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RESEARCH

D-Dimer as a Potential Prognostic Marker

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Abstract Malignant tumors are often accompanied by increased risk for procoagulant activity, thrombosis and embolism. As a marker indicating such disturbancies is D-dimer, a product of fibrinolysis. In this retrospective study almost 300 patients with malignant tumors were evaluated. During LMWH treatment (as thromboprophylaxis) the highest frequency of VTE with worst prognosis occurred in pancreatic cancer (partly due to the late discovery) followed by ovarian, colonic and breast cancers. Also, increased D-dimer level correlated with progression (stages) and high mortality rate. Furthermore, D-dimer showed very similar or better prognostic activity than the clinically widely used classic tumor markers and suggested to use it as an additional value..

Keywords cancer · LMWH · D-dimer · prognostic marker · solid tumors

Abbreviations

CEA	carcinoembryonic antigen
cP	cancer procoagulant C
DD	D-dimer
DVT	deep vein thrombosis
LMWH	low molecular weight heparin
PA	plasminogen activator
PE	pulmonal embolism
TF	tissue factor
VTE	venous thromboembolism

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Introduction

Bouillaud (1823) was the first who described deep vein thrombosis (DVT) in three tumorous patients [1]. He observed edema of the lower leg and thought that it is the consequence of a vein occlusion caused by a fibrin clot. Later Trousseau (1865) supported the connection between the tumor and thromboemboliasis (VTE), and called the symptom as phlegmasia alba dolens [2]. The Trousseau-syndrome has all signs of Virchow-trias: stasis (could be caused by tumorous compression), endothel damage (probably due to the invasion of tumor cells) and increased blood coagulation (resulted mainly by the products from the tumor cells influencing hemostasis, the cascade of blood coagulation).

Since the first observers it is accepted, that VTE is more frequent in tumorous patients (7–28 fold) [3]. On the other hand, about 10% of patients with VTE suffers from malignancy at the time of diagnosis [4]. Secondary VTE due to an already identified tumor is not infrequent, however, the frequency is dependent on the tumor types [5]. Moreover, cancer associated with VTE often has a poor prognosis.

Heparin (unfractionated) is one of the most widely used drugs to prevent hemostatic complications even in tumorous patients. Recently, heparin is mostly replaced by its relatives, low molecular weight heparins (LMWH). All LMWHs are individual drugs and not interchangable. There are—mainly preclinical—observations that beside the

antithrombotic effect these drugs can improve the patients' survival. It is proven that besides its strong anticoagulant activity heparin can interact with enzymes (e.g. heparanase), cell adhesion molecules (e.g. P- and L-selectin), growth factors, cytokines. Such molecules may influence the capacity of tumor cells to protect themselves in the circulation by attracting thrombocytes or promote the development of a fibrin-coat. These steps theoretically can interfere with the successful survival of tumor cells in the circulation as well as with the effective arrest of tumor cells on the endothelial surface in the target organ. The local effect of the heparins inside or just around the tumors is even more unknown, but most of the emphasis is given to the antiangiogenic and antimigratory capacity [6–15]. Presumably, the antitumor effect is partly independent from the effect on hemostasis.

Tumor growth, angiogenesis and the activation of the coagulation cascade are accompanied by the activation of the fibrinolytic system. It starts shortly after the activation of the coagulation, when—with the help of FXII—the proactivators of the fibrinolysis are also activated. The aim is to solubilize the fibrin and decrease the risk of thrombus formation in the tumorous vessel. The endothelial cells in the tumor tissue can produce, store and bind fibrinolytic proteins. The average fibrinolytic activity estimated from the D-dimer quantity is often elevated in the plasma [16].

D-dimer (DD) an end-product of fibrinogen and crosslinked fibrin degradation, appears in the blood as a small peptide fragment after the activation of the fibrinolytic system, but at the same time it signals the activated coagulation as well. The name is because it consists of two crosslinked D fragments of fibrinogen. As a result of the increased procoagulant activity the level of the D-dimer in the serum could be much higher in tumorous patients indicating a higher risk for thrombus formation and thromboembolic complications. (But the normal values do not rule out the presence of a thrombotic process.) In tumorous patients many factors can increase the coagulative activity, partly because the tumor cell can produce procoagulants (e.g. TF, uPA, cP), which are resistant to the normal anticoagulation. Besides the tumor progression itself, e.g. surgery, chemotherapy, which can be prevented by thromboprophylaxis. Nowadays, the most frequently used drugs for this purpose are the low molecular weight heparins (LMWHs) [12].

Many of the informations cited above provided by experiments where heparin or LMWHs was given shortly before or after the tumor inoculation. Essentially there are limited data on the effect of a "chronic" LMWH administration. The aim of this study was to estimate the efficacy of chronic LMWH treatment (e.g. nadroparin, dalteparin, enoxaparin) on the frequency of coagulative complications, how these events are reflected at the level of D-dimer, and also, the potential of D-dimer to behave as a prognostic factor, together with the well-known markers, in certain tumor types.

Materials and Methods

Patients

Two hundreds ninty nine patients were selected randomly in this retrospective study. The only selection criteria was the availability of the required parameters (at least most of them making the comparisons possible). Certain clinicopathological characteristics (age, multiple tumors, stage at the time of diagnosis) are summarized in Table 1.

Markers

Tumor markers—CEA, CA125, CA15-3, CA19-9—as well as D-dimer was measured by routine laboratory methods (ELISA, EIA) in the Central Laboratory of the hospital).

Treatment

The prophylaxis by nadroparin (Fraxiparin, GlaxoSmith Kline) was adjusted to the body weight giving once a day sc. injection, whereas enoxaparion (Clexane, Sanofi-Aventis) was given in a 40 mg/day prophylactic dose and continued according to the body weight and risk up to 60 mg/day, and 5000 IU dalteparin (Fragmin, Pfizer) was given if the treatment was started with dalteparin.

Thromboprophylaxis was used with adjuvant therapy (ususally during a 6 months treatment period and 3 months

Table 1 Clinicopathological characteristics

	Breast cc	Colon cc	Pancreatic cc	Ovarian cc
No of cases				
Male/female	3/110	35/31	15/21	–/84
Age (yr; median, range)				
Male	50 (49–62)	64 (46–83)	68 (56–77)	–
Female	56 (30–79)	66 (44–80)	67 (27–81)	64 (35–84)
Stage				
I/II	72.1%	40.7%	16.6%	34.6%
III/IV	27.9%	59.3%	83.3%	65.4%
Multiplex tumor	12/113 (10.6%)	11/66 (16.6%)[a]	2/36 (5.5%)	15/84 (17.8%)
Survival (yr)				
<2	25.6%	42.4%	84.6%	31.4%
≥2	74.4%	57.6%	15.4%	68.6%
Histology[b]	IDC, ILC	AC	AC	CAC

[a] multiplex tumor developed only in female patients (frequency in females 35.1%)

[b] only the main types were considered– *IDC* invasive ductal carcinoma, *ILC* invasive lobular carcinoma, *AC* adenocarcinoma, *CAC* cystadenocarcinoma

thereafter) or with palliative treatment (parallel with chemotherapy).

The patients were informed in details regarding the advised prophylaxis and an informed consent was agreed by them. They were also educated for sc. self-administration. The type of LMWH was decided purely on what was available. Indications for the prophylactic treatment was the malignant disease, chemotherapy, previous thrombotic and/or thromboembolic events—essentially the risk factors of Khorana was followed [17]. In case of complications (thrombocytopenia, haemorrhage) the LMWH-treatment was discontinued. Such complications were very rare (4 cases). For chemotherapy the relevant standard protocols were used.

Statistical Analysis

Variables of the selected sample were presented by medians and frequencies. Because of the nominal data obtained, chi-square test was used as the adequate statistical procedure to reveal differences between data. Calculations were performed with the use of Statistica 8.0 software (StatSoft Inc. Tulsa, OK). Statistical significance level was set to $p < 0.05$.

Results

The age distribution of the patients and the frequency of multiple malignancies showed the usual values (Table 1) The stages at the diagnosis were quite different between the tumor types. Even the breast cancers were discovered at the later stages (stage III-IV) in almost one-third of the cases. Pancreatic cancers were identified in a very advanced or metastatic (usually liver metastases) stage in more than 80%, making the surgical intervention impossible. Concerning the multiple tumors the highest incidence occurred in female patients with colonic cancer (11/31–35.1%). Although, histological or cytological diagnosis was given as usual, subtyping was not considered in this study.

Correlation Between LMWH-Treatment and Hemostasis Disturbances

Hemostasis disturbancies (vein thrombosis, arterial thrombosis, embolism—further: VTE) were present in 11.5% (24/208) (15.8% if arteria disturbances are counted) of LMWH-treated patients, with different frequency in tumor-types (e.g. breast cancer 6.6%—lowest, pancreas cancer 27.2%—highest). It is worth to mention, that the survival rate was practically even in LMWH-non treated/VTE-free patients compared to LMWH-treated/VTE-free patients (72.0% versus 63.2%) (Table 2).

It is a question whether relationship exists between the data above and the level a D-dimer (further: DD). If all tumorous cases are taken into account the increased DD-level accompanied with much worse prognosis than the normal values (alive/dead ratio: 28.6% versus 89.0%) during the 12 year observation period (Table 3A). Since in the LMWH-treated patients the incidence of the increased and the normal DD-level were almost identical, one can conclude that there is no relationship between LMWH-treatment and DD-level (Table 3B). The lack of relationship suggests, which is known, that the DD-level could be influenced by several factors. In the LMWH-treated group the DD-level increased most frequently in ovarian cancers, and less frequently in breast cancers.

Correlation Between LMWH-Treatment, DD-Level, Survival and Stages (OS)

In this evaluation (closed at November 2011) only those patients were considered who were diagnosed with the given tumor between January 2000 and December 2009. Consequently the patients were grouped—arbitrary—as survivors for less than 2 years (<2 year), or more than 2 years (>2 year). When all patients were measured just slight difference was found between the two groups. Ovarian and colonic cancers were close to the average, while patients with breast cancer showed much longer and with pancreatic cancer much shorter survival (Table 4) (The bad survival with pancreatic cancer can be explained by the late discovery.) The longer (>2 year) survival was equally represented in groups with normal or increased DD-level both in different tumor types (excluding pancreatic cancer), and in all tumors.

Stages represent the tumor progressionThe number of LMWH non-treated patients were too low to calculate correlation. However, in LMWH-treated patients the DD-levels were much higher in the advanced than in the earler stages, and these values indicate an increased risk for death of the patient. These differences are significant (Table 5). Normal DD-level was measured mainly in stages I/II, and also the risk for death was rather low. All of these strongly support that DD-level is a potential prognostic marker.

Comparison of "Classical" Tumor Markers and DD-Level

Tumor markers are well known and widely used in tumor-types participated in this study. The cases were marked (increased or normal) essentially upon the value observed at the time of diagnosis and later during progression. Most patients had several measurements and the mark was given according to the majority of the values. If the number of elevated or

Table 2 Correlation between LMWH treatment and VTE

		Breast cc	Colon cc	Pancreas cc	Ovarian cc		
						Total	
LMWH-treated patients		75	50	22	61	208	
Patients with VTE		5 (6)[a]	5 (8)	5 (5)[b]	9 (14)	24	(33)
Incidence (%)		6.6	10.0	27.2	14.8	11.5%	(15.8%)
						Total	
LMWH/VTE	+/+	4/1[c]	1/3	0/6[b]	4/8	9/18[*]	
	+/-	59/11	25/21	1/15	32/21	117/68[*^]	63.2%[d]
	-/-	5/1	6/3	1/3	6/0	18/7[*^]	72.0%[d]
	-/+	–	0/1	–	1/0	1/1	

[a] in brackets: including arterial thrombosis and embolia as well

[b] excluding v. lienalis and v. portae thrombosis

[c] number of alive/dead patients (during the observation period); (+) LMWH-treated patients or patients with VTE, (-) LMWH-non-treated patients or patients without VTE

[d] percentage of alive patients without VTE in LMWH-treated group (+/-), or int he LMWH non-treated group (-/-)

Difference is significant between total +/+ and +/-; +/+ and -/- ($p<0.05$); difference is not significant between +/- and -/- ($p=0.39$)

[*] marks significant difference ($p<0.05$), ^ not significant

normal marks were equal, the case was considered as increased (since the incerase has much higher clinical significance) Table 6. allows to make certain conclusions: (a) Both in pancreatic and ovarian cancers the increase in DD-level was similar to the elevated classical markers (CA19-9 and CA125/CEA, respectively), while the increase in DD-level was much more frequent than the increase of CEA in the colonic cancers, or the increase in CA15-3/CEA in breast cancers. (b) The same tendency was observed during progression reflected by the stages. (c) In the pancreatic and ovarian cancers where the increase in markers and DD was similar. (d) According to the values the determination of CEA in breast and ovarian cancer has very limited significance, it can be avoided.

Discussion

It is known that malignant diseases are often accompanied by hemostatic disturbances, mainly thrombotic events with or without embolism. A closer look at DVT and PE incidence

Table 3 Correlation between LMWH-treatment, VTE and DD-level

(A)		LMWH+/VTE+	LMWH+/VTE-	LMWH-/VTE-		
		No of alive/died patients			All cases	
Breast cc	DD+	0/1	10/6	1/0	11/8	
	DD-	2/0	32/0	2/0	36/2	
Colon cc	DD+	0/1	4/14	1/0	5/16	
	DD-	0/1	10/4	1/0	11/6	
Pancreas cc	DD+	0/3	0/9	0/1	0/13	
	DD-	0/0	2/2	1/1	3/3	
Ovarian cc	DD+	0/1	5/15	1/0	6/20	
	DD-	2/0	7/0	2/0	15/0	
All cases	DD+	1/10	19/44[*]	2/1[*]	22/55[*]	(28.6%)[a*]
	DD-	1/6	51/6^	8/1^	65/8[*]	(89.0%)[a*]
(B)	LMWH+					
	DD+	DD-	DD+/DD-			
Breast cc	30	44	40.5%			
Colon cc	22	19	53.7%			
Ovarian cc	32	18	64.0%			
All cases		84	81	50.1%		

[a] survival rate of patients during the 12 year observation period

Difference is signinficant between all cases in DD+and DD- groups; and DD+LMVH/VT +/- and LMVH/VT -/- ($p<0.05$)

Difference is not significant between groups of DD- LMVH/VT +/- and LMVH/VT -/- ($p=0.95$)

[*] marks significant difference ($p<0.05$), ^ not significant

Table 4 Correlation between LMWH-treatment, DD-level and survival

	Survival (yr)	LMWH-treatment		DD-level		
		Yes	No	Increased	Normal	
	No of patients					
Breast cc	<2	12	3	3	6	
	>2	19	2	15	12	55.6%[b]
	>2	76.5%[a]				
Colon cc	<2	21	1	11	12	
	>2	22	6	12	14	46.1%
	>2	51.2%				
Pancreas cc	<2	17	3	5	7	
	>2	6		2	0	–
	>2	26.0%				
Ovarian cc	<2	24	0	14	1	
	>2	36	5	12	14	46.1%
	>2	60.0%				
All cases	>2	52.6%	61.5%	55.4%	60.6%	

[a] ratio of >2 and <2 survivors in LMWH-treated patients

[b] ratio of increased DD-level and control level in the >2 group

Table 5 Correlation between LMWH-treatment, DD-level and stages

Stages	LMWH-treated			
	DD+		DD-	
	I/II	III/IV	I/II	III/IV
Breast cc				
(No of patients)	14	8	28	5
(survival rate)[a]	85.7%	37.5%	92.8%	80.0%
Colon cc	7	15	6	13
	85.7%	6.7%	83.3%	69.2%
Ovarian cc	8	25	9	8
	62.5%	32.0%	88.9%	100.0%
Pancreas cc	–	13		
	–	0%	–	50.0%
Total	29	61	43	30
	79.3%[#]	19.7% [b #]	90.7%	76.7% [b]

Results with LMWH non-treated patients due to the very low number (0–2 patients/group) are not shown

[a] percentage of patients survived during the observation period (12 year)

Difference is signinficant between total DD+I/II and DD+III/IV; DD+ III/IV and DD- III/IV ($p<0.05$)

Difference is not significant between total DD- I/II and DD- III/IV ($p=0.10$); DD+I/II and DD- I/II ($p=0.17$)

[b],# marks significant difference ($p<0.05$)

Table 6 Correlation between the increased level of "classical" markers and DD-level

	Markers	Frequency (%) all cases	Stage I/II	Stage III/IV
Breast cc	CA15.3	31.0[b]	23.6	50.0
	CEA	12.9[b]	9.0	23.3
	DD	41.6[b]	32.2	63.3
Colon cc	CEA	34.8[b]	35.7	33.3
	DD	57.7[b]	53.3	60.0
Pancreas cc	CA19-9	78.2	NE[a]	78.5
	DD	68.7	NE	66.6
Ovarian cc	CA125	63.6[b]	34.8	74.2
	CEA	15.6[b]	8.3	18.5
	DD	53.8[b]	36.3	61.5

[a] NE: non evaluable due to the low number of patients

Difference is significant in breast cc between CA15.3-CEA and DD-CEA; for ovarian cc CA125-CEA and DD CEA; for colon cc between CEA-DD ($p<0.05$)—non significant .in breast cc between CA15.3-DD, in pancreatic cancer CA19.9-DD, in ovarian cc CA125-DD

Differences at stages reflect the same significance a sin all cases

[b] marks significant difference ($p<0.05$)

rates for different histological types, though, reveals that ovarian carcinoma, primary brain tumor and lymphomas are among the tumor types with the highest VTE rates. Nevertheless, thrombosis can affect all histologic types, of all stages, and during any and all treatments. The target vessels are the veins, but it can hardly rule out that the arterial side is not involved in procoagulation. In our study the lowest frequency was observed in breast cancer and the highest in pancreatic cancer.

One of the most challenging problems is the clinically beneficial effect of LMWHs on the survival in cancerous patients. Till now the results are controversial [6–15]. While preclinical studies are in favour of this action, others, e.g. in prospective clinical trials (on NSCLC stage IIIB, prostatic cancer—receiving the treatment for 3 months as maximum) failed to support the increase of survival [18]. Previously, in a retrospective study we found, that patients treated with nadroparin at least for 6 months showed a significantly increased survival in certain subgroups (T3 and T4, as well as M1—in mainly colonic and breast cancers) [19]. Our recent retrospective study used the data of randomly selected patients with the difficulty that almost all patients received LMWH-treatment, therefore very few belonged to the untreated (i.e. control) group.

A strong support came from the correlation between DD-level and tumor progression, represented by stages. In most tumors high DD-levels indicated a more advanced stage and increased risk for death. These data raised the question: can D-dimer considered as a potential prognostic marker, similarly to the "classical" ones? This was the reason that we

compared the change in DD-level to the widely accepted tumor markers. Usually, most of these markers can serve as a tool monitoring the progression of the disease. But, neither of them are ideal due to the rather low specificity and sensitivity. As an example, the need to identify additional biomarkers to increase the performance of current biomarkers is illustrated in studies of ovarian cancer. Given that only approximately 50% of patients with early-stage ovarian cancer have elevated levels of CA125, considerable effort has been invested in discovering additional ovarian cancer markers besides CA125. To date the effort has resulted in the demonstration of the additive value of HE4/WFDC2 when combined with CA125 [20, 21]. In our study, rather similarly, DD-level increased in equal or higher percentage at an early stage of tumor growth than the "classical" markers did. With an exception of pancreas cancer (which were discovered at late stages), all the other tumor types in this study (ovarian cc, colon cc, breast cc) supported the use of DD-level as an additional prognostic marker. But even in pancreatic cancers, where the increased values—well below 100%—did not overlap in each cases, plenty of room is available to use the classical marker and DD-level together in order to improve the judgement on prognosis. Moreover, to measure CEA in breast and ovarian cancers seems to be useless.

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