

Blood Coagulation, Fibrinolysis and Cellular Haemostasis

Medical conditions increasing the risk of chronic thromboembolic pulmonary hypertension

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Summary

Chronic thromboembolic pulmonary hypertension (CTEPH) is characterized by organized thromboemboli that obstruct the pulmonary vascular bed. Although CTEPH is a serious complication of acute symptomatic pulmonary embolism in 4% of cases, signs, symptoms and classical risk factors for venous thromboembolism are lacking. The aim of the present study was to identify medical conditions conferring an increased risk of CTEPH. We performed a case-control-study comparing 109

consecutive CTEPH patients to 187 patients with acute pulmonary embolism that was confirmed by a high probability lung scan. Splenectomy (odds ratio=13, 95% CI 2.7–127), ventriculo-atrial (VA-) shunt for the treatment of hydrocephalus (odds ratio=13, 95% CI 2.5–129) and chronic inflammatory disorders, such as osteomyelitis and inflammatory bowel disease (IBD, odds ratio=67, 95% CI 7.9–8832) were associated with an increased risk of CTEPH.

Keywords

Pulmonary embolism, clinical / epidemiological studies, acquired coagulation disorders, infection / bacterial, viral

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Introduction

Chronic thromboembolic pulmonary hypertension (CTEPH) is a serious and frequently undiagnosed disorder with significant morbidity and mortality. It develops in 0.5–5% of nonfatal acute pulmonary emboli (1, 2), thus being one of the most common forms of pulmonary hypertension (3). It is characterized by a combination of fresh and organized thrombi that permanently obstruct the pulmonary vascular bed. In contrast to venous thromboembolism, no predisposing factors or underlying medical conditions have been identified, with the exception of antiphospholipid antibodies in 10–20% of patients (4, 5), and plasma factor VIII>230IU/dl in 25% of patients (6). A few case reports have suggested a link between chronic thromboembolism and prior splenectomy (7–9), ventriculo-atrial (VA-) shunt for the treatment of hydrocephalus (10–16) or chronic inflammatory conditions (17). The aim of the present study was to systematically investigate whether these conditions are associated with an increased risk of CTEPH.

Methods

Due to the low incidence of CTEPH and the length of time until clinical manifestation, a case-control design was chosen (18). The study was conducted at the Departments of Cardiology and Haematology of the University of Vienna between July 1992 and December 2003. Since pulmonary (thrombo)endarterectomy (PEA) is a long-term successful treatment option of CTEPH and this treatment is offered only at the University of Vienna, possible surgical candidacy of every CTEPH patient in Austria is evaluated at this institution.

Referring centres

The majority of patients (n=99) were from Austrian centres, including Internal Medicine (n=26), Pulmonology (n=28), Cardiology (n=22), Cardiothoracic Surgery (n=5), Haematology (n=2), and private practice (n=14). Two patients referred themselves. Three further patients were from Croatia, three from Italy, two from Germany, one from Hungary and one from Egypt.

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Patients

We studied consecutive patients with CTEPH (n=109) at the time of the first diagnostic right-heart catheterization. The diagnosis of CTEPH was established by chest X-ray, transthoracic and transesophageal echocardiography with Doppler, pulmonary function tests including arterial blood gas analysis at rest and exercise, right heart catheterization, pulmonary angiography, ventilation-perfusion scan of the lungs, multi-slice and high resolution computed tomography. A panel of cardiologists, pulmonologists, radiologists, and cardiothoracic surgeons reviewed each case. CTEPH type (19, 20) was classified according to imaging, and if available, according to the surgical specimen. All patients were referred for exertional or resting dyspnea, with 10 patients in New York Heart Association functional class II, 67 in class III, and 32 in class IV. Mean 6-minute walking distance was 280±130m. Pulmonary function tests were within the normal range in all patients. Elevated antiphospholipid antibody titers were identified in 16% of patients. The prevalence of established risk factors for thrombosis (21) was not increased compared with the general population. One patient showed a combined G20210A/ATIII deficiency, three patients had hyperhomocysteinemia, one patient had protein S deficiency. Heterozygous Factor V Leiden was present in 5 patients. Pulmonary spirometric testing was normal, except for a mildly restrictive pattern in seven patients.

Because CTEPH is a thromboembolic disorder (1, 5), but does not share any of the established plasmatic risk factors for venous thromboembolism (4), the most suitable controls were patients with idiopathic pulmonary embolism who did not develop clinical pulmonary hypertension over time. The term idiopathic pulmonary embolism was defined according to current literature as pulmonary embolism not related to malignancy, pregnancy, surgery, following medical hospitalization, associated with serious medical disease such as chronic obstructive pulmonary disease, congestive heart failure, stroke, nephritic syndrome, and coagulation defects excluding FVIII or antiphospholipid antibodies. Accordingly, 187 patients who presented consecutively between July 1992 and December 1999 with acute idiopathic venous thromboembolism and a high probability ventilation-perfusion scan of the lungs (22) served as controls. Physical examination of control patients was performed at three-month intervals during the first year after enrollment and every six months thereafter until December 2002. Dyspnea on exertion and/or rest, syncope, or chest pain were considered as clinical signs of pulmonary hypertension. Echocardiography was performed in patients who developed any of these symptoms during the observation period. A thorough medical history of study participants, specifically focused on the presence/absence of risk factors under investigation, was obtained. A history of osteomyelitis was based on the medical records reporting the disease, radiographs and extended antibiotic treatment. Patients showed variable courses of chronic active osteomyelitis. One patient with inflammatory bowel disease (IBD) was diagnosed clinically with pelvic osteomyelitis. Patient #4 suffered from infectious bone destruction in the course of meningomyelocele, while the other patients had open leg fractures requiring surgical intervention and extended antibiotic treatments. Trauma was classified as multiple fractures and/or open injuries of the lower

limbs reported in the medical histories, all of which were not clinically associated with a thromboembolic event.

The diagnosis of IBD was accepted if the clinical, endoscopic, histological and radiological criteria according to the Lennard-Jones classification were fulfilled (23). Evidence for prior thromboembolism was based on suggestive clinical symptoms, and documented phlebography/ultrasound evidence of deep vein thrombosis, or a high probability lung scan. These data were extracted from the patients' medical histories.

Statistical analysis

The prognostic relevance of putative risk factors for CTEPH was investigated by multiple logistic regression. The risk factors considered were prior splenectomy, VA-shunt, and chronic inflammatory conditions, i.e. osteomyelitis and IBD. Because osteomyelitis due to intestinal fistulas and abscesses is common in patients with IBD (24), and is difficult to diagnose in this setting, the two conditions were grouped together. To avoid a confounding effect of conditions that had led to splenectomy or osteomyelitis, hemolytic anemia and trauma were included into the analysis. Univariate analysis with risk factors adjusted only for age and sex (marginal effects) was contrasted with multivariate analysis with risk factors additionally adjusted for all other risk factors (partial effects). Results are presented as odds ratios and corresponding 95% confidence limits and p-values.

Because the occurrence of unfavourable risk factor levels was low and parameter estimates were relatively large, these odds ratios were obtained by the penalized likelihood for logistic regression (25). Furthermore, confidence limits were obtained using the profile penalized likelihood approach as implemented in a specialized computer program (26), which for the given sample gives more reliable results than the standard Wald approach.

Differences between univariate and multivariate results were explained by means of partial Kendall's τ correlations (adjusting for age and sex) of the risk factors. Furthermore, possible departures from a main effects model were checked. Continuous outcomes are presented as means±standard deviations. Chi-square analysis was used to compare frequencies in a contingency table format.

The study was approved by the ethics committee of the Medical University of Vienna.

Results

Patients

Mean age of CTEPH patients (55 females, 54 males) was 58±16 years, controls (104 females, 83 males) were 55±16 years old (p=0.50). Controls were lacking clinical evidence of pulmonary hypertension during an observation period of 36–125 months after the initial event.

Ten of 109 CTEPH patients (9%) had undergone splenectomy 24–204 months (mean 118 months) prior to CTEPH diagnosis (Table 1). In the control group there was only one patient with prior splenectomy. Six VA-shunt carriers (6%) were among the CTEPH patients, versus one in the control group. Three VA-shunt patients with CTEPH had a history of recurrent shunt infections, and documentation of right atrial thrombus (Table 1,

Table 1: Clinical characteristics of patients with CTEPH and associated medical conditions.

Patients after splenectomy	age/sex	age at splenectomy	reason for splenectomy	age at CTEPH diagnosis	CTEPH type	treatment
1	50/f	39	motor vehicle accident	43	proximal	PEA
2	59/m	41	hemolytic anemia	58	distal	mt
3	63/f	45	osteomyelofibrosis	59	distal	mt
4	62/m	52	trauma	60	distal	mt
5	55/m	40	hemolytic anemia	54	distal	mt
6	54/m	42	hemolytic anemia	52	proximal	mt
7	70/f	67	ITP	69	distal	mt
8	76/m	68	gastric cancer	74	proximal	refused PEA
9	51/f	32	lymphoma	49	distal	mt
10	61/m	48	gastric ulcer	54	proximal	refused PEA
Patients with VA-shunt	age/sex	age at VA-shunt	reason for VA-shunt	age at CTEPH diagnosis	CTEPH type	treatment
1	35/f	15	internal hydrocephalus	35	distal	PEA
2	31/f	15	internal hydrocephalus	21	proximal	deceased prior to PEA
3	74/f	62	arachnoid cyst	73	proximal	PEA
4	61/f	53	internal hydrocephalus	56	distal	PEA
5	45/f	24	internal hydrocephalus	41	proximal	PEA
6	22/f	18	internal hydrocephalus	21	proximal	PEA
Patients with chronic inflammatory conditions	age/sex	age at diagnosis	reason for chronic inflammation	age at CTEPH diagnosis	CTEPH type	treatment
1	62/m	29	Crohn's disease	59	proximal	PEA
2	58/f	48	osteomyelitis	52	proximal	PEA
3	55/f	45	ulcerative colitis	53	distal	mt
4	28/f	15	osteomyelitis	26	distal	LTX
5	53/m	48	osteomyelitis	50	proximal	deceased prior to PEA
6	36/m	18	osteomyelitis	34	proximal	PTE
7	74/m	58	osteomyelitis	67	distal	refused PEA
8	50/f	25	Crohn's disease	44	proximal	PEA
9	29/m	22	Crohn's disease	27	proximal	deceased prior to PEA
10	46/m	35	osteomyelitis	43	distal	refused PEA
11	84/m	25	osteomyelitis	77	distal	mt

PEA =pulmonary thromboendarterectomy, mt=medical therapy, ITP=idiopathic thrombocytopenic purpura, LTX=lung transplantation, proximal/distal=location of most proximal thrombus by pulmonary angiography.

cases #2, 3, and 5). Mean device implantation time was 120±87 months. Eleven patients (10%) but no control had a history of osteomyelitis of the lower limbs, or IBD. One IBD patient had a clinical history of pelvic osteomyelitis.

A history of venous thromboembolism was identified in 53 patients (48%). In the group of patients with associated con-

ditions an even greater proportion of 17/27 patients (63%) had no clinical evidence of venous thromboembolism (p=0.0001). The mean time interval between the first venous thromboembolic event and the diagnosis of CTEPH was 95 months, with a range between 7 and 408 months. In 56 patients, no evidence and symptoms for prior thromboembolism could be identified, among those were five VA-shunt carriers, eight patients with osteomyelitis/IBD and four patients after splenectomy.

Clinical, functional, laboratory, and pulmonary haemodynamic variables were not different between subgroups, with a mean pulmonary arterial pressure of 49±15mmHg, pulmonary vascular resistance of 906±426dynes.s.cm⁻⁵, and mixed venous saturation of 57±12%. As expected, splenectomy was associated with significantly (p=0.0012) elevated platelet counts (348±197G/l), but there was no difference (p=0.17) in platelet counts between CTEPH patients (242±100G/l) and controls (230± 63G/l). In multiple logistic regression, elevated platelet counts were not associated with an increased risk of CTEPH (p=0.53).

Table 2: Medical conditions and adjusted odds ratios for the risk of CTEPH.

Condition	Cases (n=109)	Controls (n=187)	Odds ratios Marginal effects (95% CI)	Odds ratios Partial effects (95% CI)
Splenectomy	10 (9%)	1 (0.5%)	14 (3.1–129)	13 (2.7–127)
Hemolytic anaemia	3 (3%)	0 (0%)	12 (1.1–1625)	1.5 (0.06–265)
Trauma	5 (5%)	6 (3%)	1.5 (0.4–4.7)	0.7 (0.07–3.4)
VA-shunt	6 (6%)	1 (0.5%)	10 (2.0–96)	13 (2.5–129)
Chronic inflammation	11 (10%)	0 (0%)	46 (5.9–5991)	67 (7.9–8832)

Risk factors for CTEPH

Splenectomy, VA-shunt and chronic inflammatory states, i.e. osteomyelitis and IBD, remained independent risk factors for CTEPH in the multivariate analysis, with splenectomy increasing the likelihood of CTEPH 13-fold ($p=0.015$), VA-shunt 13-fold ($p=0.0014$), and chronic inflammation 67-fold ($p<0.0001$). Chronic inflammation and trauma were positively correlated ($t=0.30$) and so were splenectomy and hemolytic anemia ($t=0.38$), which explains why some risk factors lost their significance in the multivariate analysis (Table 2). No interactions of the prognostic factors were observed.

Discussion

Our study demonstrates that prior splenectomy, VA-shunt, osteomyelitis and IBD are risk factors for CTEPH. Regarding CTEPH as an extreme variant of venous thromboembolism, with a reported 3.8% prevalence after an episode of symptomatic idiopathic pulmonary embolism (1), these medical conditions must be considered as novel risk factors for venous thrombosis.

As CTEPH is an enigmatic disease with mostly asymptomatic pulmonary thromboembolism that is ongoing over years to decades (5), one methodological difficulty in the present study was to define an appropriate control group, with controls having sufficient time to develop symptomatic pulmonary hypertension within the observation period. According to echocardiographic studies, systolic pulmonary arterial pressure increases and right ventricular function declines in the years following acute pulmonary embolism (2). As exercise capacity is tightly associated with right ventricular function (27), it was assumed in the present study that the observation period is sufficient to develop symptoms of pulmonary hypertension. However, although it is unlikely, we cannot exclude that a proportion of patients in the control group had silently developed CTEPH. When matched non-anticoagulated patients coming for general admission to the cardiology department were chosen as controls, the same results were obtained.

Splenectomy was more frequently associated with distal types of CTEPH (19, 20) (Table 1), which explains why only one patient in this group underwent PEA. In previous work, splenectomy has been found associated with thromboembolic disease (7–9), but also with idiopathic pulmonary hypertension (IPAH) (28). Thrombus location in sub-segmental pulmonary artery branches and beyond is a diagnostic challenge in an area where the diagnoses of CTEPH and IPAH are overlapping (19).

An important observation that contrasts the findings in acute venous thromboembolism is the frequent lack of clinically significant thromboembolic events in CTEPH despite massive pulmonary arterial thrombus material. More than 50% of CTEPH patients did not manifest a clinical venous thromboembolic event in the course of their disease, while an even greater propor-

tion of 17/27 patients (63%) in the group with associated conditions remained free of clinical thromboembolic events. The observation of a long time interval between an initial event and the diagnosis is in accordance with previous data indicating that decades may lie between an initial thromboembolic event and clinical manifestation of CTEPH (29).

CTEPH risk factors are apparently unrelated clinical conditions. The pathogenesis of CTEPH is still unclear but it is widely accepted that the disease is determined by thromboembolic obliteration triggering an abnormal process of vascular remodeling due to a variety of factors, such as shear stress, pressure, inflammation, and the release of cytokines and vasculotrophic mediators. The observation of an association of CTEPH with prior splenectomy, VA-shunt, osteomyelitis and IBD provokes the hypothesis that bacterial infection of the thrombus precipitates fibrous organization, rendering the thrombus resistant to classical thrombolysis, and a surface for secondary *in situ* thrombosis. For example, three of the six VA-shunt carriers reported a history of recurrent shunt infections, one of them with a staphylococcus aureus-positive atrial thrombus. In previous studies VA-shunt infection has been reported in 27% of shunt carriers (30). Although none of the patients under investigation had a history of systemic infection after splenectomy, we cannot exclude episodes of silent, clinically inapparent infection. In fact, Ejstrud demonstrated an 8-fold increased risk of bacteremia in splenectomized patients (31). The concept that chronic inflammatory conditions might impair the pulmonary arterial fibrinolytic system in CTEPH patients, is weakened by previous data documenting that both plasminogen activator inhibitor-1 on the thrombus surface was demonstrated (34), pointing to the possibility that inflammatory processes within the thromboemboli which originate from peripheral sites, could underlie a very localized fibrinolytic dysregulation that may prevent thrombus resolution.

Despite the limitations that are inherent to a case-control study design and the difficulties to define an appropriate control group for a CTEPH cohort, this is the first study to describe medical conditions that are to be considered novel risk factors for chronic thrombosis. Since early treatment of pulmonary hypertension is associated with a clear survival benefit (35), identification of patient groups at increased risk is important. For example, HIV infection, anorexigen intake, liver disease, etc. (36, 37), have been linked to the development of non-thromboembolic pulmonary hypertension. Screening of these established risk populations has recently been recommended by the World Health Organization (38) and has become part of the American College of Chest Physicians' practice guidelines (39). Similarly, CTEPH risk populations may greatly benefit from early recognition and specific treatment in the future.

References

1. Pengo V, Lensing AW, Prins MH, et al. Incidence of chronic thromboembolic pulmonary hypertension after pulmonary embolism. *N Engl J Med* 2004; 350: 2257–64.
2. Ribeiro A, Lindmarker P, Johnsson H, et al. Pulmonary embolism: one-year follow-up with echocardiography doppler and five-year survival analysis. *Circulation* 1999; 99: 1325–30.
3. Lang IM. Chronic thromboembolic pulmonary hypertension—not so rare after all. *N Engl J Med* 2004; 350: 2236–8.
4. Wolf M, Boyer-Neumann C, Parent F, et al. Thrombotic risk factors in pulmonary hypertension. *Eur Respir J* 2000; 15: 395–9.
5. Fedullo PF, Auger WR, Kerr KM, et al. Chronic thromboembolic pulmonary hypertension. *N Engl J Med* 2001; 345: 1465–72.

6. Bonderman D, Turecek PL, Jakowitsch J, et al. High prevalence of elevated clotting factor VIII in chronic thromboembolic pulmonary hypertension. *Thromb Haemost* 2003; 90: 372–6.
7. Cappellini MD, Robbiolo L, Bottasso BM, et al. Venous thromboembolism and hypercoagulability in splenectomized patients with thalassaemia intermedia. *Br J Haematol* 2000; 111: 467–73.
8. Stewart GW, Amess JA, Eber SW, et al. Thromboembolic disease after splenectomy for hereditary stomatocytosis. *Br J Haematol* 1996; 93: 303–10.
9. Chou R, DeLoughery TG. Recurrent thromboembolic disease following splenectomy for pyruvate kinase deficiency. *Am J Hematol* 2001; 67: 197–9.
10. Favara BE, Paul RN. Thromboembolism and cor pulmonale complicating ventriculo-venous shunt. *JAMA* 1967; 199: 668–71.
11. Unnithan RR, Bahuleyan CG, Sambasivan M, et al. Ventriculo-atrial shunt producing pulmonary hypertension. *J Assoc Physicians India* 1984; 32: 1000–1.
12. Trowitzsch E, Ostrejz M, Evers D, et al. Echocardiographic proof of pulmonary hypertension with irreversible increased resistance in the pulmonary circulation as a complication after placement of a ventriculo-atrial shunt for internal hydrocephalus. *Eur J Pediatr Surg* 1992; 2: 361–4.
13. Haasnoot K, van Vught AJ. Pulmonary hypertension complicating a ventriculo-atrial shunt. *Eur J Pediatr* 1992; 151: 748–50.
14. Drucker MH, Vanek VW, Franco AA, et al. Thromboembolic complications of ventriculoatrial shunts. *Surg Neurol* 1984; 22: 444–8.
15. Pascual JM, Prakash UB. Development of pulmonary hypertension after placement of a ventriculoatrial shunt. *Mayo Clin Proc* 1993; 68: 1177–82.
16. Rao PS, Molthan ME, Lipow HW. Cor pulmonale as a complication of ventriculoatrial shunts. Case report. *J Neurosurg* 1970; 33: 221–5.
17. Ralston DR, St John RC. Progressive shortness of breath in a 50-year-old man with ulcerative colitis. *Chest* 1996; 110: 1608–10.
18. Schlesselman JJ. Case control studies: design, conduct, analysis. In: Press OU, ed. New York, Oxford, 1982.
19. Jamieson SW. Pulmonary thromboendarterectomy [editorial]. *Heart* 1998; 79: 118–20.
20. Thistlethwaite PA, Mo M, Madani MM, et al. Operative classification of thromboembolic disease determines outcome after pulmonary endarterectomy. *J Thorac Cardiovasc Surg* 2002; 124: 1203–11.
21. Seligsohn U, Lubetsky A. Genetic susceptibility to venous thrombosis. *N Engl J Med* 2001; 344: 1222–31.
22. Value of the ventilation/perfusion scan in acute pulmonary embolism. Results of the prospective investigation of pulmonary embolism diagnosis (PIOPED). The PIOPED Investigators. *JAMA* 1990; 263: 2753–9.
23. Lennard-Jones J, Shivananda S. Clinical uniformity of inflammatory bowel disease at presentation and during the first year of disease in the north and south of Europe. *Eur J Gastroenterol Hepatol* 1997; 9: 353–9.
24. Freeman HJ. Osteomyelitis and osteonecrosis in inflammatory bowel disease. *Can J Gastroenterol* 1997; 11: 601–6.
25. Heinze G, Schemper M. A solution to the problem of separation in logistic regression. *Stat Med* 2002; 21: 2409–19.
26. Heinze G, Ploner M. Fixing the nonconvergence bug in logistic regression with SPLUS and SAS. *Comput Methods Programs Biomed* 2003; 71: 181–7.
27. Miyamoto S, Nagaya N, Satoh T, et al. Clinical correlates and prognostic significance of six-minute walk test in patients with primary pulmonary hypertension. Comparison with cardiopulmonary exercise testing. *Am J Respir Crit Care Med* 2000; 161: 487–92.
28. Hoepfer MM, Niedermeyer J, Hoffmeyer F, et al. Pulmonary hypertension after splenectomy? *Ann Intern Med* 1999; 130: 506–9.
29. Moser KM, Auger WR, Fedullo PF. Chronic major-vessel thromboembolic pulmonary hypertension. *Circulation* 1990; 81: 1735–43.
30. Schoenbaum SC, Gardner P, Shillito J. Infections of cerebrospinal fluid shunts: epidemiology, clinical manifestations, and therapy. *J Infect Dis* 1975; 131: 543–52.
31. Ejstrup P, Kristensen B, Hansen JB, et al. Risk and patterns of bacteraemia after splenectomy: a population-based study. *Scand J Infect Dis* 2000; 32: 521–5.
32. Olman MA, Marsh JJ, Lang IM, et al. Endogenous fibrinolytic system in chronic large-vessel thromboembolic pulmonary hypertension. *Circulation* 1992; 86: 1241–8.
33. Lang IM, Marsh JJ, Olman MA, et al. Parallel analysis of tissue-type plasminogen activator and type 1 plasminogen activator inhibitor in plasma and endothelial cells derived from patients with chronic pulmonary thromboemboli. *Circulation* 1994; 90: 706–12.
34. Lang IM, Marsh JJ, Olman MA, et al. Expression of type 1 plasminogen activator inhibitor in chronic pulmonary thromboemboli. *Circulation* 1994; 89: 2715–21.
35. Rich S, Kaufmann E, Levy PS. The effect of high doses of calcium-channel blockers on survival in primary pulmonary hypertension. *N Engl J Med* 1992; 327: 76–81.
36. Abenheim L, Moride Y, Brenot F, et al. Appetite-suppressant drugs and the risk of primary pulmonary hypertension. International Primary Pulmonary Hypertension Study Group. *N Engl J Med* 1996; 335: 609–16.
37. Humbert M, Nunes H, Sitbon O, et al. Risk factors for pulmonary arterial hypertension. *Clin Chest Med* 2001; 22: 459–75.
38. McGoon MD. Task force on diagnosis and assessment, Third World Symposium on Pulmonary Arterial Hypertension, Venice, 2003.
39. McGoon M, Gutterman D, Steen V, et al. Screening, early detection, and diagnosis of pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines. *Chest* 2004; 126 (Suppl.): 14S-34S.