharge	29 (58.0)	19 (9.7)	10 (7.3)	0.426
After discharge [n = 261]*	21 (8.1)	12 (7.8)	9 (8.3)	0.886
< 90 days after discharge [n = 261]*	11 (22.0)	7 (4.6)	4 (3.7)	0.730
> 90 days after discharge [n = 240]°	10 (20.0)	5 (3.7)	5 (4.9)	0.749

^ Patients at risk of a thromboembolic event at 3 months after transplant.

***** Patients at risk of a thromboembolic event after hospital discharge.

° Patients at risk of a thromboembolic event 90 days after hospital discharge.

BMI*:** body mass index; ***COPD: chronic obstructive pulmonary disease; ***BO***: bronchiolitis obliterans; ***LAM***: lymphangioleiomyomatosis; ***VTE***: venous thromboembolism; ***CMV***: cytomegalovirus.

The study group (90-day extended prophylaxis) comprised 138 patients, and the control group 195 patients. The demographic characteristics of both cohorts are shown in ***Table 8***.

Older age, interstitial lung disease as the underlying disease, diabetes mellitus prior to transplantation, and hemodynamic instability (defined as the need for vasoactive drugs) were more frequent in the study cohort. However, the incidence of thromboembolic events was not significantly different between the protocols (***Table 1***). Thirteen patients in the study group did not receive the prophylaxis protocol due to diverse reasons, but none of them developed VTE.

Thromboembolic events

Fifty patients developed VTE and 52 died during the first year after LT. The cumulative incidence of VTE during this period was 15.3% (95% CI: 11.6-19.4) in the entire group; 16.1% (95% CI: 11.3-21.6) in the cohort receiving conventional prophylaxis and 14.1% (95% CI: 8.9-20.6) in cohort receiving 90-day extended prophylaxis, with no differences between the two cohorts (***Figure 1***). The events are classified in ***Table 9***.

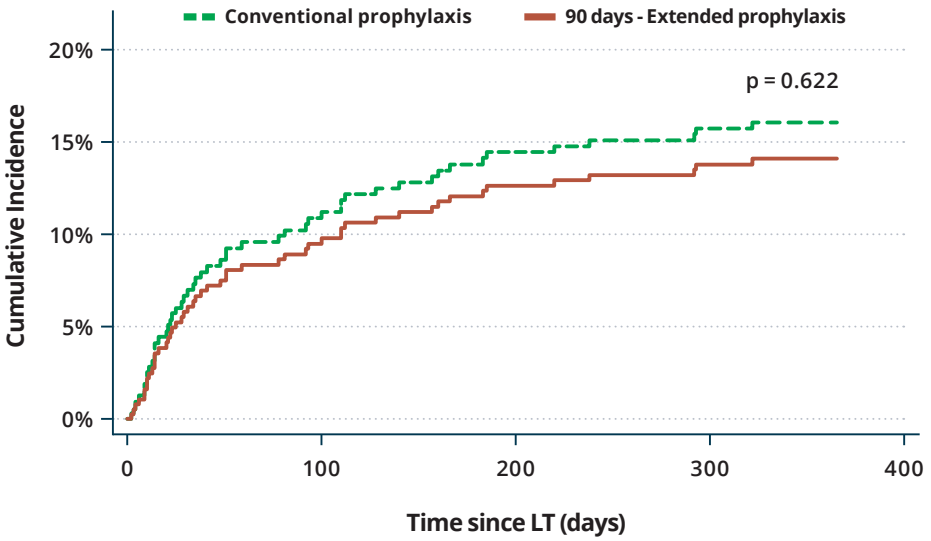

Figure 1: Cumulative incidences of thromboembolic event according to anticoagulant prophylaxis (n = 333)

Table 9: Classification of the thromboembolic events recorded during the first year after LT

	Patients with a thromboembolic event n = 50 (%)
Deep venous thrombosis (DVT) of the lower extremities	2 (4.0)
Pulmonary embolism (PE)	35 (70.0)
PE + DVT	5 / 35
Treated upper extremity thrombosis	12 (24.0)
Atrial thrombosis	1 (2.0)

Median time from transplant to the event was 40 days (p25-p75 14-112). Twenty-nine events (58%) took place before hospital discharge (***Table 1*** and ***Figure 2***); of the other 21 events, 11 (22%) were within the first 90 days after LT and 10 (20%) were between post-operative day 90 and the first year (***Table 1***). There were 22 events in single LT patients, 9 (41%) of them localized in the graft.

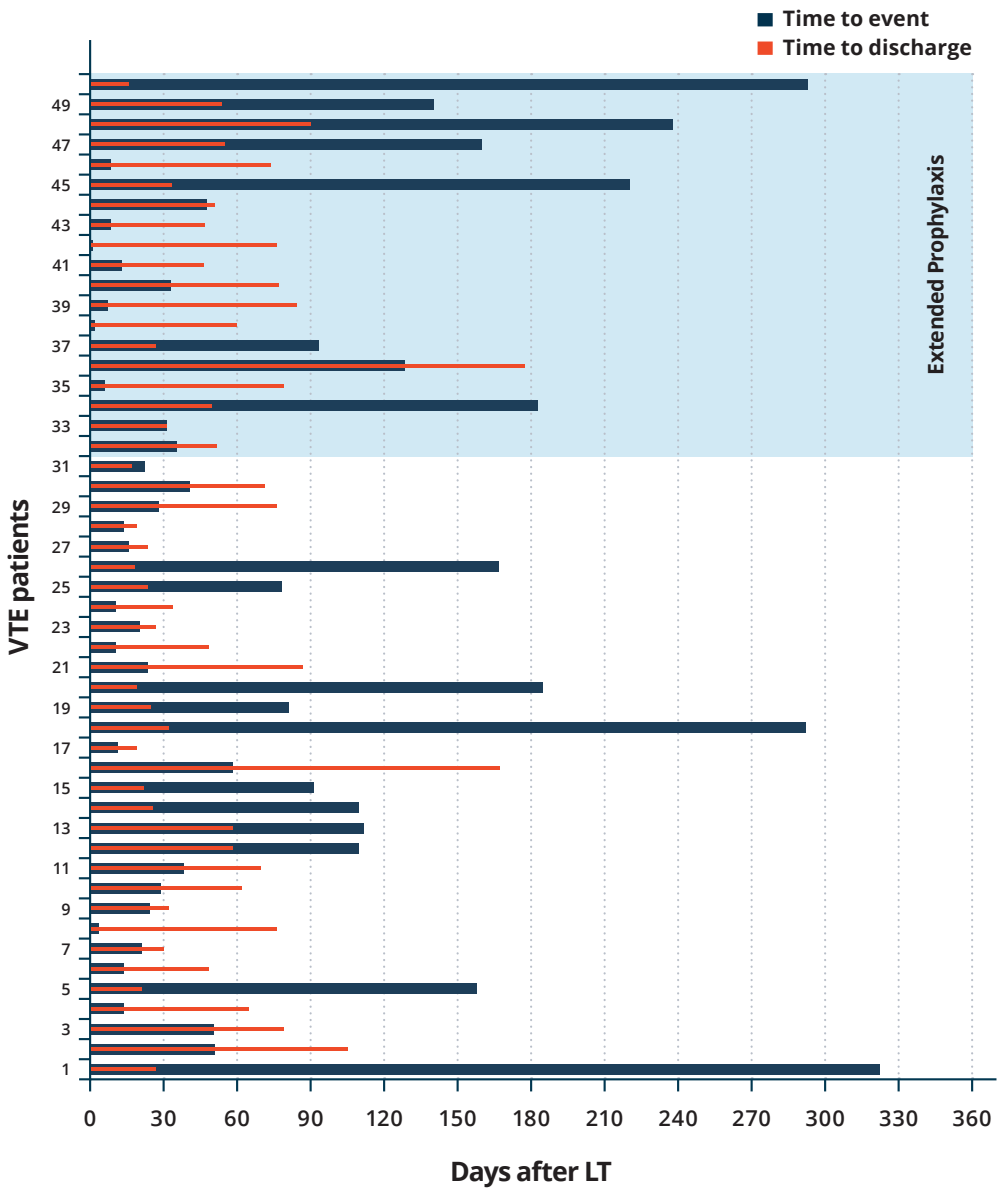

Figure 2: Time to event and hospital discharge of all patients with VTE

Pulmonary embolism and deep venous thrombosis of the lower extremities

Forty-one PE and 2 DVT were diagnosed during the first year after LT. We considered 6 asymptomatic subsegmental PE to be incidental events that were not treated; therefore, they were not taken into account for the purposes of the study.

The final sample was 35 PE. The clinical characteristics of the events are shown in ***Figure 3***.

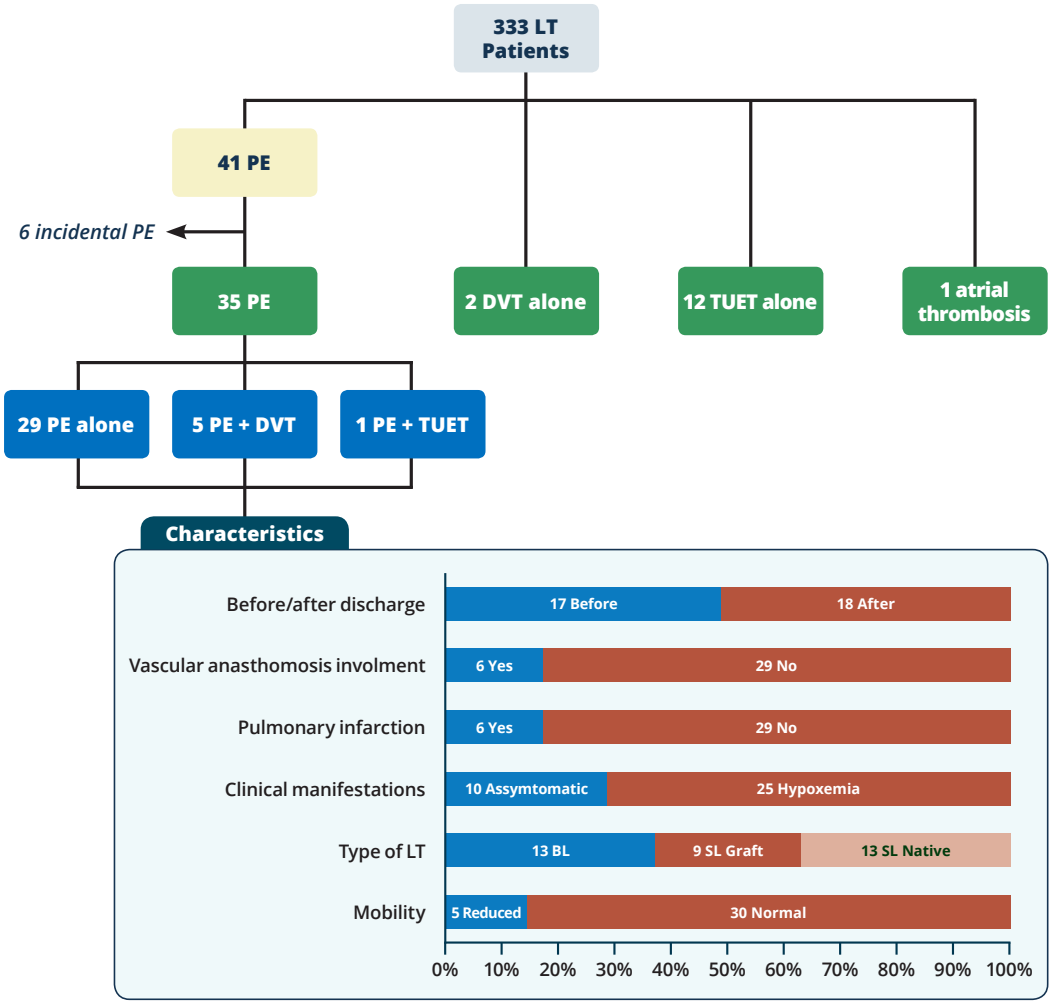

Figure 3: Flow chart and characteristics of the thrombotic events

Four single LT recipients due to interstitial lung disease did not have CT angiography to confirm the diagnosis. In 3 out of these 4 cases, a high probability VPS was considered enough for the diagnosis of PE. The last case had an intermediate probability VPS coinciding with a femoral vein DVT.

No patients fulfilled the criteria for massive PE. Thirteen patients underwent echocardiography close to the event, and 3 presented right ventricular dilatation. Coagulation assays revealed 1 lupus anticoagulant–positive patient and 1 patient with mild factor VII deficiency.

In 24 (68.6%) of the 35 patients with PE, VPS was performed between 7 and 857 days after the event (median time of 172 days). Partial reperfusion of the defects was reported in 10 patients and persistent perfusion defects in 2. One patient had a new perfusion defect (detected by VPS 145 days after the event) No patients developed pulmonary hypertension after PE.

There were 12 deaths (32.4%), 11 of which were considered not directly related to the thrombotic event. The causes of death were chronic allograft dysfunction (4 cases), respiratory infection (3 cases), sepsis (2 cases), melanoma (1 case), and multiple organ failure (1 case). During the follow-up (median 659 [p25-p75 138-1337] days), 8 of the 37 patients (21.6%) developed chronic allograft dysfunction (5 cases of bronchiolitis obliterans syndrome and 3 cases of restrictive allograft syndrome).

Treated upper extremity thrombosis

TUET alone was diagnosed in 12 patients. Since it was related to intravenous devices, most cases were diagnosed in the ICU. Only 1 patient presented at the emergency department 5 days after discharge with swelling of the left arm that had begun 3 days earlier. All the cases were symptomatic, and thrombus extension was evaluated using ultrasound to confirm the diagnosis and the need for anticoagulant treatment.

Safety

There were 2 mild bleeding events in patients under prophylactic doses of low-molecular-weight heparin. Only one patient in the study cohort receiving

anticoagulation at treatment doses suffered a massive epistaxis with airway obstruction that required invasive mechanical ventilation and admission to intensive care unit.

In the control cohort there were 4 bleeding events in patients receiving anticoagulation treatment: hemothorax (2 cases), hematoma (1 case) and thrombocytopenia (1 case).

4.1.2. Risk factors for VTE after LT

Table 10 shows the influence of the variables included in the univariate Cox analysis on the incidence of VTE.

Table 10: Univariate predictors of VTE (Univariate Cox models)

	Hazard ratio (95%CI)	p
Pretransplant variables		
Age (years)	1.01 (0.99-1.04)	0.322
Sex: Male	3.00 (1.45-6.18)	0.003
BMI (kg/m^2)	0.99 (0.93-1.06)	0.822
Interstitial lung disease	2.40 (1.34-4.27)	0.003
COPD, bronchiectasis, or BO	0.58 (0.31-1.09)	0.092
Cystic fibrosis	0.22 (0.03-1.62)	0.138
Pulmonary arterial hypertension	0.45 (0.06-3.24)	0.426
LAM	2.47 (0.34-17.9)	0.371
Pretransplant diabetes mellitus	0.70 (0.30-1.66)	0.426
Pretransplant VTE	1.24 (0.30-5.13)	0.760
Pretransplant CMV serology (positive)	1.46 (0.62-3.42)	0.386
Peritransplant variables		
Single lung transplant	1.67 (0.96-2.91)	0.069
Extracorporeal circulation	0.85 (0.41-1.75)	0.663
Reintervention	1.12 (0.40-3.10)	0.830
Surgical complications	1.10 (0.93-3.05)	0.860
Hemodynamic instability	1.25 (0.69-2.24)	0.461
Days on mechanical ventilation	0.99 (0.99-1.00)	0.953
Days of hospitalization	1.00 (0.99-1.00)	0.453
Primary graft dysfunction	1.44 (0.82-2.53)	0.208
Post-transplant variables		
Treatment with mTOR	0.84 (0.12-6.01)	0.860
Reduced mobility 3 months after transplant ^	0.89 (0.26-3.01)	0.848
CMV disease	0.95 (0.29-3.04)	0.927
Extended prophylaxis	0.87 (0.49-1.54)	0.628

^ Patients at risk of a thromboembolic event at 3 months after transplant n = 258

BMI: body mass index; ***COPD***: chronic obstructive pulmonary disease; ***BO***: bronchiolitis obliterans; ***LAM***: lymphangioleiomyomatosis; ***VTE***: venous thromboembolism; ***CMV***: cytomegalovirus.

Multivariable Cox proportional hazards analysis revealed that male gender (HR 2.72; 95%CI 1.25-4.03; p = 0.007) and interstitial lung disease (HR 2.25; 95%CI 1.25-4.03; p = 0.007) were significantly related to VTE, after adjusting for type of anticoagulation prophylaxis (***Table 11***). Ninety-day extended prophylaxis did not seem to protect from VTE in the study population.

Table 11: Best Model of Independent Predictors of VTE after LT by Stepwise Multivariate Cox Regression Analysis, after adjusting for type of anticoagulation prophylaxis

	Hazard ratio (95%CI)	p
90d-Extended prophylaxis	0.77 (0.43-1.38)	0.386
Sex (male)	2.72 (1.25-4.03)	0.007
Diagnosis (ILD)	2.25 (1.25-4.03)	0.007

4.2. STUDY 2: COAGULATION PROFILES AFTER LUNG TRANSPLANTATION

Table 12: Clinical characteristics of 48 lung transplant recipients

	All n = 48
Age (years)	53.1 (12.8)
Sex (males)	32 (66.7)
Diagnosis	
Interstitial lung disease	26 (54.2)
COPD	14 (29.2)
Cystic fibrosis	4 (8.4)
Bronchiectasis	2 (4.1)
PAH	2 (4.1)
Type of lung transplant	
Bilateral lung transplant	30 (62.5)
Single lung transplant	18 (37.5)
Extracorporeal circulation	11 (22.9)
Primary graft dysfunction	
Grade 0	39 (81.3)
Grade 1	1 (2.1)
Grade 2 and 3	8 (16.7)
Days in the ICU	16 (7-42)
Days on mechanical ventilation	6 (2-36)
Days of hospitalization	28 (24-57)

Data are presented as n (%), mean (SD), or median (p25-p75), as appropriate.
COPD: chronic obstructive pulmonary disease; ***PAH***: pulmonary arterial hypertension; ***ICU***: intensive care unit.

Demographic data, including type of LT, primary graft dysfunction, and hospital length of stay are shown in ***Table 12***.

4.2.1. Baseline coagulation profile

At baseline, median (p25-p75) values of almost all coagulation factors were within the normal range, except for FVIII, which was above the normal range (158% [125-195]). Nevertheless, if we analyze coagulation parameters individually (***Table 13***) several patients presented procoagulation abnormalities. As FVIII was the most prevalent abnormality, we changed the cut-off point using the 90th percentile of our laboratory, which is 224.12% and indicates the highest thrombotic risk: only 3 patients (6.3%) had levels above this threshold. Besides these factors, 1 patient presented anticardiolipin antibody IgM positivity, 1 patient had a heterozygous factor V Leiden mutation, and 1 patient had a heterozygous prothrombin mutation.

Table 13: Coagulation parameters measured before transplantation

Coagulation parameters	Pre-transplant n = 48
D-dimer, n (%)	
≤ 243	40 (83.3)
> 243	8 (16.7)
vWF, n (%)	
≤ 176	26 (54.2)
> 176	22 (45.8)
FVIII, n (%)	
≤ 100	5 (10.4)
> 100	43 (89.6)
Factor VIII p90, n (%)	
< 224.12	45 (93.7)
> 224.12	3 (6.3)
ACT PC, n (%)	
≥ 70	46 (95.8)
< 70	2 (4.2)
PS, n (%)	
≥ 74.1	42 (89.4)
< 74.1	5 (10.6)

vWF: Von Willebrand factor; ***FVIII***: factor VIII activity; ***ACT PC***: protein C activity; ***PS***: free protein S.

4.2.2. Coagulation profiles after LT

Patients presented increased median values of D-dimer up to 2 weeks after transplantation, with subsequent normalization (***Figure 4a***). vWF and FVIII increased 24 hours after transplantation and remained high at 1 year after transplantation (***Figures 4b*** and ***c***). As for anticoagulant proteins, PS decreased after transplantation, with normalization at 4 months (***Figure 4d***). As for fibrinolysis abnormalities, low plasminogen was seen at 24 hours, with subsequent normalization (***Figure 4e***). The only anticoagulation abnormality was decreased α2-antiplasmin at 24 hours, which normalized at 2 weeks.

Broken line indicates upper limit of our laboratory normal range in D-dimer and von Willebrand factor;
lower limit of our laboratory normal range in Protein S and Plasminogen;
upper limit of the 90th percentile of our laboratory in factor VIII.

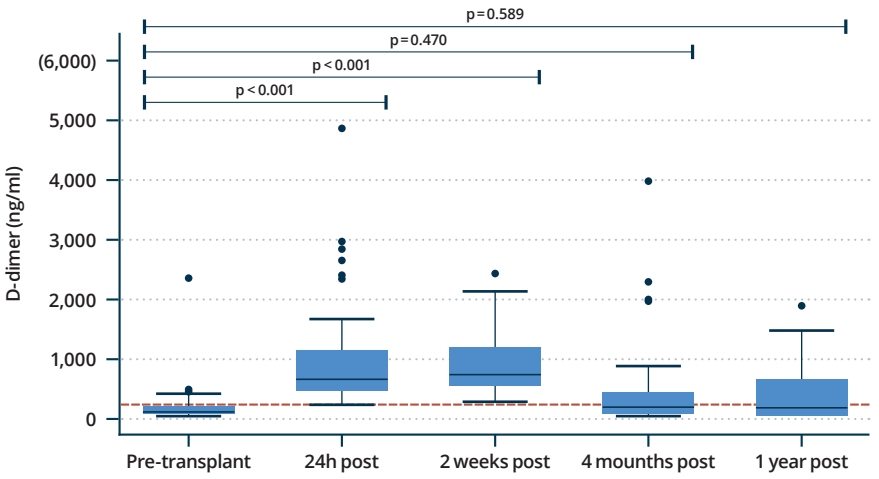

Figure 4a: D-dimer before and at different time-points post-LT

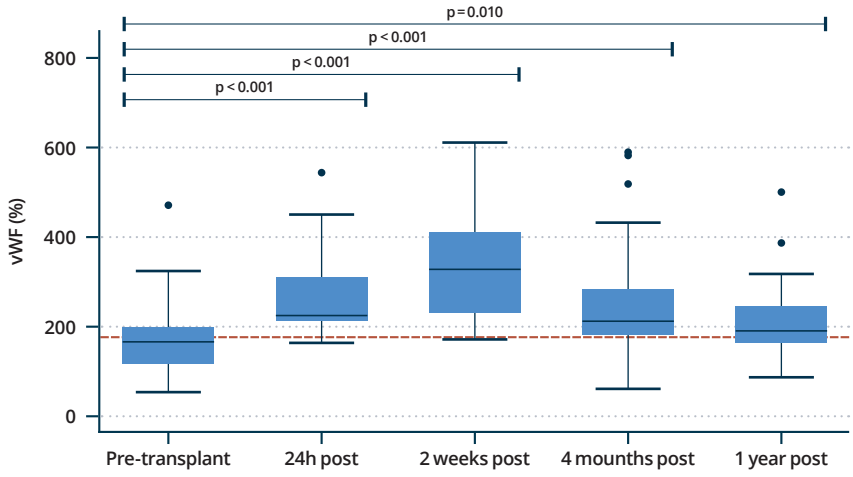

Figure 4b: vWF before and at different time-points post-LT

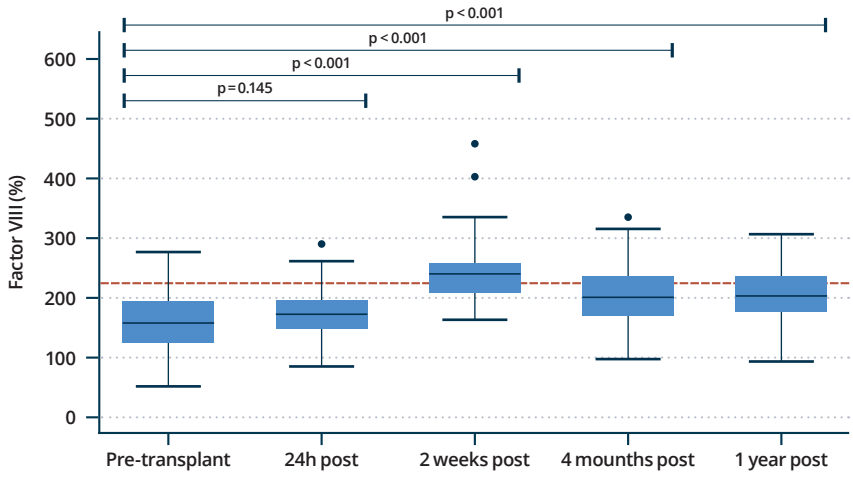

Figure 4c: Factor VIII before and at different time-points post-LT

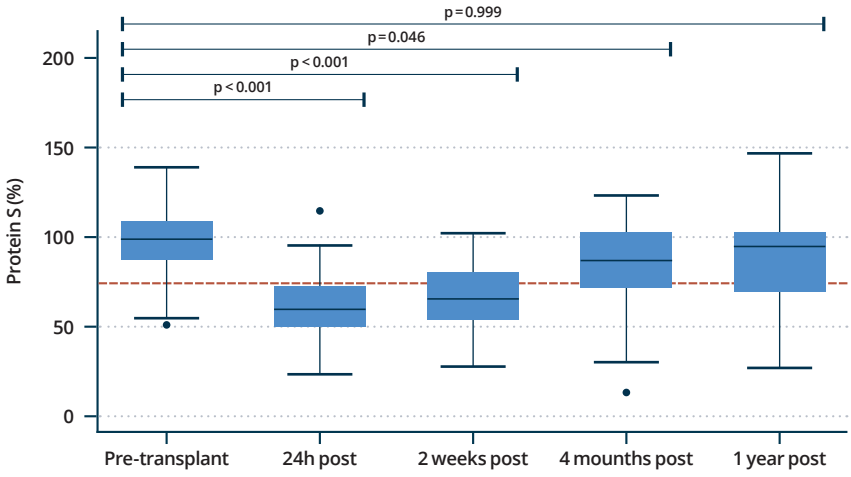

Figure 4d: Protein S before and at different time-points post-LT

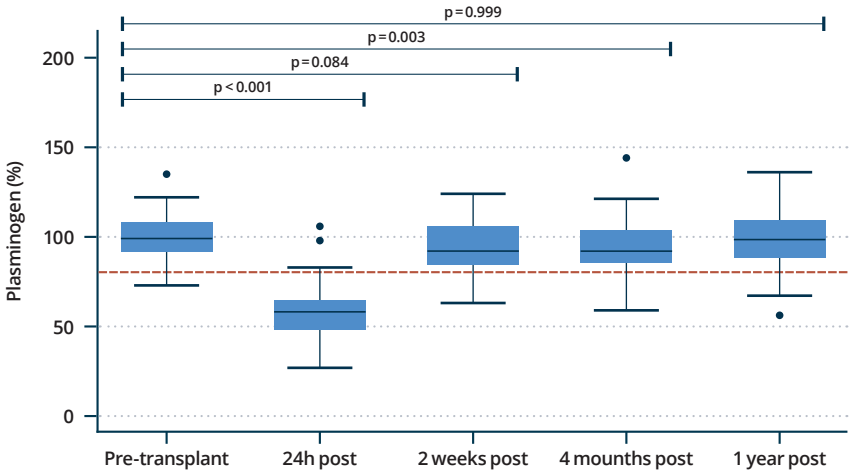

Figure 4e: Plasminogen before and at different time-points post-LT

If we analyze the coagulation profile individually (***Table 14***) at 1 year after LT, some patients still have not recovered a normal coagulation profile and up to 29.4% have FVIII above the 90th percentile.

Table 14: Coagulation parameters measured after LT

	24 h post-LT n = 48	2 w post-LT n = 48	4 mo post-LT n = 46	1 Yr post-LT n = 34	p1	p2	p3	p4
D-dimer, n (%)								
≤243	1 (2.1)	0 (0.0)	24 (53.3)	20 (58.8)	<0.001	<0.001	0.0215	0.0645
>243	47 (97.9)	48 (100)	21 (46.7)	14 (41.2)				
vWF, n (%)								
≤176	2 (4.4)	1 (2.2)	10 (22.7)	12 (35.3)	<0.001	<0.001	0.002	0.460
>176	43 (95.6)	44 (97.8)	34 (77.3)	22 (64.7)				
FVIII, n (%)								
≤100	2 (4.4)	0 (0.0)	1 (2.3)	1 (2.9)	0.999	0.310	0.625	0.625
>100	43 (95.6)	45 (100)	43 (97.7)	33 (97.1)				
FVIII p90, n (%)								
<224.12	39 (86.7)	20 (44.4)	31 (70.5)	24 (70.6)	0.508	<0.001	0.002	0.004
>224.12	6 (13.3)	25 (55.6)	13 (29.5)	10 (29.4)				
PC, n (%)								
≥70	31 (68.9)	45 (100)	44 (100)	32 (94.1)	0.010	0. 999	0.999	0.999
<70	14 (31.1)	0 (0.0)	0 (0.0)	2 (5.9)				
PS, n (%)								
≥74.1	11 (24.4)	16 (35.6)	31 (70.4)	25 (73.5)	<0.001	<0.001	0.010	0.999
<74.1	34 (75.6)	29 (64.4)	13 (29.6)	9 (26.5)				

vWF: Von Willebrand factor; ***FVIII***: factor VIII activity; ***ACT PC***: protein C activity; ***PS***: free protein S; ***p1***: 24 hours post-transplant vs. pre-transplant; ***p2***: 2 weeks post-transplant vs. pre-transplant; ***p3***: 4 months post-transplant vs. pre-transplant; Values are missing for some variables, as follows: 3 in D-dimer, vWF, Factor VIII, ACT PC, Protein S both at 24 hours and 2 weeks post-transplant; 4 in D-dimer, vWF, factor VIII, ACT PC, protein S, plasminogen, and α2-antiplasmin 4 months post-transplant.

4.2.3. Comparison of coagulation profiles according to VTE status

At 4 months, 10 (20.8%) patients had presented a thrombotic event. There were no differences in clinical characteristics between the 2 groups (***Table 15***). The median time to the thrombotic event was 22 (20-46) days, distributed as follows: 1 event before 2 weeks, 6 events between 2 weeks and 1 month, 2 events between 1 and 2 months, and 1 event between 2 and 3 months. All of the patients were treated after the event, and at 4 months, 8 were still under anticoagulant treatment.

Table 15: Clinical characteristics of 48 lung transplant recipients according to occurrence of VTE during 4 months follow-up

	All n = 48	VTE n = 10	No VTE n = 38	p
Age (years)	53.1 (12.8)	58.0 (9.2)	51.8 (13.4)	0.122
Sex (males)	32 (66.7)	5 (50.0)	27 (71.1)	0.267
Diagnosis of interstitial lung disease	26 (54.2)	3 (30.0)	23 (60.5)	0.152
Single lung transplant	18 (37.5)	4 (40.0)	14 (36.8)	0.999
Extracorporeal circulation	11 (22.9)	3 (30.0)	8 (21.1)	0.675
Primary graft dysfunction (grade >1)	8 (16.7)	3 (30.0)	5 (13.2)	0.336
Days in the ICU	16 (7-42)	33 (11-44)	13 (6-41)	0.127
Days on mechanical ventilation	6 (2-36)	16 (1-42)	6 (2-28)	0.949
Days of hospitalization	28 (24-57)	41 (25-55)	27 (23-58)	0.309

Data are presented as n (%), mean (SD) or median (p25-p75), as appropriate.
VTE: venous thromboembolism; ***ICU***: intensive care unit.

Patients with VTE presented procoagulant abnormalities: at 2 weeks, FVIII was higher than in patients without VTE (***Figure 5***), and PS was significantly decreased at 4 months. The accuracy of FVIII at 2 weeks post-LT for predicting VTE at 4 months was good (***Figure 6***). The cutoff point corresponding to the 90th percentile had good sensitivity but low specificity and positive predictive value (sensitivity, 100%; specificity, 51.4%; positive predictive value, 30.8%; and negative predictive value, 100%).

Moreover, we found that 60% of patients who had experienced VTE at 4 months had abnormal values of D-Dimer, vWF, factor VIII, PC, and PS at 2 weeks, whereas only 23.7% of patients without VTE had these alterations (p = 0.051).

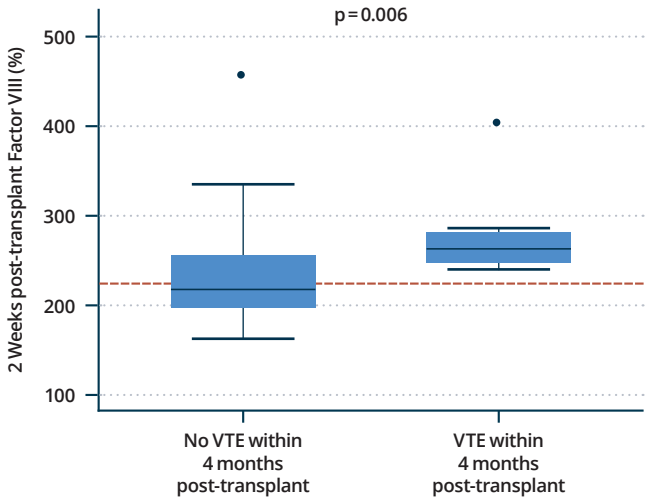

Figure 5: Post-transplant factor VIII at 2 weeks according to VTE status

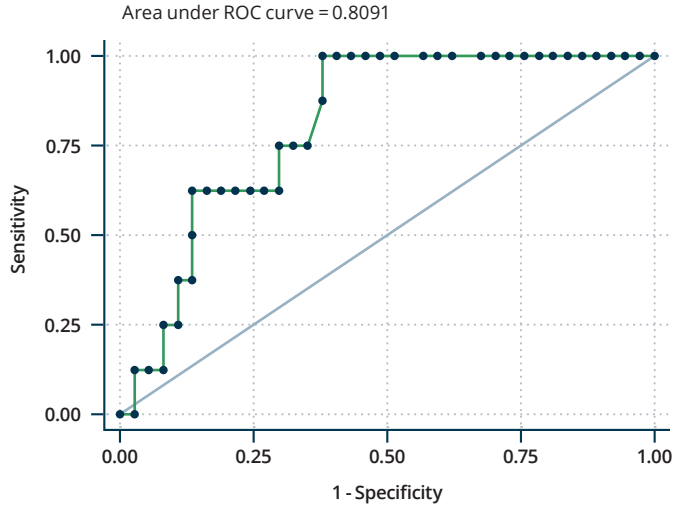

Figure 6: ROC curve of 2-week post-LT FVIII cutoff points for prediction of VTE at 4 months

4.2.4. Comparison of coagulation profiles according to status of interstitial lung disease

In our cohort, 26 patients had interstitial lung disease (ILD) as the primary pulmonary disease. At baseline, this group presented abnormal median vWF and FVIII levels that were higher than those of the other patients. They also presented higher P-selectin levels, although these were within the normal range (***Table 16***).

Table 16: Baseline coagulation parameters according to diagnosis of ILD in the 48 lung transplant recipients

Coagulation Factors	ILD diagnosis n = 26	Non ILD diagnosis n = 22	p
PT (ratio)	0.99 (0.95-1.06)	0.97 (0.94-1.03)	0.258
APTT (ratio)	1.04 (0.99-1.11)	1.08 (1.02-1.16)	0.181
Fibrinogen (g/L)	3.91 (3.40-4.36)	3.55 (3.28-4.03)	0.109
D-dimer (ng/mL)	146 (80-233)	100 (81-172)	0.291
vWF (%)	194 (158-204)	120 (95-174)	**<0.001**
FVIII (%)	172 (140-201)	135 (109-167)	**0.002**
PC (%)	116 (102-132)	121 (107-136)	0.456
PS (%)	98.8 (89.9-105.9)	97.9 (79.7-110.7)	0.983
Plasminogen (%)	98.0 (87.0-106.0)	102.5 (93.0-109.0)	0.390
α2-Antiplasmin (%)	108 (98-115)	105 (100-112)	0.514
AT (%)	100.5 (91.0-119.0)	107.0 (89.0-117.0)	0.796
Homocysteine (µmol/L)	10.7 (7.4-15.4)	11.4 (6.7-14.8)	0.642
ADAMTS 13 (IU/mL)	1.01 (0.94-1.31)	1.05 (0.79-1.34)	0.889
P-Selectin (ng/mL)	64.90 (49.90-76.26)	52.68 (37.08-66.73)	**0.035**

Data are presented as median (p25-p75).

PT: prothrombin time; ***APTT***; activated partial thromboplastin time; ***vWF***: Von Willebrand Factor antigen; ***FVIII***: factor VIII activity; ***PC***: protein C activity; ***PS***: free protein S; ***AT***: antithrombin III.

Red: procoagulant abnormalities; ***yellow:*** normal values.

At 4 months, ILD patients presented higher median levels of D-dimer (289 ng/mL vs. 137 ng/mL; p = 0.017), vWF (259.3% vs. 205.4%; p = 0.011), and FVIII (225.2% vs. 189.2%; p = 0.005). No statistically significant differences were seen in median levels of coagulation parameters between ILD patients and non-ILD patients at 24 hours, 2 weeks, and 1 year.

Individually, differences were seen at baseline, with a higher proportion of patients showing elevated vWF (65.4% vs. 22.7%; p = 0.003) and FVIII (100% vs. 77.3%; p = 0.015), and at 4 months, with a higher proportion of patients having FVIII above the 90th percentile (50% vs. 9.1%; p = 0.003).

Discussion

5

The main feature of our study is its novelty. The results show that VTE is a relevant complication after LT, although it is rarely addressed in the literature. Male gender and IPF should be regarded as specific risk factors. We found that 90-day extended prophylaxis did not reduce the incidence of VTE; therefore, studies evaluating other prophylactic protocols will be needed. As for the mechanistic pathways, we found that some transplant recipients had persistent abnormalities in coagulation profile at 1 year after LT.

VTE is a relevant complication after LT

We describe a 15% incidence of VTE in our cohort. This is within the range reported in other studies (8-30%) [20–25,122–125]. The wide range of incidence reported may be explained by differences in the methodology of the studies, which hamper comparison between them. The 3 aspects that differ between studies are follow-up time, VTE screening protocol and prophylactic treatment.

With respect to follow-up time, the main difference is that few studies focus on the postoperative period, whereas most consider an extended period during the first year owing to a known higher risk in the first 4 to 6 months.

Only 2 previous studies in the literature report data on patients under VTE surveillance. In 1995, *Kroshus et al.* [20] reviewed 116 consecutive LT patients, all of whom had a VPS between POD 7 and POD 10 and a follow-up VPS at 6 and 12 months. Suspicion of PE led VPS to be repeated. The authors report a 12.1% incidence of VTE, with 5 cases of PE that were all symptomatic. In another study, *Evans et al.* [123] performed DVT diagnostic tests based on clinical suspicion, whereas all patients underwent a postoperative imaging study (VPS or CT angiography) per protocol. With a similar protocol to ours, the overall incidence of PE was also comparable (15%), although, unfortunately, information on the incidence of asymptomatic events is not available. While these data make it

difficult to assess the usefulness of a screening protocol for thrombotic complications, the relevance of this problem led *Evans et al.* to change their protocol and, since then, they routinely assess patients for DVT.

Finally, prophylactic treatment is not mentioned in some of the studies published in LT [21–23]. However, when specified, it usually consists of compression stockings combined with subcutaneous heparin during post-LT hospitalization [120,121]. *Yegen et al.* [25] only used 1 of these measures, whereas *Kroshus et al.* [20] prolonged prophylaxis with antiplatelet agents after discharge. There are no data about bleeding complications, and, despite using antithrombotic strategies, some of these studies show an incidence of VTE in the higher range.

Given the impossibility of comparing studies with such different methodologies, larger, multicenter, randomized, clinical trials will be needed to elucidate the best strategy.

Malegender and IPF as risk factors

In our series, 2 risk factors were related to VTE, namely, male gender and IPF.

Consistent with our findings, men are known to have a higher risk for recurrence of VTE. Moreover, when female reproductive risk factors are taken into account, men also have a higher risk for the first event [126]. The causes of this are unclear, although it seems that genetic differences may play a role [127]; specifically, height seems to explain in part the association between gender and thrombotic risk. Another explanation could be a sex chromosome–linked mutation, although this has not yet been explored.

In the first study, we also described IPF as a risk factor for VTE after LT; furthermore, the second study showed a procoagulant state in this population before LT.

A higher risk for VTE in IPF patients has been reported [22,23]. Although the reasons are not well known, age has been proposed as a confounding factor; we did not find differences regarding age as a risk factor for VTE in the first study or in the coagulation profile in our second study when patients were studied according to median age. Consistent with our findings, other authors [45] have reported that a prothrombotic state and high FVIII levels are more likely in IPF patients. Nevertheless, clinical trials with anticoagulation in IPF patients have shown conflicting results, with 1 study showing a beneficial effect of decreasing mortality [50], whereas in another [51], increased mortality in the anticoagulation group prompted early termination. Although we describe a procoagulant state in IPF patients before LT, this abnormality tends to disappear after surgery, and significant differences are not present at 1 year after LT. In fact, LT may counteract the effect of the coagulation on lung injury; however, considering that these patients constitute a high-risk population, implementing specific prophylactic strategies during the first months after LT could be beneficial.

Extended prophylaxis with enoxaparin is not protective

In the first study, we evaluated 90-day extended prophylaxis with enoxaparin and found that it did not protect against VTE. To our knowledge, this is the first study to evaluate a prophylaxis protocol after LT. While the protocol did not reduce the incidence of VTE, it did not increase bleeding complications, which are the main concern in the implementation of prophylactic strategies. This is even more the case if we give prophylaxis to all patients, not only to those considered at higher risk. The lack of effect of this strategy and the fact that we observed up to 20% of the thrombotic events in our population between POD 90 and the first year prompted us to study coagulation profiles; our hypothesis was that the failure of the prophylactic strategy could be due to persistent coagulation abnormalities beyond 4 months. Taking into account the limitations of our study and the lack of data in the literature, we think that a reasonable

option would be to prolong prophylaxis in patients considered at high risk, that is, prolonged immobilization or coagulation abnormalities at 2 weeks post-LT. If we look at data regarding other types of SOT, the strategy of treating only high-risk patients has been used in RT [110–112], with special emphasis on bleeding risk that is significant. What is yet to be determined is whether it is best to use antiplatelet agents or heparin for such an indication, although studies seem to favor the antiplatelet option owing to the reduced frequency of bleeding complications [113–117].

Coagulation status remains abnormal in some patients at 1 year after LT

To our knowledge, this is the first time that coagulation profiles have been studied in LT patients. The persistent procoagulant state found in our population would explain the relevance of VTE as a complication after LT, not only early, but also in the long term after surgery. Although similar results have been described in RT and liver transplantation [89–92,98–100], persistent coagulation abnormalities seem to be more significant in LT, maybe owing to the greater endothelial damage associated with transplantation itself.

Our study was not designed to explore the possible causes of this procoagulant state; the fact that it is common to all kind of grafts and that it persists at 1 year suggests that factors shared between various organs, such as immunosuppressive treatment, may play a major role. However, studying the effect of each drug on the coagulation system has proven difficult owing to the need to use them in combination. The same problem arises when trying to elucidate the specific role of the multiple risk factors that accumulate in this population.

The importance of describing a persistent procoagulant state after LT relies not only on the fact that VTE is a highly prevalent complication (with the result that better prophylactic strategies are needed in affected patients), but also on the

possibility that it affects graft survival. The effect of the coagulation cascade on lung injury has been described in acute respiratory distress syndrome and IPF, although there is also evidence relating it to lung allograft dysfunction. Thus, *Christie et al.* [128] reported that lower postoperative PC and higher PAI-1 levels are associated with primary graft dysfunction. Moreover, in a prospective study with 46 patients [129], D-dimer levels were associated with FEV_1, the 6-minute walk test result and oxygen consumption in the cardiopulmonary exercise test. Therefore, this study points to 2 potential research lines: establishing the best prophylactic strategy for VTE after LT and studying the effect of coagulation profiles on lung graft function.

In our more in-depth study of prophylaxis, we tried to find a signal that identifies high-risk patients early after surgery. In our study, patients who presented a VTE at 4 months had an abnormal coagulation test result at 2 weeks more frequently than non-VTE patients (60% vs. 23.7%; p = 0.051). This observation could be used as a marker to identify high-risk patients and implement more intense prophylactic strategies. In order to simplify this screening process and reduce costs, we looked for a single-parameter test. We chose FVIII and, specifically, the 90th percentile cutoff point, because it has been related directly to thrombotic risk. This test presented good sensitivity and negative predictive value, thus supporting intensification of anticoagulation in patients with the highest risk. As the strength of our study is limited by the small size of our sample, future studies will be needed to evaluate whether a coagulation study at 2 weeks (specifically FVIII) could be a good tool for stratifying risk in these patients.

Finally, while our study aimed to draw attention to a problem that is usually underdressed in the literature, it is subject to limitations. The inherent limitations of a single-center study hamper generalization of our conclusions. In the first study, comparison of 2 different eras makes it impossible to dismiss a possible

bias towards suspicion of VTE in the study cohort, owing to the same concern that led us to perform the study in the first place. In contrast, because we do not have a specific VTE screening protocol, we may have under-diagnosed thrombotic events, and the relatively low number of VTE events may have limited the detection of risk factors. Another limitation is that the 2 consecutive cohorts that were compared differed in terms of age, number of patients with ILD as their underlying disease, number of patients with diabetes and peritransplant hemodynamic instability. Finally, we did not track the use of compressive stockings in the intensive care unit. The second study is limited by its sample size, which prevents us from providing conclusive results, especially when the cohort is studied according to VTE or ILD status. We did not have a control group receiving immunosuppression or undergoing other cardiothoracic surgery that would have helped to identify the causes of this procoagulant state.

In conclusion, a persistent procoagulant state after LT probably explains the increased incidence of VTE in this population, not only in the early postoperative period but also in the long term after surgery. The causes are still unknown, and an effective prophylactic strategy has yet to be determined. Larger studies will be needed to resolve these issues and to explore the effect of these coagulation abnormalities on the graft.

Conclusions

6

VTE is a major complication after LT, with a cumulative incidence of 15.3% in our series.

Male gender and ILD as the underlying disease are risk factors for this complication. Patients with ILD present a procoagulant state before transplantation that tends to normalize after surgery.

Ninety-day extended prophylaxis with enoxaparin is not able to prevent VTE after LT.

Most markers of a procoagulant state normalize at 2 weeks after lung transplantation; however, abnormal values of FVIII and vWF persist at 1 year after LT in some patients. Patients who present a thrombotic complication at 4 months after transplantation more frequently present coagulation abnormalities at 2 weeks after LT.

Future lines of investigation

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New lines of investigation derived from our study include the following:

- We would like to study coagulation profiles in other cohorts of lung transplant patients to confirm our results.
- Moreover, we would like to study the effect of persistent coagulation abnormalities on graft function.
- Finally, an important feature derived from our work is the necessity of better prophylaxis protocols in this population. Multicenter trials will be needed to answer this question and to define which patients are at highest risk.

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Annexes

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ANNEX I: PUBLICATIONS

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Deep vein thrombosis and pulmonary embolism after solid organ transplantation: an unresolved problem

Berta Sáez-Giménez [a,b,*], Cristina Berastegui [a,b], Karina Loor [a,b], Manuel López-Meseguer [a,b], Víctor Monforte [a,b], Carlos Bravo [a,b], Amparo Santamaría [c], Antonio Roman [a,b]

[a] Pulmonology Service, Lung Transplant Program, Hospital Universitari Vall d'Hebrón, Passeig Vall d'Hebrón 119-129, 08035 Barcelona, Spain
[b] Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid, Spain
[c] Hemostasis and thrombosis Unit, Department of Hematology, Hospital Univeristari Vall d'Hebrón, Passeig Vall d'Hebrón 119-129, 08035 Barcelona, Spain

A B S T R A C T

Venous thromboembolism (VTE) is a major complication after solid organ transplantation (SOT), with an incidence that ranges from 2 to 34%. Besides genetic risk factors such as inherited thrombophilia, other specific risk factors for VTE in SOT recipients include impairment of fibrinolysis produced by corticosteroids, *in vitro* procoagulant effects of calcineurin inhibitors, endothelial damage due to cytomegalovirus infection, and specific surgical factors. Prevention strategies have not been systematically studied. Therefore, it is mandatory for the international scientific community to conduct large, multicenter, randomized clinical trials to define strategies for the prevention of VTE in SOT recipients.

1. Introduction

The incidence of venous thromboembolism (VTE) in the general population is estimated at between 1 and 2 cases per 1000 people per year [1]. VTE, which includes deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common complication of surgery and a major cause of morbidity and mortality in various medical conditions. The overall risk for VTE is a combination of environmental and genetic factors. Solid organ transplantation (SOT) has been recognized as complex environmental factor which includes the inherent risk associated to other facts such as surgery or immunosuppressive treatment.

The risk of VTE differs with the type of surgery performed. Approximately half of all patients undergoing orthopedic surgery without prophylaxis develop VTE [2]; when prophylaxis is implemented, the incidence decreases to around 18 per 1000 cases per year [3]. Cancer is also a risk factor for VTE, and 8% of patients experience VTE either within the first year after diagnosis or during the course of their disease [4]. Other conditions that involve an intermediate risk for VTE are included in Table 1.

Nevertheless no solid epidemiologic data are available on the real incidence of this complication following SOT and its prevention. The objective of the present paper is to review the epidemiology, risk factors, and prevention of VTE after SOT.

2. Venous thromboemblism in solid organ transplantation: epidemiological data

Table 2 summarizes data about the epidemiology of VTE in SOT.

2.1. Renal transplantation

Few data are available on VTE in renal transplantation during the precyclosporine era [5]. Since the introduction of calcineurin inhibitors, the incidence of VTE after renal transplantation has been reported to range between 2 and 14% [6]. In 1987, Allen et al. [7] published a descriptive retrospective study of 480 kidney recipients and reported an incidence of 8.3%, which peaked within the first 4 months after transplantation. In a retrospective study including 1833 kidney recipients from 1985 to 1995, Humar et al. [8] found a slightly lower incidence (4.2%), with a peak between the third and fifth months after transplantation.

The risk of late VTE was also assessed in a study based on the Medicare database [9]; the study population comprised 28,924 kidney recipients and the incidence of late VTE (occurring 1.5 to 3 years after transplantation) was 1.5%. In summary, these data strongly suggest a high incidence of VTE after kidney transplantation.

2.2. Liver transplantation

The introduction of calcineurin inhibitors as immunosuppressive treatment for SOT considerably improved the outcome of liver recipients. In this context, VTE after liver transplantation has been described as a relevant complication with an incidence of between 3 and 5% and significant morbidity and mortality both during surgery and during the early postoperative period. In their analysis of 495 liver recipients between 2004 and 2006, Sakai et al. [10] reported PE in 20 patients (i.e., a 4% incidence of intraoperative PE).

Ishitani et al. [11] reported a 3.7% incidence of clinically symptomatic VTE during a 6-year follow-up, with a peak of incidence during the first 2 months after transplantation. The VTE prophylactic protocol included treatment with intermittent pneumatic leg compression devices until

* Corresponding author at: Hospital Universitari Vall d Hebrón. Passeig de la Vall d Hebrón 119-129, 08035, Barcelona. Tel.: +34 618403192; fax: +34 9 3 274 60 65.
E-mail address: bsaez@vhebron.net (B. Sáez-Giménez).

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Table 1
Incidence of VTE in different populations.

Population studied	Incidence
General population [1]	0.1–0.2%
After orthopedic surgery [3]	
Without prophylaxis	50%
With prophylaxis	1.1–10.6%
Cancer [4]	1–8%
Abdominal surgery [66]	0.6–3.1%
Low-risk: appendectomy, cholecystectomy, or lysis of adhesions	0.6%
Intermediate-risk: gastrointestinal tract	1.8%
High-risk: splenectomy	3.1%
Thoracic surgery	
Lobectomy/pneumonectomy [67]	0.18%
Cardiac surgery [68,69]	0.56%
Renal transplantation	2–14%
Liver transplantation	3–5%
Heart transplantation	18–34%
Lung transplantation	8–29%

patients were able to sit. Salami et al. [12] later conducted a similar retrospective study and reported an incidence of VTE of 4.6%. Diagnostic procedures were carried out when VTE was clinically suspected, and there were no prespecified prophylaxis protocols.

2.3. Heart transplantation

Several retrospective studies on the epidemiology of VTE in heart recipients, including arterial and venous thrombotic events, reported an incidence of VTE events of between 15 and 34%. It is important to emphasize that these studies included episodes of acute myocardial infarction, stroke, and occlusion of retinal vessels as thrombotic events. Forrat el al. [13] reviewed 285 heart transplant patients on low-dose aspirin (250 mg/d) and found 97 cardiac and noncardiac thromboembolic complications (34%), of which 33 (11.6%) were DVT or PE events. Miriuka et al. [14] analyzed the frequency of thrombotic events, which were mainly related to the coronary artery tree, but which also included DVT and PE. A total of 22 patients (26.2%) had at least 1 complication of VTE, including 13 DVT and 5 PE. Finally, Garcia-Herrera et al. [15] reported 25 thrombotic complications in 164 patients (15%) who received a heart transplant between 1984 and 1999.

In summary, the literature shows a high incidence of thromboembolic complications after heart transplantation despite the use of antiplatelet agents as an almost standard prophylactic strategy.

2.4. Lung transplantation

VTE after lung transplantation is a common finding in clinical practice and has been reported to occur in 8 to 27% of lung recipients in retrospective studies (Table 2). In 1995, Kroshus et al. [16] reported an incidence of VTE complications of 12.1% between 10 days and 36 months after surgery. All patients underwent screening ventilation–perfusion scanning in the first week, at 6 months, and at 1 year after transplantation. Doppler ultrasonography of the legs was performed to exclude DVT only when the condition was clinically suspected. Patients also received prophylaxis with perioperative and postoperative subcutaneous heparin until discharge, as well as pneumatic compression garments until they were able to walk again. Patients then received antiplatelet agents. Although this study was retrospective, both the diagnostic and the predefined prophylactic strategy were thorough and strongly support the relevance of VTE in lung recipients.

Izbicki et al. [17] published similar findings in a retrospective cohort, with an incidence of PE of 8.6% between 4 and 24 months after the intervention. Similar findings on the incidence of PE were reported by Nathan et al. (8.3%) [18], who emphasized that all the PE events were diagnosed in the subgroup of patients with idiopathic pulmonary fibrosis. This subgroup was identified as susceptible in another study [19], but with a lower incidence (only 1.78%).

In 2004, Burns and Iacono [20] reported an incidence of 27% for PE in postmortem examination of lung and heart-lung transplant patients. This incidence was higher in patients who died during the first month after transplantation, suggesting that PE may be under-diagnosed as a complication contributing to respiratory failure in the early postoperative period.

Finally, Yegen et al. [21] performed a case-control study in a cohort of 121 patients who underwent lung transplantation between 2001 and 2005. All the patients received prophylaxis with heparin, enoxaparin, or pneumatic compression devices during their post-transplant stay. Asymptomatic patients were not routinely screened for VTE. The authors diagnosed 27 cases of VTE (incidence of 22%).

Table 2
Epidemiologic data on VTE in SOT.

Organ	Author	Year of transplantation	*n*	Incidence	Monitoring VTE	Prophylaxis	Symptomatic events
Kidney	Allen et al. [7]	1974–1984	480	8.3%	None	No	100%
Kidney	Humar et al. [8]	1985–1995	1833	4.5%	None	GCS (since 1990)	100%
Kidney	Poli et al. [50]	1990–2005	538	9.1%	Periodical DUS	LMWH 3 months	61%
Kidney	Todeschini et al. [51]	2008–2011	284	9.0%	Periodical DUS	LMWH 1 week followed by anti-platelet agent	100%
Kidney	Moscarelli et al. [46]	1991–2010	769	12.5%	Periodical DUS	LMWH 3 months	61%
Kidney	Abbott et al. [9]	1996–2000	28942	1.5%	Not known	Not known	Not available
Liver	Sakai et al. [10]	2004–2006	495	4.0%	Perioperative period		100%
Liver	Ishitani et al. [11]	1989–1995	299	3.7%	None	GCS	100%
Liver	Salami et al. [12]	1995–2010	917	4.6%	None	Not mentioned	100%
Liver	Cherian et al. [65]	1982–2007	2149	0.37%[a]	None	GCS and LMWH until discharge.	100%
Heart	Forrat et al. [13]	1984–1988	285	11.6%	None	Aspirin 250 mg/d until 1992	Not available
Heart	Miriuka et al. [14]	1986–1997	84	21.4%	Periodical CA	Not mentioned	Not available
Heart	Garcia-Herrera et al. [15]	1984–1999	164	15%	None	Antiplatelet agent	Not available
Lung	Kroshus et al. [16]	1986–1993	116	12.1%	Periodical V/Q	GCS and LMWH until discharge. Later antiplatelet agents.	100%
Lung	Izbicki et al. [17]	1997–2003	70	8.6%	None	Not mentioned	100%
Lung	Nathan et al. [18]	1996–2002	72	8.3%	None	Not mentioned	100%
Lung	Garcia-Salcedo et al. [19]	1999–2009	280	1.78%[b]	None	Not mentioned	100%
Lung	Burns et al. [20]	1990–2002	126	27%	*Postmortem examination*		Not available
Lung	Yegen et al. [21]	2001–2005	121	22%	None	LMWH or GCS during hospitalization	100%
Lung	Kahan et al. [27]	1994–2006	153	29%	None	GCS ± LMWH	100%

n: sample size; GCS: graduated compression stockings; DUS: color duplex ultrasonography; CA: coronary angiography; LMWH: low-molecular-weight heparin. V/Q: xenon ventilation-perfusion scanning.
[a] Incidence of pulmonary embolism presented within 90 days of transplant.
[b] Incidence of pulmonary embolism.

In summary, the available information shows that there is an unacceptable incidence of VTE after SOT. Therefore, development of preventive strategies is mandatory.

3. Pathophysiology of VTE after SOT

A hypercoagulable state has been described in transplant recipients. In kidney recipients, impaired fibrinolysis and protein C activation have been reported [22]; some studies [23] demonstrated activation of the extrinsic pathway of coagulation and a higher fibrinogen level in kidney recipients, both in patients with comorbidities (e.g. diabetes, cardiovascular disease, and nephrotic syndrome) and in transplant recipients without comorbidities. Although hypercoagulability persists indefinitely, it involves a maximum risk of thrombosis during the first 6 months [22]. It has been suggested that candidates for renal transplantation should be screened for thrombophilia, although the causative role of this condition in hypercoagulability in transplant recipients remains unclear. In 1998, Fischereder et al. [24] reported a prevalence of around 14% and associated the high risk of thrombophilia with an increased risk of graft loss at 1 year as a result of thrombotic and rejection-related complications. Moreover, an excess prevalence of hyperhomocysteinemia [6] due to impaired renal function has been reported; it has also been suggested that immunosuppressive drugs such as CsA can modify homocysteine metabolism.

Liver recipients have traditionally been considered to be in a hypocoagulable state, as evidenced by laboratory tests such as platelet count and prothrombin time and by the massive bleeding reported as a complication during transplantation. Nowadays, however, transfusion-free liver transplant surgery is common, and awareness of thrombotic complications has increased [25]. The hemostatic system of cirrhotic patients is altered because of thrombocytopenia, although this alteration is offset by elevated levels of von Willebrand factor. After liver transplantation, levels of von Willebrand factor remain elevated, and multiple biochemical processes lead to increased platelet adhesion. This prothrombotic imbalance constitutes a potential risk for postoperative VTE events.

Other findings suggest that despite the prolonged coagulation tests such as prothrombin time, reduced levels of anticoagulation proteins lead to normal coagulation potential. This coagulation potential remains comparable to that of healthy people, even during the intervention, and increases in the postoperative period (5 to 10 days after surgery) owing to an increased rate of thrombin generation [26].

In lung transplantation, Izbicki et al. [17], described a hypercoagulable state in patients who developed PE that was characterized by increased levels of coagulation factors or the presence of antiphospholipid antibodies. Whether this state was acquired or due to congenital factors was unclear; however, none of the patients had a previous history of VTE events. The findings of other groups that have tried to identify coagulation abnormalities were not statistically significant [27].

4. Prothrombotic state in solid organ transplantation

Risk factors for VTE in the general population include major and orthopedic surgery, previous VTE, older age, malignancy, obesity, renal impairment, prolonged immobility, presence of central venous lines, pregnancy and puerperium, oral contraceptives, as well as the presence of inherited and acquired thrombophilia [2].

SOT recipients are at high risk for VTE, not only because of classic factors, but also because of risk factors directly related to transplantation itself. These factors will be addressed below and are summarized in Fig. 1.

4.1. Immunosuppressive treatment

4.1.1. Calcineurin inhibitors

The risk of VTE complications associated with cyclosporine (CsA) has been a subject of debate. Procoagulant effects have been demonstrated *in vitro,* but when research is transferred to patients, it is difficult to prove that these changes are attributable only to CsA, without taking into account concurrent immunosuppressive agents.

Thus, in the field of renal transplantation, many studies compare the incidence of VTE events between patients treated with CsA and patients treated with azathioprine (Aza). The results are controversial. Vanrenterghem et al. [28] retrospectively compared the incidence of complications of VTE in 90 kidney recipients recruited between 1983 and 1984 and treated with CsA and low-dose corticosteroids and in the same number of cadaveric kidney allograft recipients treated with Aza, antilymphocyte globulin, and high-dose corticosteroids. Patients received dipyridamole 300 mg/d or ticlopidine as prophylaxis. The authors reported 17 VTE events in the CsA group and only 1 event in the Aza group. By contrast, in their prospective study performed between 1980 and 1984, Brunkwall et al. [29] did not find an increased frequency of DVT in 97 patients receiving CsA and low-dose corticosteroids compared with a similar group of 83 patients receiving a regimen comprising Aza and high-dose corticosteroids. The authors reported an incidence of 9.3% in the CsA group. This value was significantly lower than in the Aza group, which had an incidence of 24.1%.

Furthermore, larger studies have failed to support a significant difference in VTE events between patients treated and not treated with CsA. Gruber et al. [30] examined 224 kidney recipients who were prospectively randomized and stratified by risk to treatment with CsA and prednisone or Aza, prednisone, and antilymphocyte globulin between 1980 and 1983. The incidence of renal vein thrombosis was 0.9% in the Aza group and 1.7% in the CsA group.

Penny et al. [31] subsequently reported a 2.2% incidence of renal graft thrombosis in a series of 6153 renal transplants between 1980 and 1992. These patients were compared with institutional controls and with the contralateral cadaveric donor kidney in each case. No association was demonstrated between CsA and renal graft thrombosis. Multiple logistic regression also revealed that CsA was not a risk factor.

The role of tacrolimus has not received the same attention as CsA. *In vitro* studies showed antithrombotic effects by inhibition of platelet activity and coagulation. However, once again, study results are contradictory. Pirsch et al. [32] conducted an open-label, randomized, multicenter study to assess the efficacy and safety of tacrolimus with that of CsA for primary immunosuppression in kidney recipients. Between 1993 and 1995, a total of 412 patients were randomized to tacrolimus ($n = 205$) or CsA ($n = 207$) and followed for 1 year. DVT was more frequent in the tacrolimus group (11 patients) than in the CsA group (1 patient). However, risk factors for VTE were identified in all patients, and 5 of the 11 cases in the tacrolimus group were reported by a single center.

In conclusion, there is no clear association between the use of calcineurin inhibitors and the risk of VTE.

4.1.2. Corticosteroids

Corticosteroids are prothrombotic because they enhance the synthesis of von Willebrand factor and impair fibrinolysis [33]. In kidney recipients, long-term treatment with corticosteroids has been found to create a hypercoagulable state similar to that observed in patients with Cushing's disease. Patrassi et al. [34] performed a study where the fibrinolytic potential of 19 kidney recipients was evaluated and compared with that of controls and other patients with Cushing disease. The venous occlusion test was used to stimulate fibrinolysis, and several coagulation tests were performed before and after the venous occlusion test. A hypofibrinolytic state was recorded in 68.4% of kidney recipients, suggesting that this imbalance could significantly contribute to an increased risk of VTE in kidney recipients. To confirm this hypothesis of an association between corticosteroids and increased risk of VTE, Sartori et al. [33] studied fibrinolytic capacity after withdrawal of corticosteroids in 28 kidney recipients taking stable immunosuppressive therapy with CsA, Aza, and methylprednisolone. The prevalence of impaired fibrinolytic capacity was as high as 83.3% during corticosteroid treatment and dropped to 16.7% after withdrawal of the drug.

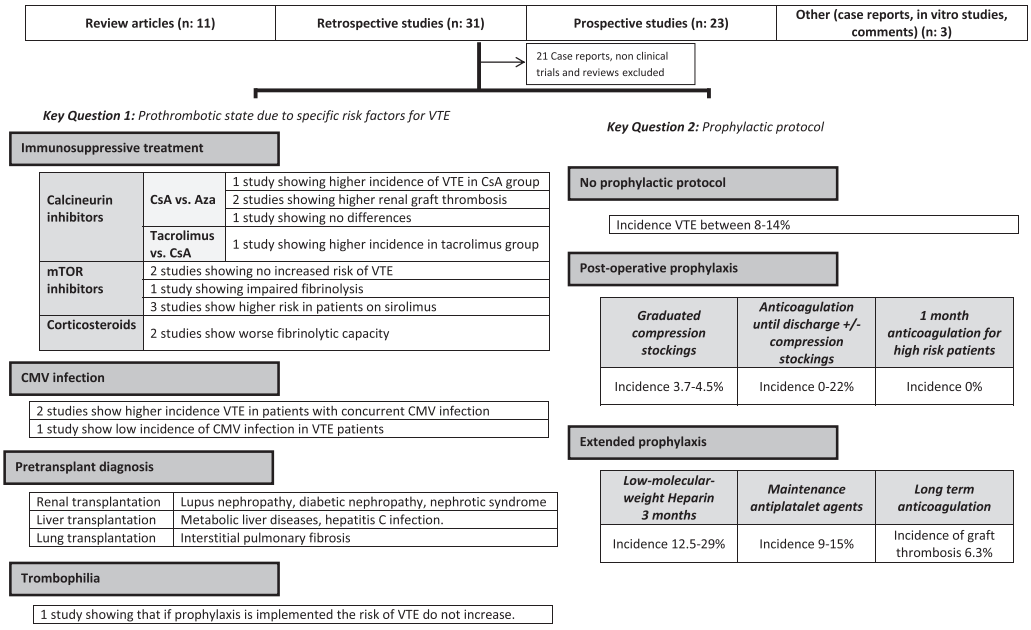

Fig. 1. Summary of evidence on VTE after SOT. Flow chart that summarizes the search and selection of articles. 68 articles were reviewed responding two key questions: which are the specific risk factors for VTE in SOT and which prophylactic protocol should be used.

4.1.3. mTOR inhibitors

Therapy with mTOR inhibitors has been associated with increased endothelial activation, thrombin formation, and impaired fibrinolysis. The prothrombotic effect has limited the use of mTOR in transplant recipients, mainly because of the possible association with hepatic artery thrombosis among liver recipients [35] that led to an FDA warning in 2002.

Many subsequent studies in liver recipients have been unable to confirm these findings on *de novo* use of mTOR inhibitors and show either reduced incidence with sirolimus [36,37] or no difference [38,39] compared with controls.

Two studies that focused on the role of mTOR inhibitors in VTE after renal transplantation did not find a greater incidence of thrombosis. Langer et al. [35] retrospectively evaluated 2 cohorts of renal transplant recipients, the first with 136 patients treated with CsA and prednisone plus Aza in 16 cases, and a second cohort with 354 patients treated with sirolimus, CsA, and short courses of prednisone. DVT and PE were clinically suspected and confirmed by Doppler ultrasonography or lung scintigraphy. The incidence of VTE was similar in both groups (5.1 vs. 5.6%). By contrast, Baas et al. [40] performed a single-center study where coagulation and fibrinolysis parameters were measured in 16 kidney recipients taking everolimus and compared with those of 20 kidney recipients taking a calcineurin inhibitor and/or mycophenolate sodium. The use of everolimus was associated with increased endothelial activation, thrombin formation, and impaired fibrinolysis, thus suggesting an increased risk of thrombotic events. Also, in heart transplantation, Thibodeau et al. [41] retrospectively reviewed 67 recipients taking sirolimus and 134 matched heart recipients not taking sirolimus and found a higher rate of VTE in patients taking sirolimus (12 vs. 7%); this association persisted after adjusting for body mass index but not after adjusting for total cholesterol.

In lung transplantation two studies showed an association between sirolimus and an increased rate of VTE. Lingaraju et al. [42] performed a retrospective review of lung recipients at the University of Pennsylvania from 2000 to 2006. A total of 202 patients survived 90 days; sirolimus was used in 59 (29.2%). This mTOR inhibitor was more frequently used in patients with VTE (56.7 vs. 24.4%, $p < 0.001$). Ahya et al. [43] enrolled 181 lung recipients in a prospective, multicenter, randomized, open-label trial. A significantly increased risk of VTE was noted in association with sirolimus: the group of patients receiving an immunosuppressive regimen with sirolimus, tacrolimus, and prednisone had an incidence of 17% compared with 3% in the group receiving a traditional immunosuppressive regimen (Aza, tacrolimus, and prednisone).

In summary, the limitations of these studies, which are mostly retrospective, with no assessment of potential risk factors for VTE and with a limited number of patients, show that the role of mTOR inhibitors in increasing risk of thrombosis remains controversial. Furthermore, since edema is frequent in patients receiving sirolimus, who are more likely to be closely monitored for DVT, a risk of bias is unavoidable.

4.1.4. Mycophenolate mofetil (MMF)

Although the association between MMF and VTE events has not been established, several studies have shown this agent to have a better profile than other immunosuppressive agents, with fewer unfavorable effects on platelets [44]. MMF can decrease *in vitro* aggregation of platelets in non transplanted patients, but also in kidney recipients.

4.2. Cytomegalovirus infection

Cytomegalovirus (CMV) has a particular tropism for endothelial cells, and its ability to produce vascular injury has been described both in immunocompetent patients and in transplant recipients. Endothelial cell activation by CMV infection results in the increased platelet reactivity and procoagulant capacity of these cells [26]. Nevertheless, results in the literature are inconsistent. Kazory et al. [45] reviewed 218 kidney

recipients and found 13 patients with acute CMV infection who presented a VTE event co-occurring with infection in 7 cases (53.8%); remarkably, the 7 patients did not have predisposing risk factors for thrombosis. In this series, the incidence of VTE was 4.2% in patients who did not have CMV infection, compared with 9% in those who had presented an episode of acute CMV infection. A much higher incidence was subsequently reported by Moscarelli et al. [46], who evaluated the incidence of VTE in 769 kidney recipients; acute CMV infection was reported in 154 patients (20%), 37 of whom (38%) also had VTE. By contrast, Yegen et al. [21] studied risk factors for VTE in 121 lung recipients, 27 of whom developed VTE; only 1 patient (4%) had CMV disease, compared with an incidence of around 7% in the control group.

4.3. Pretransplant diagnosis

It seems plausible that the underlying disease that generates the need for transplantation may lead to increased susceptibility to thrombosis. Furthermore, this increased risk of VTE could well continue after transplantation.

4.3.1. Renal transplantation

In lupus nephropathy [6], it is important to determine the presence of antiphospholipid antibodies as an acquired thrombophilia, which increases the risk of thrombotic complications and early graft loss. In the absence of controlled trials, it has been proposed that lupus patients with antiphospholipid antibodies and a history of recurrent thrombosis who undergo renal transplantation should be treated with anticoagulation therapy. In addition, diabetic nephropathy is thought to increase the risk of thrombosis due to diabetic angiopathy and atherosclerosis, although this hypothesis has not been corroborated in case-control studies [47].

Since nephrotic syndrome carries an increased risk of thromboembolic complications, it should be taken into account, especially in patients with recurrent glomerulonephritis [6].

4.3.2. Liver transplantation

Patients who receive a transplant for metabolic liver diseases such as familial amyloid polyneuropathy or acute intermittent porphyria appear to have an increased risk of thrombosis, and although no explanations have been given for this increase, patients may be predisposed because they maintain normal liver function and, therefore, an intact hemostatic system. In addition, patients with hepatitis C infection have an increased level of antiphospholipid antibodies, which places them at risk for thrombosis [11].

4.3.3. Heart and lung transplantation

The available literature shows that the initial cause of heart failure is not predictive of complications of VTE [13]. Lung recipients with interstitial pulmonary fibrosis are thought to have a higher risk of thrombosis due to unknown predisposing factors. This finding has been replicated in various studies; thus, in the retrospective reviews by Garcia-Salcedo et al. [19] and Nathan et al. [18], all the PE events occurred in the subgroup of patients with interstitial pulmonary fibrosis. Some authors [21] have suggested that age could be a confounding factor in the association between pulmonary embolism and interstitial pulmonary fibrosis.

4.4. Thrombophilia

There are few data regarding the incidence of inherited or acquired thrombophilia in SOT. Morrissey et al. [48] reviewed the outcome of 8 patients with a previous history of VTE and thrombophilia. Patients with a hypercoagulable disorder received perioperative heparin and postoperative oral anticoagulation and were compared with other renal transplant recipients who had a theoretically low risk for VTE and did not receive anticoagulation therapy. No allograft thrombosis was observed in the high-risk group, although 2 patients in the low-risk group experienced DVT; 2 of the 8 patients who received anticoagulants had bleeding complications requiring intervention.

5. Prevention of venous thromboembolism

It is not clear whether SOT patients should receive anticoagulation prophylaxis. Bleeding and thrombosis are major complications that compromise both the graft and the patient's life. Lack of consensus on which of the 2 is a greater threat hampers the design of a preventive strategy. This diversity in clinical practice was reflected in a study by Ripert et al. [49], who performed a telephone survey of all renal transplantation centers in France. Four cases were considered by each center, and very different prophylaxis protocols were proposed in each of them, thus reflecting a wide variety of practices. Table 3 summarizes the published literature on preventing VTE in SOT patients.

In renal transplantation, studies evaluating epidemiology do not show differences between those patients receiving no prophylactic treatment and those with prophylaxis (whether heparin followed by anti-platelet agents or heparin for 3 months) that reported an incidence between 9 and 12.5% [50,51].

Friedman et al. [52] developed an algorithm to identify high-risk patients who were initially treated with intravenous sodic heparin and later with warfarin, achieving a 2.6-fold reduction in the expected incidence of allograft thrombosis according to data from a historical control group; in contrast, a high rate of hemorrhagic complications was reported, with an incidence of 60% within the first 30 days after transplantation.

In another study [53], low-risk patients were treated with dalteparin during hospitalization only (daily intravenous dose of 2500 or 5000), and high-risk patients were treated for at least 1 month, with no thrombotic or hemorrhagic events. Murashima et al. [54] performed a retrospective study including 48 kidney recipients with a hypercoagulable state defined by prior venous thromboembolism, multiple vascular access thromboses, or identifiable thrombophilia. Sixteen received different anticoagulation prophylaxis regimens: heparin followed by warfarin in 12 cases (9 of them indefinite), heparin followed by aspirin indefinitely in 3 cases, and heparin for only 3 days in 1 case. The rate of allograft thrombosis was not significantly lower in the group that received anticoagulation therapy (6.3 vs. 18.8%), with an incidence of perirenal hematoma of 31.3 and 6.3%, respectively.

Two studies randomized patients to subcutaneous heparin compared with no anticoagulant treatment, although the results were conflicting. In 1989, Ubhi et al. [55] randomized 70 kidney recipients to receive either no heparin or subcutaneous calcium heparin (5000 IU twice daily for 7 days or until the patient was fully mobile) and reported 6 events in the heparin-free group compared with no thrombotic events in the heparin group. No significant increase in bleeding complications was observed. In 2012, Bakkalaglu et al. [56] enrolled 25 kidney recipients who were prescribed a prophylactic dose of LMWH for 1 week, and 25 other recipients who did not receive heparin; no thrombotic events were reported in either group, and only 1 patient in the heparin group had massive postoperative bleeding. The authors concluded that heparinization may not be necessary if no risk factors are identified, but the small sample size could be an important limitation of this study.

The efficacy of aspirin for preventing renal allograft thrombosis has also been tested. Robertson et al. [57] performed a retrospective study comparing the rate of allograft thrombosis in patients receiving aspirin 75 mg/d for 1 month to historical controls from the Oxford Transplant Centre Database. The rate of allograft thrombosis was 5.6% before prophylaxis with aspirin and 1.2% after prophylaxis ($p < 0.01$). The Leicester group [58] reviewed a consecutive series of 105 kidney recipients treated with aspirin 150 mg daily for 3 months and compared them with an untreated historical control group. A lower rate of primary allograft thrombosis was observed in the treated group, where no cases were identified, than in the control group, where the rate was 5%. No major bleeding complications were recorded in either group. These results were reproduced in 2007 by Stechman et al. [59], who treated 401

Table 3
Prophylactic protocols evaluated in the literature.

	First author	Prophylactic strategy	Results	Complications
Kidney transplantation	Friedman et al. [52]	Control (*n*: 346): no anticoagulation High risk (*n*: 502): W after NFH	2.6-fold reduction in GT incidence	60% hemorrhagic complications
	Alkhunaizi et al. [53]	Low risk (*n*: 65): LMWH (hospitalization) High risk (*n*: 55): LMWH (1 month)	No thrombotic events	No hemorrhagic events
	Morrissey et al. [48]	Low risk (*n*: 227): no anticoagulation High risk (*n*: 8): W after perioperative heparin	Low risk: 2 DVT (0.8%) High risk: no events.	2 hemorrhagic events (25%)
	Murashima et al. [54]	No anticoagulation (*n*: 32) Anticoagulation (NFH + W (*n*: 12), NFH + AAS (*n*: 3) or NFH alone (*n*: 1).	Lower GT rate (6.3 vs. 18.8%)	Higher incidence of renal hematoma (31.3 vs. 6.3%)
	Ubhi et al. [55]	No anticoagulation (*n*: 37) vs. SCaH for 7 days or until fully mobile (*n*: 32)	6 thrombotic events in the heparin-free group (14%)	No increase
	Bakkalaglu et al. [56]	No anticoagulation (*n*: 25)vs. LMWH (*n*: 25) for 1 week	No thrombotic events	1 case of massive bleeding in the heparin group
	Robertson et al. [57]	Controls (*n*: 475) vs. aspirin 75 mg/d for 1 month (*n*: 480)	Lower GT rate (5.6 vs. 1.2%)	
	Murphy et al. [58]	Controls (*n*: 121) vs. aspirin 150 mg/d for 3 months (*n*: 105)	Lower GT rate (5 vs. 0%)	No hemorrhagic events
	Stechman et al. [59]	Controls (*n*: 396) vs. aspirin 75 mg/d for 28 days (*n*: 401)	Lower GT rate (5.8 vs. 0.25%)	
Liver transplantation	Kaneko et al. [61]	Dalteparin 2 days followed by heparin (adjusted by ACT) (*n*: 128)	3% thrombotic events	15% major bleeding events
	Uchikawa et al. [62]	Fixed LMWH dose + FFP (*n*: 32) vs. LMWH (adjusted by ACT) + FFP (*n*: 10)	No thrombotic events in LMWH adjusted by ACT	No bleedings in LMWH adjusted by ACT
	Vivarelli et al. [63]	No prophylaxis (*n*: 602) vs. aspirin 100 mg/d (*n*: 236)	Lower GT rate (2.2 vs. 0.4%)	
	Shay et al. [64]	No prophylaxis (*n*: 304) vs. aspirin 325 mg/d (*n*: 165).	Lower early GT rate (3.6 to 0%)	No increase

OAC: oral anticoagulation; GT: graft thrombosis; DVT: deep vein thrombosis; SCaH: subcutaneous calcium heparin; LMWH: low-molecular-weight heparin; NFH: non fractionated heparin; W: warfarin; AAS: aspirin; ACT: activated clotting time; FFP: fresh frozen plasma.

kidney recipients with aspirin 75 mg/d for 28 days. Only 1 case of venous thrombosis was recorded (0.25%), representing a significant reduction in the unit's historical incidence (5.8%) ($p < 0.001$).

Treatment with heparin and aspirin has been associated with a significant rate of severe bleeding complications. Thus, Shullo et al. [60] described a 69% incidence of major bleeding events. Consequently, the authors recommend using only aspirin as a prophylactic treatment in kidney recipients.

Although standard prophylaxis with LMWH might help to avoid thrombotic events in liver recipients, it has not been widely used because of the stronger anticoagulant activity described in cirrhotic patients. Supporting this statement, Kaneko et al. [61] performed a study of 128 liver recipients treated with dalteparin for 2 days after transplantation and later heparin (unfractionated heparin sodium, 5000 U/d) adjusted according to activated clotting time. Four patients experienced a

Table 4
Treatment and outcome of VTE after SOT.

Organ	Author	Year transplantation	Treatment		Bleeding complications	Mortality attributable to VTE	Graft disfunction
			Drug	Duration			
Kidney	Allen et al. [7]	1974–1984	Hep followed by W	3 months	Intracerebral hematoma 1 case (2.5%)	10%	5%
Kidney	Humar et al. [8]	1985–1995	Hep iv followed by W	3–6 months	Not available	8.5%	2.6%
Kidney	Poli et al. [50]	1990–2005	NFH iv followed by OAC or LMWH	3 months	Bleeding rate 12.2 per 100 patient-years.	No deaths	Not available
Kidney	Todeschini et al. [51]	2008–2011	NFH followed by W	3–6 months	Not available	Not available	Not available
Kidney	Moscarelli et al. [46]	1991–2010	NFH	Not available	Not available	Not available	Not available
Kidney	Abbott et al. [9]	1996–2000	Not available			3-year survival 75% DVT; 50% PE	2.5%
Liver	Sakai et al. [10][1]	2004–2006	Hep iv.1case tPA.	Not available	Not available	30%	Not available
Liver	Ishitani et al. [11]	1989–1995	Hep iv followed by W or VCF	Long term	No complications	7.1%	Not available
Liver	Salami et al. [12]	1995–2010	W	3–6 months	No complications	9.5%	Not available
Liver	Cherian et al. [65]	1982–2007	W	Long term	Not available	25%	Not available
Heart	Forrat et al. [13]	1984–1988	Not available			5.1% TE deaths[2]	Not available
Heart	Miriuka et al. [14]	1986–1997	Not available			No deaths	Not available
Heart	Garcia-Herrera et al. [15]	1984–1999	Hep followed by W	Not available			
Lung	Kroshus et al. [16]	1986–1993	Hep followed by W or VCF. 3 urokinasa	Not available		7.14%	7.14%
Lung	Izbicki et al. [17]	1997–2003	Not available				
Lung	Nathan et al. [18]	1996–2002	Hep iv followed by W or VCF. 1 thrombectomy. 4 TPA	Not available	Not available	14%	Not available
Lung	Garcia-Salcedo et al. [19]	1999–2009	LMWH followed by W.	Not available		No deaths	Not available
Lung	Burns et al. [20]	1990–2002	Not available				
Lung	Yegen et al. [21]	2001–2005	Not available				
Lung	Kahan et al. [27]	1994–2006	Not available				

DVT: deep vein thrombosis; PE: pulmonary embolism; Hep: heparin; W: warfarin; OAC: oral anticoagulation; LMWH: low-molecular-weight heparin; NFH: non fractionated heparin; VCF: vena cava filter;tPA: tissue plasminogen activator. 1: Intraoperative VTE. 2: TE deaths are deaths related to cardiac and noncardiac TE complications.

thrombotic event despite the anticoagulation protocol, but 19 patients had a major bleeding event. These findings contrast with those of Uchikawa et al. [62], who recruited 42 liver recipients and divided them into 2 groups. The first received a fixed dalteparin dose and a large amount of fresh frozen plasma, while the second was treated with an appropriate dosage of dalteparin to maintain activated clotting time from 140 to 150 seconds and a small amount of fresh frozen plasma. Neither thrombotic nor hemorrhagic complications were observed for anticoagulation therapy with adjusted dalteparin based on activated clotting time.

In 2006, Vivarelli et al. [63] performed a study of 838 liver recipients to evaluate antiplatelet prophylaxis. Patients were divided in 2 groups, one treated with aspirin 100 mg/d and the other without prophylaxis. Late hepatic artery thrombosis occurred in 1 out of 236 (0.4%) patients who continued to receive antiplatelet treatment and in 13 out of 592 (2.2%) who did not receive prophylaxis. In a cross-sectional study, Shay et al. [64] reviewed 165 liver recipients taking aspirin (325 mg/d) and 304 liver recipients who did not receive antiplatelet treatment. The incidence of early hepatic artery thrombosis leading to graft loss decreased significantly from 3.6 to 0%.

There are no studies comparing different prophylactic protocols in lung transplantation. Retrospective studies evaluating epidemiology of VTE after lung transplantation under different prophylactic protocols showed an incidence between 12 and 29%, similar to those observed without specific prophylaxis [16,27].

6. Treatment and outcome

Regarding VTE treatment, no specific protocols have been designed. Heparin followed by warfarin for 3 to 6 months is the most used protocol in the studies reviewed, as showed in Table 4. Only two groups [11,65] treated the patients with long term anticoagulation. Bleeding complications have been occasionally notified; Allen et al. [7] reported one case of intracerebral hematoma and Poli et al. [50] described a bleeding rate of 12.2 per 100 patient-years (two cases of muscle hematomas and 3 gastrointestinal bleedings). By contrast, two other studies [11,12] have not described any bleeding complications after implementation of anticoagulation.

There is scarcity of data regarding the prognosis of the graft after VTE, but some series [7–9,16] report an incidence of graft dysfunction in these patients around 2.5–5% in renal transplantation and 7% in lung transplantation. The mortality rate attributable to this complication is significant and ranges between 0 and 30%.

7. Conclusion

In conclusion, this review of the literature on thrombotic events after SOT highlights the importance of this complication. VTE is frequent after SOT, mainly early after the intervention, although the risk seems to remain high during long-term follow up.

Despite general concern surrounding VTE, no clear data support a specific and effective prophylactic strategy. There are few data evaluating the presence of acquired or inherited thrombophilia in SOT patients in order to establish high risk patients, and protocols vary from center to center with contradictory results. Large, multicenter, randomized, clinical trials are necessary to define the best prophylactic protocol in each type of SOT.

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ORIGINAL ARTICLE

Prophylaxis with enoxaparin for prevention of venous thromboembolism after lung transplantation: a retrospective study

Berta Sáez-Giménez[1], Cristina Berastegui[1], Helena Sintes[1], Javier Perez-Miranda[1], Ana Figueredo[1], Manuel López Meseguer[1], Víctor Monforte[1,2], Carlos Bravo[1,2], Amparo Santamaría[3], Maria Antonia Ramon[1,2], Susana Gómez-Ollés[1,2] & Antonio Roman[1,2]

1 Pulmonology Service, Lung Transplant Program, Hospital Universitari Vall d'Hebrón, Universitat Autònoma de Barcelona, Barcelona, Spain
2 Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid, Spain
3 Hemostasis and Thrombosis Unit, Department of Hematology, Hospital Universitari Vall d'Hebrón, Barcelona, Spain

Correspondence
Susana Gómez-Ollés, Pulmonology Department, Hospital Universitari Vall d'Hebrón, Pg. de la Vall d'Hebrón, 119-129, 08035 Barcelona, Spain.
Tel.: +34934894048;
fax: +34934894049;
e-mail: susana.go@vhir.org

SUMMARY

Venous thromboembolism (VTE) is a frequent complication after solid organ transplantation (SOT) and, specifically, after lung transplantation (LT). The objectives of this study were to evaluate prophylaxis with enoxaparin and to describe risk factors for VTE after LT. We retrospectively reviewed the clinical records of 333 patients who underwent LT in our institution between 2009 and 2014. We compared two consecutive cohorts: one that received enoxaparin only during post-transplant hospital admissions and a second cohort that received 90-day extended prophylaxis with enoxaparin. Cumulative incidence function for competing risk analysis was used to determine incidence of VTE during the first year after transplantation. Risk factors were analyzed using a Cox proportional hazards regression model. The cumulative incidence of VTE was 15.3% (95% CI: 11.6–19.4). Median time from transplant to the event was 40 (p25–p75, 14–112) days. Ninety-day extended prophylaxis did not reduce the incidence of VTE. In this study, the risk factors associated with VTE were male gender and interstitial lung disease. VTE is a major complication after LT, and 90-day extended prophylaxis was not able to prevent it. Large, multicenter, randomized clinical trials should be performed to define the best strategy for preventing VTE.

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Introduction

Venous thromboembolism (VTE) is a major complication after surgery. A previous review of the literature highlights the importance of this issue after solid organ transplantation (SOT) [1]. The incidence of VTE ranges between 2% and 14% in kidney transplantation [2], between 3% and 5% in liver transplantation [3–7], between 18% and 34% in heart transplantation, and between 8% and 29% in lung transplantation (LT) [8–14]. Different prevention strategies have been used in some of these studies, which have not been systematically studied.

Solid organ transplantation is a complex environmental risk factor for VTE and carries an inherent risk arising from surgery itself, immunosuppressive treatment,

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cytomegalovirus (CMV) infection, underlying disease, and thrombophilia. In LT, the factors associated with a higher risk of VTE include traditional factors, such as older age, diabetes mellitus, pneumonia [12], and surgery-related risk factors such as need for bypass and time to discharge [13]. The role of idiopathic pulmonary fibrosis [10] and sirolimus [15,16] has yet to be established, although both factors seem to increase the risk of VTE. The specific burden of each factor remains unknown.

No consensus has been reached on prophylactic strategies, and several protocols are used in clinical practice. Findings from the field of liver and kidney transplantation suggest that either aspirin alone [17–21] or heparin adjusted for activated clotting time [22] could be useful. Various empirical protocols are used in LT, although there is no evidence to support one over the others.

We describe the impact of a specific prophylaxis protocol with enoxaparin on the incidence of VTE and the risk factors associated with VTE in a population of LT recipients.

Materials and methods

Study population

We retrospectively reviewed the computerized clinical records of 333 consecutive adult patients who underwent LT in our institution between January 2009 and December 2014. We recorded prophylaxis, age, gender, body mass index, diabetes mellitus, previous thrombotic events, CMV status, underlying disease, transplant type, need for cardiopulmonary bypass, mechanical ventilation, length of stay, primary graft dysfunction, medication, and mobility. The study protocol was approved by the institutional Ethics board.

We identified patients with any thrombotic event during the first year after LT, including deep venous thrombosis (DVT), pulmonary embolism (PE), and treated upper extremity thrombosis (TUET). Patients receiving lifetime anticoagulation therapy prior to LT were excluded. Incidental PE (untreated subsegmental perfusion defects without clinical repercussion) was not taken into account.

As part of our standard protocol, all patients underwent a ventilation–perfusion scan (VPS) following lung transplantation immediately before discharge. No subsequent screening for VTE was performed. Patients who presented with symptoms suggestive of DVT were further studied using Doppler ultrasound (US). If PE was suspected, patients underwent either VPS or computed tomography (CT) pulmonary angiography.

Between 2009 and 2012 prophylaxis with the low-molecular-weight heparin (LMWH) enoxaparin (40 mg subcutaneously) was given once daily to all patients admitted to hospital after LT (control cohort). The prophylaxis began on postoperative day 1 if there were no formal contraindications. In January 2013, concern over the high incidence of VTE led us to change our standard protocol, and patients have since been receiving prophylactic enoxaparin up to day 90 or until full mobility is recovered (study cohort). Dosing of enoxaparin was adjusted in renal impairment and according to patient weight. We did not use any protocol to monitor enoxaparin treatment.

Data analyses

Qualitative variables are expressed as absolute numbers and percentages. Normally distributed quantitative variables are expressed as the mean and standard deviation; non-normally distributed variables are expressed as the median and interquartile range (p25–p75).

The demographic and clinical variables of patients receiving conventional prophylaxis and those receiving 90-day extended prophylaxis after LT were compared using the chi-square test for qualitative variables (or the Fisher exact test when one of the expected effects was less than 5). Normally distributed quantitative variables were compared using an unpaired *t*-test; non-normally distributed quantitative variables were compared using the Mann–Whitney test.

Cumulative incidence function for competing risk analysis was used to determine incidence of VTE through the formulas proposed by Gooley *et al.* [23] and Greewood (cited in Marubini *et al.* [24]) using the STATA syntax *stcompet*; both for the entire group and according to type of anticoagulant prophylaxis. Death and retransplant without previous VTE were treated as competing risks.

Cox proportional hazards regression was applied by modeling time from LT to the first event, with VTE as the outcome measure. We first conducted univariate analyses based on the Cox proportional hazards model using each of the potential predictors of VTE as independent variables and VTE as the dependent variable. Then, we performed a stepwise multivariable Cox regression with a backward elimination (*P*-value criterion of 0.20) fitted with all candidate variables, after adjusting for type of anticoagulant prophylaxis.

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Data were analyzed using STATA software (StataCorp. 2011, release 12.1 College Station, TX, USA: StataCorp LP).

Results

Comparison between study and control cohorts

The study group (90-day extended prophylaxis) comprised 138 patients, and the control group 195 patients. The demographic characteristics of both cohorts are shown in Table 1. Older age, interstitial lung disease as the underlying disease, diabetes mellitus prior to transplantation, and hemodynamic instability (defined as the need for vasoactive drugs) were more frequent in the study cohort. However, the incidence of thromboembolic events was not significantly different between the protocols (Table 1). Thirteen patients in the study group did not receive the prophylaxis protocol due to diverse reasons, but none of them developed VTE.

Thromboembolic events

Fifty patients developed VTE and 52 died during the first year after LT. The cumulative incidence of VTE during this period was 15.3% (95% CI: 11.6–19.4) in the entire group; 16.1% (95% CI: 11.3–21.6) in the cohort receiving conventional prophylaxis and 14.1% (95% CI: 8.9–20.6) in cohort receiving 90-day extended prophylaxis, with no differences between the two cohorts (Fig. 1). The events are classified in Table 2. Median time from transplant to the event was 40 days (p25–p75 14–112). Twenty-nine events (58%) took place before hospital discharge (Table 1 and Fig. 2); of the other 21 events, 11 (22%) were within the first 90 days after LT and 10 (20%) were between postoperative day 90 and the first year (Table 1). There were 22 events in single LT patients, nine (41%) of them localized in the graft.

Pulmonary embolism and deep venous thrombosis of the lower extremities

Forty-one PE and two DVT were diagnosed during the first year after LT. We considered six asymptomatic subsegmental PE to be incidental events that were not treated; therefore, they were not taken into account for the purposes of the study. The final sample was 35 PE. The clinical characteristics of the events are shown in Fig. 3.

Four single LT recipients due to interstitial lung disease did not have CT angiography to confirm the diagnosis. In three of these four cases, a high probability VPS was considered enough for the diagnosis of PE. The last case had an intermediate probability VPS coinciding with a femoral vein DVT.

No patients fulfilled the criteria for massive PE. Thirteen patients underwent echocardiography close to the event, and three presented right ventricular dilatation. Coagulation assays revealed one lupus anticoagulant-positive patient and one patient with mild factor VII deficiency.

In 24 (68.6%) of the 35 patients with PE, VPS was performed between 7 and 857 days after the event (median time of 172 days). Partial reperfusion of the defects was reported in 10 patients and persistent perfusion defects in two. One patient had a new perfusion defect (detected by VPS 145 days after the event) No patients developed pulmonary hypertension after PE.

There were 12 deaths (32.4%), 11 of which were considered not directly related to the thrombotic event. The causes of death were chronic allograft dysfunction (four cases), respiratory infection (three cases), sepsis (two cases), melanoma (one case), and multiple organ failure (one case). During the follow-up [median 659 (p25–p75 138–1337) days], eight of the 37 patients (21.6%) developed chronic allograft dysfunction (five cases of bronchiolitis obliterans syndrome and three cases of restrictive allograft syndrome).

Treated upper extremity thrombosis

Treated upper extremity thrombosis alone was diagnosed in 12 patients. As it was related to intravenous devices, most cases were diagnosed in the ICU. Only one patient presented at the emergency department 5 days after discharge with swelling of the left arm that had begun 3 days earlier. All the cases were symptomatic, and thrombus extension was evaluated using ultrasound to confirm the diagnosis and the need for anticoagulant treatment.

Safety

There were two mild bleeding events in patients under prophylactic doses of LMWH. Only one patient in the study cohort receiving anticoagulation at treatment doses suffered a massive epistaxis with airway obstruction that required invasive mechanical ventilation and admission to intensive care unit.

In the control cohort there were four bleeding events in patients receiving anticoagulation treatment: hemothorax (two cases), hematoma (one case), and thrombocytopenia (one case).

Table 1. Characteristics of lung transplantation (LT) recipients according to anticoagulant prophylaxis. Bold values indicate statistically significant correlations.

	All *n* = 333	Control cohort *n* = 195	Study cohort *n* = 138	*P*
Pretransplant variables				
Age, mean (SD)	52.0 (11.4)	50.2 (11.8)	54.5 (10.5)	**<0.001**
Sex: male, *n* (%)	201 (60.4)	119 (61.0)	82 (59.4)	0.768
BMI, *n* (%) [*n* = 327]				
<20 kg/m^2, *n* (%)	33 (10.1)	23 (12.1)	10 (7.3)	0.199
20–24.9 kg/m^2, *n* (%)	110 (33.6)	58 (30.5)	52 (38.0)	
≥25 kg/m^2, *n* (%)	184 (56.3)	109 (57.4)	75 (54.7)	
Diagnosis, *n* (%)				
Interstitial lung diseases (ILD)	143 (42.9)	74 (38.0)	69 (50.0)	**0.029**
COPD, bronchiectasis, or BO	123 (36.9)	73 (37.4)	50 (36.2)	0.823
Cystic fibrosis	25 (7.5)	16 (8.2)	9 (6.5)	0.566
Pulmonary arterial hypertension	17 (5.1)	13 (6.7)	4 (2.9)	0.138
LAM	5 (1.5)	3 (1.5)	2 (1.5)	0.989
Other	20 (6.0)	16 (8.2)	4 (2.9)	0.989
Pretransplant diabetes mellitus, *n* (%)	52 (15.6)	22 (11.3)	30 (21.7)	**0.010**
Pretransplant VTE, *n* (%)	10 (3.0)	8 (4.1)	2 (1.5)	0.205
Pretransplant CMV serology (positive), *n* (%)	282 (84.7)	161 (82.6)	121 (87.7)	0.201
Peritransplant variables				
Type of lung transplant, *n* (%)				
Bilateral	201 (60.4)	116 (59.5)	89 (64.6)	0.699
Single	132 (39.6)	79 (40.5)	53 (38.4)	
Extracorporeal circulation, *n* (%)	75 (22.5)	49 (25.1)	26 (18.8)	0.176
Reintervention, *n* (%)	32 (9.6)	15 (7.7)	17 (12.3)	0.158
Surgical complications, *n* (%)	38 (11.4)	23 (11.8)	15 (10.9)	0.794
Hemodynamic instability, *n* (%)	213 (64.0)	135 (69.2)	78 (56.5)	**0.017**
Days on mechanical ventilation, median (p25–p75)	13 (2–37)	13 (2–42)	12 (2–36)	0.571
Days of hospitalization, median (p25–p75)	37 (25–60)	38 (25–65)	36 (24–56)	0.228
Primary graft dysfunction, *n* (%)	114 (34.3)	71 (36.4)	43 (31.4)	0.343
Post-transplant variables				
Treatment with mTOR, *n* (%)	7 (2.1)	3 (1.5)	4 (2.9)	0.454
Reduced mobility 3 months post-transplant, *n* (%) [*n* = 258]*	41 (15.9)	19 (12.7)	22 (20.4)	0.169
CMV disease, *n* (%)	20 (6.0)	14 (7.2)	6 (4.4)	0.284
Incidence of thromboembolic events				
Total thromboembolic events, *n* (%)	50 (15.0)	31 (15.9)	19 (13.7)	0.592
Before discharge	29 (58.0)	19 (9.7)	10 (7.3)	0.426
After discharge [*n* = 261]†	21 (8.1)	12 (7.8)	9 (8.3)	0.886
<90 days after discharge [*n* = 261]†	11 (22.0)	7 (4.6)	4 (3.7)	0.730
>90 days after discharge [*n* = 240]‡	10 (20.0)	5 (3.7)	5 (4.9)	0.749

BMI, body mass index; COPD, chronic obstructive pulmonary disease; BO, bronchiolitis obliterans; LAM, lymphangioleiomyomatosis; VTE, venous thromboembolism; CMV, cytomegalovirus.

*Patients at risk of a thromboembolic event at 3 months after transplant.

†Patients at risk of a thromboembolic event after hospital discharge.

‡Patients at risk of a thromboembolic event 90 days after hospital discharge.

Study of risk factors

Table 3 shows the influence of the variables included in the univariate Cox analysis on the incidence of VTE. Multivariable Cox proportional hazards analysis revealed that male gender (HR 2.72; 95% CI 1.25–4.03; $P = 0.007$) and interstitial lung disease (HR 2.25; 95% CI 1.25–4.03; $P = 0.007$) were significantly related to VTE, after adjusting for type of anticoagulation prophylaxis (Table 4). Ninety-day extended prophylaxis did not seem to protect from VTE in the study population.

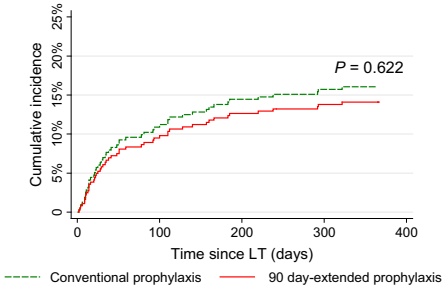

Figure 1 Cumulative incidences of thromboembolic event according to anticoagulant prophylaxis (n = 333).

Table 2. Classification of the thromboembolic events recorded during the first year after lung transplantation (LT).

	Patients with a thromboembolic event n = 50 (%)
Deep venous thrombosis (DVT) of the lower extremities	2 (4.0)
Pulmonary embolism (PE)	35 (70.0)
PE + DVT	5/35
Treated upper extremity thrombosis	12 (24.0)
Atrial thrombosis	1 (2.0)

Discussion

Our results show that VTE is a relevant complication following LT, mainly in the early postoperative period and that 90-day extended prophylaxis seems unable to prevent it.

The 15% incidence of VTE in our cohort is similar to that reported for LT recipients in the literature, namely 8–29% [8–11,13,25,26]. The single case of atrial thrombosis in 333 LT patients we recorded is within the expected range; we interpreted this problem as being directly related to the surgical procedure. It is difficult to explain the high incidence of VTE in LT, although the various potentially involved factors include increased vascular trauma, higher levels of immunosuppression, and worse preoperative functional status [13]. The variations in incidence found in epidemiologic studies may be due to differences in methodology (i.e., follow-up time, VTE screening protocol, and prophylactic treatment), which hamper comparison between studies. One study [8] reports data on patients under periodical surveillance by VPS, but there is no information regarding the incidence of asymptomatic events. In our study, 10 of the 35 PE were asymptomatic events detected in the protocol VPS, thus highlighting the role of this test to assess graft vasculature after surgery. DVT is not routinely screened for, although some groups have recently implemented routine assessment of DVT and 3-month prophylaxis with enoxaparin after reporting a 39% incidence of DVT and 15% incidence of PE in patients where only suspected events were investigated [26]. Less is known about the impact of VTE on survival. From the few small series published, mortality attributable to VTE seems to be low (between 7% and 14%), [8–10] as is the frequency of chronic graft allograft dysfunction following VTE (7–15%) [8–12]. Nevertheless, VTE seems to be more frequent in frail patients and therefore is associated with poorer prognosis. Following this line of argument, Evans *et al.* [26] described DVT as a risk factor for patient survival (HR 2.43; 95% CI, 1.29–4.64), and Lingaraju *et al.* [15] found VTE to be associated with poorer survival 3 months after LT.

Data on risk factors in LT in the literature are inconsistent. The present study found male patients and patients with interstitial lung disease to be more susceptible to VTE in the adjusted analysis. Nathan *et al.* [10] pointed out the role of idiopathic pulmonary fibrosis as a risk factor in a small cohort of 72 lung recipients. The authors detected 7 VTE events, all of them in patients with idiopathic pulmonary fibrosis. Susceptibility was attributed to inherent disease factors, because other circumstances (i.e., functional status and length of stay) were similar in both groups. This possibility was also explored by Navaratnam *et al.* [27] in a study that compared 211 incident cases of idiopathic pulmonary fibrosis and 256 age- and sex-matched controls. The authors found that a prothrombotic state—defined as any acquired or inherited clotting defect—was four times more common in idiopathic pulmonary fibrosis patients than in controls. Although other authors have suggested age as a confounding factor in the association between idiopathic pulmonary fibrosis and VTE [12], the results of Navaratnam *et al.* support the idea of pretransplant idiopathic pulmonary fibrosis as a risk factor for VTE.

In our study, we evaluated 90-day extended prophylaxis with enoxaparin and found that this strategy did not protect against VTE. To our knowledge, this is the first study to evaluate a prophylaxis protocol in LT. Prophylaxis protocols have been evaluated in kidney and liver transplantation, although most studies were retrospective and analyzed few patients, and only two are randomized [28,29]. These studies showed

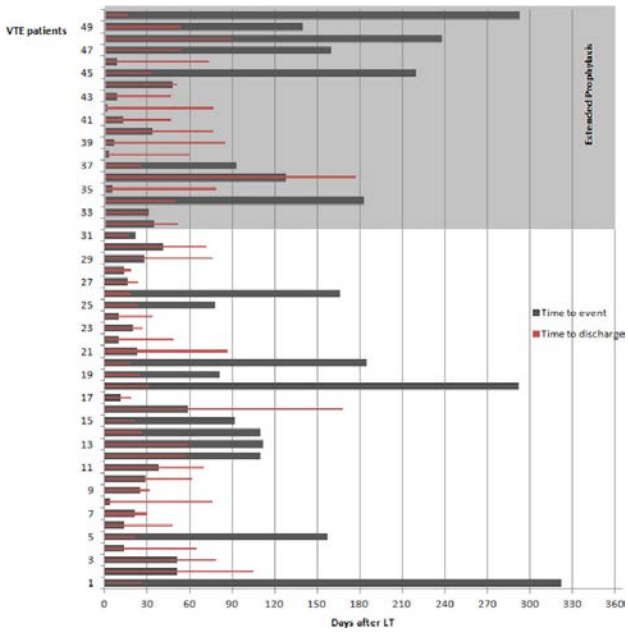

Figure 2 Time to event and hospital discharge of all patients with venous thromboembolism (VTE).

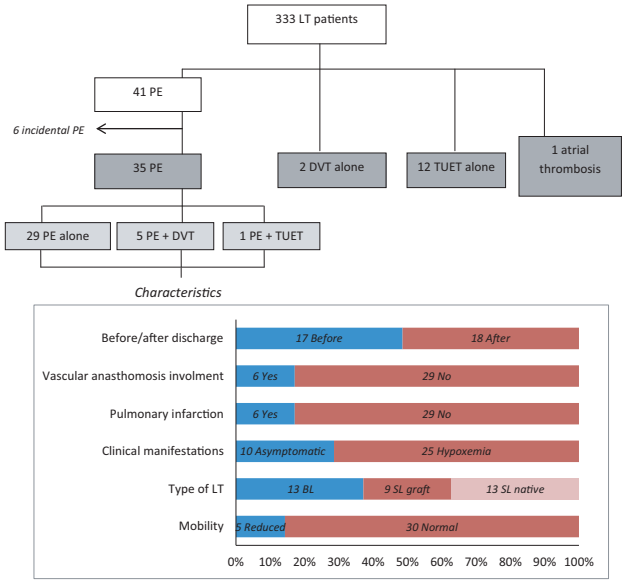

Figure 3 Flow chart and characteristics of the thrombotic events.

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Table 3. Univariate predictors of VTE (univariate Cox models).

	Hazard ratio (95% CI)	*P*
Pretransplant variables		
Age (years)	1.01 (0.99–1.04)	0.322
Sex: male	3.00 (1.45–6.18)	0.003
BMI (kg/m^2)	0.99 (0.93–1.06)	0.822
Interstitial lung disease	2.40 (1.34–4.27)	0.003
COPD, bronchiectasis, or BO	0.58 (0.31–1.09)	0.092
Cystic fibrosis	0.22 (0.03–1.62)	0.138
Pulmonary arterial hypertension	0.45 (0.06–3.24)	0.426
LAM	2.47 (0.34–17.9)	0.371
Pretransplant diabetes mellitus	0.70 (0.30–1.66)	0.426
Pretransplant VTE	1.24 (0.30–5.13)	0.760
Pretransplant CMV serology (positive)	1.46 (0.62–3.42)	0.386
Peritransplant variables		
Single lung transplant	1.67 (0.96–2.91)	0.069
Extracorporeal circulation	0.85 (0.41–1.75)	0.663
Reintervention	1.12 (0.40–3.10)	0.830
Surgical complications	1.10 (0.93–3.05)	0.860
Hemodynamic instability	1.25 (0.69–2.24)	0.461
Days on mechanical ventilation	0.99 (0.99–1.00)	0.953
Days of hospitalization	1.00 (0.99–1.00)	0.453
Primary graft dysfunction	1.44 (0.82–2.53)	0.208
Post-transplant variables		
Treatment with mTOR	0.84 (0.12–6.01)	0.860
Reduced mobility 3 months after transplant*	0.89 (0.26–3.01)	0.848
CMV disease	0.95 (0.29–3.04)	0.927
Extended prophylaxis	0.87 (0.49–1.54)	0.628

BMI, body mass index; COPD, chronic obstructive pulmonary disease; BO, bronchiolitis obliterans; LAM, lymphangioleiomyomatosis; VTE, venous thromboembolism; CMV, cytomegalovirus.

*Patients at risk of a thromboembolic event at 3 months after transplant $n = 258$.

Table 4. Best model of independent predictors of venous thromboembolism (VTE) after lung transplantation (LT) by stepwise multivariable Cox regression analysis, after adjusting for type of anticoagulation prophylaxis.

	Hazard ratio (95% CI)	*P*
90-day extended prophylaxis	0.77 (0.43–1.38)	0.386
Sex (male)	2.72 (1.25–4.03)	0.007
Diagnosis (ILD)	2.25 (1.25–4.03)	0.007

inconsistent results for the balance between bleeding risk and thrombotic events. Heparin implies a significant increase in bleeding complications in both kidney and liver transplantation [28–33], but treatment with dalteparin adjusted for activated clotting time in liver transplantation seems to be safe [22]. Prophylaxis of renal or liver allograft thrombosis with aspirin alone seems to be another good option that does not increase the frequency of bleeding complications [17–21], although doses differ between studies. Furthermore, one of the strengths of our study is the long follow-up of our patients, out to 1 year.

In our study, we did not use any protocol to monitor enoxaparin prophylaxis or treatment. Guidelines about antithrombotic treatment for VTE recommend enoxaparin monitorization in pregnancy, children, and renal impairment and only when receiving anticoagulation treatment, not prophylaxis [34]. There are three studies assessing this issue in solid organ transplantation [35–37]. All of them use antifactor Xa to monitor enoxaparin activity in transplanted patients receiving therapeutic anticoagulation. Standard dosing of enoxaparin was associated with high incidence of supratherapeutic anti-Xa levels in all three studies. Although we do not have evidence regarding the monitorization of prophylactic protocols, these studies suggest that standard doses of enoxaparin might be supratherapeutic and, thus, the lack of effect of our protocol might not be dose-related.

Venous thromboembolism is more frequent in the postoperative period; however, we did not find an association between VTE and surgical factors, such as need for bypass and time to discharge, as reported in Kahan *et al.* [13]. We were unable to replicate the results reported by Yegen *et al.* [12], who showed an association between traditional risk factors such as older age, diabetes mellitus, and pneumonia and a higher risk of VTE.

Therapy with mammalian target of rapamycin (mTOR) inhibitors has been considered a risk factor for thrombosis since the United States Food and Drug Administration warning in 2002, which alerted to a possible association with hepatic artery thrombosis among liver recipients [38]. In kidney transplantation, Baas *et al.* [39] reported increased endothelial activation, thrombin formation, and impaired fibrinolysis with everolimus. Subsequent studies have been unable to reproduce these results, either in liver or kidney transplantation [40–44]. Two studies report an increased risk of VTE in LT recipients with sirolimus [15,16]. Ahya *et al.* [16] performed a prospective, multicenter, randomized, open-label trial comparing the incidence of VTE in 181 LT patients who received a regimen based on tacrolimus, sirolimus, and prednisone or on

tacrolimus, azathioprine, and prednisone. The higher incidence of VTE in the sirolimus cohort persisted after adjusting for pretransplant diagnosis and after stratifying by transplant center. As only seven patients in our study received mTOR inhibitors during the first year after LT, it is not possible to draw conclusions.

Our study is subject to a series of limitations. First, it was a retrospective analysis performed in a single center. Second, we did not track the use of mechanical devices for thromboprophylaxis in the intensive care unit. Third, as no routine VTE screening was performed (US was performed only in symptomatic patients), we may have under-diagnosed thrombotic events, and the relatively short number of VTE events may have limited the detection of risk factors. Moreover, the effect of our protocol could have been diminished by the fact that we compared two consecutive cohorts that differed in terms of age and number of patients with pretransplant interstitial lung disease and diabetes and peritransplant hemodynamic instability. Although the two eras of our study did not differ in terms of the diagnostic protocol, it is not possible to dismiss a higher suspicion in the study cohort that could have lead to a higher detection rate and, consequently, to an infraestimation of the effect of the prophylactic treatment.

We conclude that VTE is a major complication after LT and that 90-day extended enoxaparin prophylaxis seems unable to prevent it. In our study, males and patients with interstitial lung disease were at higher risk of thrombotic events, even if no association was detected between this complication and other classic risk factors, surgical factors, or the use of mTOR inhibitors. Despite its limitations—retrospective and comparing two different eras—this study highlights the relevance of this complication, and the need for randomized clinical trials to identify the best strategies for preventing VTE in LT recipients.

Authorship

BSG: researched the data and wrote the article. JP and AF: participated in the data collection. CB, HS, MLM, VM, CD and AS: critically reviewed the article. MAR: performed the statistical analysis and reviewed the article. SG and AR: designed the study.

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Conflict of interest

The authors declare no conflict of interests.

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ANNEX II: LIST OF NATIONAL AND INTERNATIONAL PRESENTATIONS

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B. Saez-Gimenez, V. Cortina, M. Ramón, H. Sintes, C. Berastegui, M. Lopez-Meseguer, V. Monforte, C. Bravo, A. Santamaria, A. Roman. A Prospective Study of Coagulation Profile in Lung Transplantation. International Society for Heart and Lung Transplantation International Meeting and Scientific Sessions. Nice, April 2017.

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ANNEX III: GRANTS AND FUNDING

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