Thrombosis Research 163 (2018) 92–99

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

The lectin pathway and coagulation in lung cancer patients undergoing lobectomy – A randomised controlled trial

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ARTICLE INFO

Keywords:
Complement pathway, mannose-binding lectin
Complement system protein
Neoplasms
Heparin, low molecular weight heparin
Blood coagulation

ABSTRACT

Background: Lectin pathway proteases activate coagulation and may theoretically play a role in the increased thrombosis risk in cancer, which is especially high during surgery.
Aims: To investigate lectin pathway proteins during lung cancer surgery, the influence of low molecular weight heparin (LMWH) on lectin pathway proteins, and correlations between lectin pathway proteins and coagulation.
Methods: Fifty lung cancer patients undergoing video-assisted thoracoscopic surgery lobectomy were randomised to LMWH, $n = 26$, or no anticoagulant (control), $n = 24$. Pre-, intra- and postoperative lectin pathway protein concentrations (mannose-binding lectin (MBL), H– and M-ficolin, collectin liver-1, MBL-associated serine protease (MASP)-1, -2 and -3, MBL-associated proteins MAp44 and MAp19) were assessed using a time-resolved immunofluorometric assay, and fibrinogen, fibrin d-dimer, and thrombin generation were analysed.
Results: For all proteins except MASP-1, concentrations decreased during surgery in both groups; most markedly M-ficolin which decreased 49% (median: 2836 [quartiles:2297–3505] to 1424 [1187–2199] ng/ml) (LMWH group) and 43% (2974 [2539–3510] to 1685 [1391–2076] ng/ml) (control group), while MBL decreased 12% (1936 [823–2801] to 1702 [676–2830] ng/ml) (LMWH) and 23% (1526 [250–2412] to 1175 [229–1947]) (controls). No differences in postoperative change were observed between groups except for MAp19 ($p = 0.03$) which decreased 9% (401 [337–467] to 364 [288–416] ng/ml) (LMWH) *vs* 28% (370 [272–468] to 268 [212–379] ng/ml) (controls). No correlation was found between lectin pathway proteins and coagulation ($r = -0.23$–0.28, $p > 0.06$) except for M-ficolin and fibrinogen ($r = 0.29$–0.36, $p = 0.01$–0.04).
Conclusion: Lectin pathway proteins were influenced by surgery but not by LMWH. No consistent correlation was found between lectin pathway proteins and coagulation.

1. Introduction

The complement system is an important arm of the innate immune system. The lectin pathway of the complement system is the most recently described of the complement pathways, and new functions for it are still discovered [1]. The lectin pathway can be activated by the pattern recognition molecules mannose-binding lectin (MBL), H-, L- and M-ficolins and the collectins CL-L1 and CL-K1, complexed with the MBL-associated serine proteases (MASP)-1, -2 and -3 [2]. The pattern recognition molecules recognise carbohydrate structures on microbial surfaces [3] and altered expression of surface molecules of human apoptotic cells [4] and cancer cells [5,6].

Changes in lectin pathway protein serum concentrations in cancer patients have been reported in several studies. Higher concentrations of MBL [7,8], the ficolins [9,10] and MASP-2 [7] were found in colorectal and ovarian cancer patients, and lower concentrations of CL-L1 and MAp44 were reported in colorectal cancer patients [9]. Furthermore, high MASP-2 levels in colorectal cancer were associated with a poor prognosis in one study [11]. The mechanisms behind these alterations in lectin pathway proteins in cancer patients are at present unclear, as is the pathophysiological relevance.

Cancer patients often undergo surgical tumour removal, and the

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https://doi.org/10.1016/j.thromres.2018.01.042
Received 28 September 2017; Received in revised form 11 January 2018; Accepted 24 January 2018

lectin pathway may possibly be even further altered by the acute phase response induced by surgery. However, only few studies have investigated the influence of surgery on the lectin pathway in cancer patients. Notably, the previous published studies report contradictive results, as both a postoperative increase [12] and decrease [13–15] of lectin pathway proteins as well as no influence of surgery [16] have been found.

Cancer patients also have a well recognised risk of venous thromboembolism (VTE) [17–19], which has a negative impact on treatment [20] and mortality [21]. This VTE risk is further increased when cancer patients undergo surgery and can be considerable, depending on both the localisation and stage of cancer and the type of surgery as recently reviewed by Htun and Lee [22]. Several studies report that MASP-1 and -2 can activate the coagulation cascade and impair fibrinolysis *in vitro,* leading to increased fibrin deposition [23–26]. Thus, lectin pathway activation may in theory contribute to the increased thrombosis risk in cancer patients. However, this association has not been investigated previously *in vivo*.

Low molecular weight heparin (LMWH) is widely used for thromboprophylaxis. *In vitro* studies demonstrated that heparins can inhibit both lectin pathway activation [27] and activation of the common complement pathway *via* complement factor (C) 3 [28,29] through potentiation of antithrombin and C1-inhibitor. This indicates that heparin may not only act as an anticoagulant but also as a complement inhibitor. This could have a potential beneficial effect in complement-mediated diseases, but on the other hand, inhibition of complement activation in certain patient groups may have harmful effects, *e.g.* in infectious diseases. However, little is known about whether LMWH treatment in prophylactic doses influences lectin pathway activation *in vivo*.

In this study, we investigated the lectin pathway in lung cancer patients undergoing video-assisted thoracoscopic surgery and the influence of LMWH treatment on lectin pathway proteins in plasma as well as the association with biochemical coagulation parameters. The aims of the study were 1) to assess lectin pathway protein concentrations in plasma in lung cancer patients before, during and after surgery, 2) to investigate the effect of LMWH treatment on lectin pathway protein concentrations and 3) to investigate the correlation between lectin pathway protein concentrations and haemostatic activation in these patients. Our hypotheses were that 1) lectin pathway protein plasma concentrations increase during surgery as part of the surgical acute phase response 2) LMWH treatment attenuates changes in lectin pathway protein concentrations during surgery, and 3) lectin pathway protein levels are positively associated with coagulation markers.

2. Methods and materials

2.1. Design and study population

The present study was a sub-study of a previously published randomised controlled trial [30] (EudraCT no. 2012-002409-23, clinicaltrials.gov identifier: NCT01741506). Briefly, patients with non-small cell pulmonary cancer stage I-II (T1a-2bN0M0; localised tumour < 7 cm and no lymph node or remote metastases) who were eligible for tumour resection with Video-Assisted Thoracoscopic Surgery (VATS) lobectomy were included at Aarhus University Hospital, Odense University Hospital and Copenhagen University Hospital, Denmark, between 2012 and 2014 as previously described [30]. In the present project, only samples from Aarhus University Hospital were included. Inclusion criteria were age above 18 years, ability to give written informed consent, and for women, the use of effective contraception. Exclusion criteria were thrombotic events three months or less prior to inclusion, current pregnancy or breastfeeding, antiplatelet treatment, anticoagulant treatment with a vitamin K antagonist, direct oral thrombin antagonist or direct oral factor Xa antagonist, and previous adverse reactions to treatment with LMWH.

The patients were randomised to LMWH (dalteparin (Fragmin®, Pfizer Inc., New York, USA), 5000 IE/day) or no treatment (control group) from the day prior to surgery and until discharge. The randomisation procedure was described in detail previously [30]. Blood samples were obtained at four time points: 1) preoperatively, *i.e.* on the day prior to surgery, before the first LMWH dose, 2) intraoperatively, *i.e.* after tumour resection but before closing the wound, 3) on the first postoperative day at 8 a.m. and 4) on the second postoperative day at 8 a.m. In the present study, the pre-, intra- and first postoperative samples were analysed.

The present study was approved by the Danish Data Protection Agency (file no. 1-16-02-410-16) and the Central Denmark Region Committees on Health Research Ethics (file no. 1-10-72-141-16) in accordance with the Helsinki Declaration, and all participants gave written informed consent.

2.2. Laboratory analyses

Blood was drawn from the antecubital vein into citrated tubes (sodium citrate 3.2%). Blood for lectin pathway and thrombin generation analysis was centrifuged at 3000*g* for 25 min to obtain citrated platelet-poor plasma. Plasma was frozen immediately and stored at −80 °C until analysis. Blood for fibrinogen and fibrin d-dimer was centrifuged within one hour at 3000*g* for 10 min and analysed within 4 h.

2.2.1. Lectin pathway proteins

MBL, MASP-1, -2 and -3, MAp19, MAp44, H- and M-ficolin and CL-L1 were analysed with in-house time-resolved fluorometric assays (TRIFMAs) at the Department of Biomedicine, Aarhus University, as previously described [13,15,31–36]. Briefly, plasma was thawed, diluted in assay buffers and added to microtiter wells coated with relevant antibodies. After incubation and wash, biotin-labelled antibodies were added to the wells, and after a second incubation and wash, europium-labelled streptavidin was added. After another wash, enhancement solution (Ampliqon, Odense, Denmark) was added and the europium was detected with a fluorometer (Victor X5®, PerkinElmer, Waltham, MA, USA). The signals obtained in the wells were compared to a standard curve of known protein content, and each microtiter plate contained three quality controls. The person performing the analysis was blinded with regards to LMWH treatment status and coagulation results.

2.2.2. Coagulation analyses

Plasma-fibrinogen, fibrin d-dimer and thrombin generation were analysed as described previously [30]. Briefly, thrombin generation was analysed using calibrated automated thrombography (Thrombinoscope® BV, Maastricht, the Netherlands) with 5 pM recombinant tissue factor and 4 μM phospholipids (final concentrations). Fibrinogen (Clauss method, Siemens Dade reagent) and fibrin d-dimer (Siemens INNOVANCE® D-Dimer reagent) were analysed using a CS2100i (Sysmex, Kobe, Japan).

2.2.3. Other clinical and laboratory variables

Intraoperative fluid therapy was registered systematically from the patients' medical records. Preoperative leukocyte counts and pre- and postoperative C-reactive protein (CRP), platelet count, haemoglobin and international normalised ratio (INR) were analysed according to routine protocols at the Department of Clinical Biochemistry, Aarhus University Hospital, Denmark.

2.3. Statistics

A normal distribution could not be obtained for MBL, CL-L1, MASP-3 and MAp19. Therefore, median and quartiles were used for descriptive statistics and figures for all lectin pathway proteins. A mixed model repeated measures analysis of variance (ANOVA) was performed