Detection of a Hypercoagulable State in Patients With Pancreatic Cancer Using a Clot Waveform Analysis

Mayu Kobayashi , Hideo Wada * , Shunsuke Fukui , Yasuaki Shimada , Yuuichi Nakazawa , Hiroki Mizutani , Yuhuko Ichikawa , Yuuki Nishiura , Isao Moritani , Yutaka Yamanaka , Hidekazu Inoue , Motomu Shimaoka , Hideto Shimpo , Katsuya Shiraki *

Posted Date: 1 June 2023

doi: 10.20944/preprints202306.0031.v1

Keywords: cancer; pancreatic cancer; CWA; APTT; sTF/FIXa; thrombin time

Article

Detection of a Hypercoagulable State in Patients with Pancreatic Cancer Using a Clot Waveform Analysis

Mayu Kobayashi [1], Hideo Wada [2,*], Shunsuke Fukui [1], Yasuaki Shimada [1], Yuuichi Nakazawa [1], Hiroki Mizutani [1], Yuhuko Ichikawa [3], Yuuki Nishiura [1], Isao Moritani [1], Yutaka Yamanaka [1], Hidekazu Inoue [1], Motomu Shimaoka [4], Hideto Shimpo [5] and Katsuya Shiraki [1,2]

[1] Department of Gastroenterology, Mie Prefectural General Medical Center,Yokkaichi 510-8561, Japan; xsvaria1010@gmail.com (MK), shunsuke-fukui@mie-gmc.jp (SF), yasuaki-shimada@mie-gmc.jp (YS); yuuichi- nakazawa@mie-gmc.jp (YN), n279828658b79ku@gmail.com (HM), yuuki-nishiura@mie-gmc.jp (YN), isao-moritani@mie-gmc.jp (IM), yutaka-yamanaka@mie-gmc.jp (YY), hidekazu-inoue@mie-gmc.jp (HI)

[2] Department of General and Laboratory Medicine, Mie Prefectural General Medical Center, Yokkaichi 510-8561, Japan. katsuya-shiraki@mie-gmc.jp (SK)

[3] Department of Central Laboratory Medicine, Mie Prefectural General Medical Center, Yokkaichi 510-8561, Japan. ichi911239@yahoo.co.jp (YI),

[4] Department of Molecular Pathobiology and Cell Adhesion Biology, Mie University Graduate School of Medicine, Tsu 514-8507, Japan. motomushimaoka@gmail.com (MS)

[5] Mie Prefectural General Medical Center, Yokkaichi 510-8561, Japan. hideto-shimpo@mie-gmc.jp (HS)

* Correspondence: e-mail@e-mail.com; katsuya-shiraki@mie-gmc.jp (SK); wadahide@clin.medic.mie-u.ac.jp (HW); Tel.: +81-59-345-2321

Abstract: Background: The patients with cancer, especially pancreas cancer, are frequently associated with thrombosis which is one of causes for poor outcome, and hypercoagulability exists in cancer patients. Hypercoagulability is considered to be caused by thrombin burst. Methods: Activated partial thromboplastin time (APTT), small amount of tissue factor induced FIX activation (sTF/FIXa) assays and thrombin time (TT) using clot waveform analysis (CWA) were performed in 138 patients with malignant neoplasm including pancreas cancer and 66 non-cancer patients. Results: Thrombosis was frequently associated in pancreas cancer and was observed in stage I. CWA-APTT showed that the peak times and heights were markedly long and heigh, respectively, in cancer patients, and the peak times were significantly longer in pancreas cancer patients than benign pancreas diseases, and the 1st DPH was significantly higher in pancreas cancers than other cancers or benign pancreas diseases. CWA-sTF/FIXa showed that the peak times and heights were longer and higher, respectively, in patients with cancer than those without cancers, and that the 1st DPH was significantly higher in pancreas cancers than in other cancers. CWA-TT showed that the peak times were significantly shorter in cancer patients than in healthy volunteers and that the peak heights were significantly higher in cancer than in benign pancreas diseases. Conclusions: Cancer patients including pancreas cancer were frequently associated with thrombosis due to hypercoagulability with thrombin burst detected by CWA.

Keywords: cancer; pancreatic cancer; CWA; APTT; sTF/FIXa; thrombin time

1. Introduction

Thrombotic complications such as venous thromboembolism (VTE), acute cerebral infarction (ACI) and acute coronary syndrome (ACS) have often been reported and studied as cancer-associated thrombosis (CAT) by many physicians, and researchers [1–3]. The incidence of VTE is <0.1 % in the general population and it was reported that approximately 20% of the overall VTE incidence might be caused by active cancer [4]. CAT including among ACS, ACI and VTE, is considered a main cause of noncancer death in patients with cancer [5]. VTE is one of the most important thrombotic complications in patients with cancer [6,7], and ACS and ACI are arterial thromboses that are mainly caused by platelet activation and atherosclerosis [8,9]. Trousseau syndrome [10,11] is a well-known thrombotic syndrome in cancer patients. CAT, including Trousseau syndrome is associated with

 doi:10.20944/preprints202306.0031.v1

various thrombotic diseases and has various factors, including leukocytosis, thrombocytosis, physical factors and increased coagulation factors, which have been proposed as risk factors for CAT [1].

Hypercoagulability including increased levels of coagulation factors, such as tissue factor (TF) and clotting factor VIII (FVIII), activated coagulation factors and TF-positive microparticles was proposed as a risk factor for CAT [1]. Activation of the coagulation cascade and platelets which is related with tumor growth and metastasis, may cause CAT [12,13]. Therefore, an early diagnosis of hypercoagulability and prophylaxis for CAT may be important in management of cancer [10].

Although increased D-dimer or soluble fibrin (SF) levels are useful for detecting thrombosis in patients with CAT [14–16], routine coagulation tests, such as activation partial thromboplastin time (APTT) and prothrombin time (PT) cannot detect the hypercoagulable state [17]. A clot waveform analysis (CWA) of APTT (CWA-APTT) has been developed to analyze to evaluate hemostatic abnormalities in patients with bleeding tendency and to monitor anticoagulant therapy [17–19]. Furthermore, the use of a small amount of TF induced FIX activation (sTF/FIXa) (CWA-sTF/FIXa) assay can evaluate the hemostatic abnormalities including platelet abnormalities [17]. Hypercoagulability is considered to be caused by a thrombin burst [20,21] which is evaluated by thrombin time using a small amount of thrombin with a CWA (CWA-TT) [22].

In the present study, hypercoagulability and complications of thrombosis in patients with malignant neoplasms including pancreatic cancer were investigated using a CWA-APTT, CWA-sTF/FIXa and CWA-TT in comparison to patients without cancer or those with benign pancreatic diseases.

2. Materials and Methods

The study population included 138 patients with cancer and 66 patients without cancer who were managed at Mie Prefectural General Medical Center from August 21, 2020 to December 31, 2022. Cancer types were pancreatic cancer (n=30), uterine cancer (n=4), prostate cancer (n=7), hepatocellular carcinoma (n=15), malignant lymphoma (n=9), leukemia and myelodysplastic syndrome (leukemia/MDS) (n=11), lung cancer (n=11), breast cancer (n=5), biliary tract cancer (n=11), colon cancer (n=10), stomach cancer (n=12), esophageal cancer (n=5) and other cancer (n=8; origin unknown, n=3; bladder cancer, n=2; kidney cancer, n=2 and ovarian cancer, n=1) (**Table 1**). Noncancer patients included those with chronic hepatitis (n=31), anemia (n=9), bone marrow proliferative disease (n=6), pregnancy loss (n=3), fatty liver (n=3), carrier of hepatitis B virus (n=4), alcoholic liver damage (n=2), hepatic hemangioma (n=2), pancreatic cyst (n=3), benign pancreatic tumor (n=2) and pancreatitis (n=1). CWA was also performed in 20 healthy volunteers as controls. Disseminated intravascular coagulation (DIC) was diagnosed using the Japanese Ministry of Health Labor and Welfare criteria for DIC [23]. Acute cerebral infarction was diagnosed using computed tomography or magnetic resonance imaging, acute coronary syndrome was diagnosed using coronary angiography, electrocardiography and was based on elevated troponin levels, venous thromboembolism was diagnosed using computed tomography or venous ultrasound [24]. The stage of pancreatic cancer was determined in accordance with guidelines [25].

The study protocol (2019-K9) was approved by the Human Ethics Review Committee of Mie Prefectural General Medical Center, and informed consent was obtained from each participant. This study was carried out in accordance with the principles of the Declaration of Helsinki.

The CWA-APTT was performed using APTT-SP®, which uses silica as an activator of FXII and synthetic phospholipids (Instrumentation Laboratory; Bedford, MA, USA) and platelet-poor plasma (PPP), with an ACL-TOP® system (Instrumentation Laboratory) as previously reported [17,26]. The CWA- sTF/FIXa assay was performed using platelet-rich plasma (PRP) and 2,000-fold diluted HemosIL RecombiPlasTin 2G (TF concentration <0.1 pg/ml; Instrumentation Laboratory) [27]. Three curves are shown on the monitor of this system [17], one shows the changes in the absorbance observed while measuring the APTT, corresponding to the fibrin formation curve (FFC), the second (first derivative peak 1st DP), corresponds to the coagulation velocity and the third (second derivative peak 2nd DP) corresponds to the coagulation acceleration. The respective height and time of the 1st

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 June 2023 doi:10.20944/preprints202306.0031.v1

DP, 2nd DP and fibrin formation are called the 1st DP height (1st DPH) and 1st DP time (1st DPT), 2nd DPH and 2nd DPT, and fibrin formation height (FFH) and fibrin formation time (FFT), respectively (**Figure 1)**. PRP was prepared by centrifugation at 900 rpm for 15 minutes (platelet count, 40 x 10^{10} /L), and PPP was prepared by centrifugation at 3,000 rpm for 15 minutes (platelet count, <0.5 x 10^{10} /L) [28].

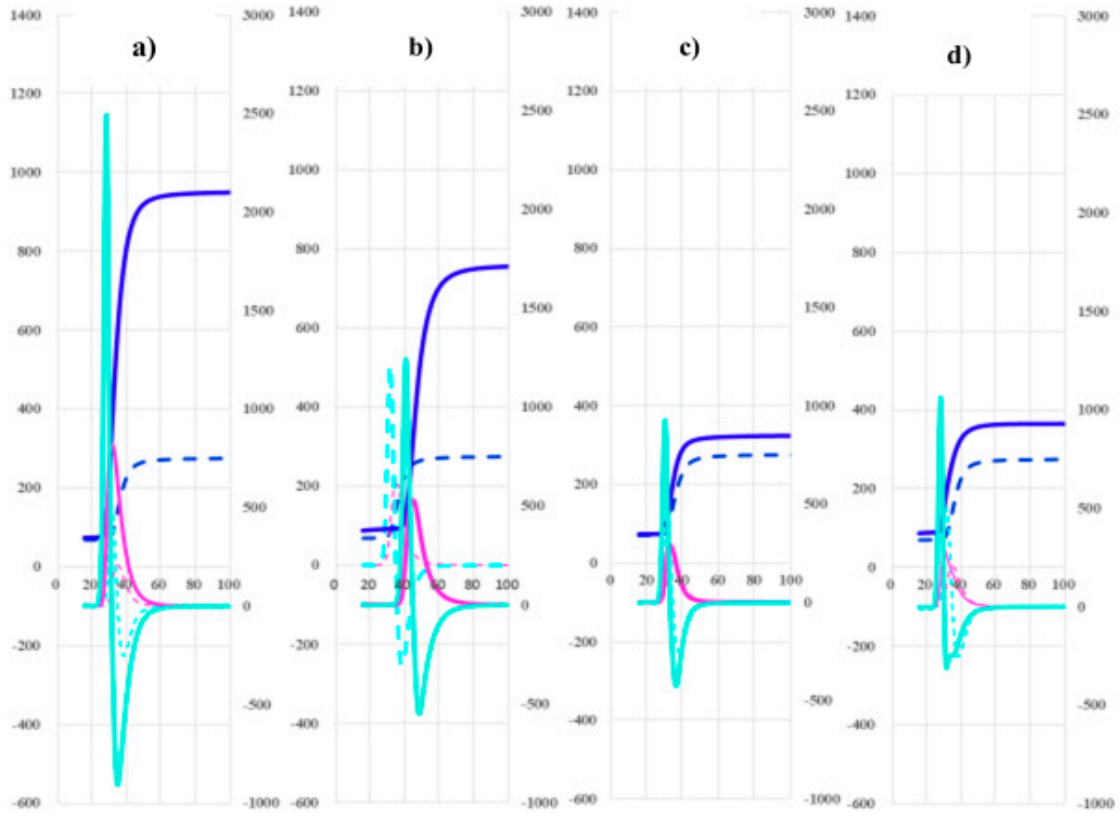

Figure 1. CWA-APTT in patient with pancreatic cancer a), esophageal cancer b), chronic hepatitis c) or pancreatic cyst d).

Table 1. Subjects' details.

	N	Age (years)	Sex (F:M)	Thrombosis
Pancreatic cancer	30	70.3±10.7	12:18	11/30 (36.7%)
Uterine cancer	4	60.5±9.7	4:0	1/4 (25.0%)
Prostate cancer	7	76.9±6.3	0:7	3/7 (43.0%)
Hepatocellular carcinoma	15	72.3±8.1	0:15	3/15 (20.0%)
Malignant lymphoma	9	68.2±19.1	6:3	4/9 (44.4%)
Leukemia/myelodysplastic syndrome	11	74.3±14.1	4:7	1/11 (6.7%)
Lung cancer	11	69.6±11.0	3:8	3/11 (27.3%)
Breast cancer	5	69.8±11.9	5:0	0/5 (0%)
Biliary tract cancer	11	78.7±6.2	6:5	1/11 (9.1%)
Colon cancer	10	73.6±11.6	4:6	0/10 (0%)
Gastric cancer	12	76.8±10.9	0:12	3/12 (25.0%)
Esophageal cancer	5	66.8±9.5	0:5	1/5 (20.0%)
Others	8	77.4±10.5	1:7	3/8 (37.5%)
Total	138	72.4±11.4	45:93	34/138 (24.6%)

The CWA-TT was measured using 0.5 IU thrombin (Thrombin 500 units Mochida Pharmaceutical CO., LTD, Tokyo, Japan) with an ACL-TOP® system (Instrumentation Laboratory)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 June 2023 doi:10.20944/preprints202306.0031.v1

[17]. Three types of curves are shown on this system monitor [22]. The 1st DP shows two peaks and the second peak height of the 1ST DP (1st DPH-2) reflects the thrombin burst.

Statistic alanalyses

The data are expressed as the median (range). The significance of differences between groups was examined using the Mann-Whitney *U-test*. *P* values of <0.05 were considered to indicate a statistical significance. All statistical analyses were performed using the Stat-Flex software program (version 6; Artec Co., Ltd, Osaka, Japan).

3. Results

The frequency of thrombotic complications was high in cases of pancreatic cancer (36.7 %), prostate cancer (43.0 %), malignant lymphoma (44.4 %) and others (37.5 %) (**Table 1**). Thrombotic complications were seen in 18 patients with VTE (pancreatic cancer 8 and other cancer 10), 6 patients with acute cerebral infarction (pancreatic cancer 1 and other cancer 5), 4 patients with DIC (pancreatic cancer 1 and other cancer 3), one patient with acute coronary syndrome (pancreatic cancer) and one patient with thrombotic microangiopathy (other cancer). The frequency of thrombosis tended to be higher in patients with pancreatic cancer than in those with other cancers (19.4 %), although this difference was not significant. The thrombotic complications in patients with other cancers was gradually increased as the stage of cancer progressed; however, a high frequency of thrombotic complications (60%) was observed in stage I pancreatic cancer (**Table 2**).

Figure 1 shows the results of the CWA-APTT in a patient with pancreatic cancer (a), esophageal cancer (b), chronic hepatitis (c) and pancreas cyst (d). The peak heights of the FFC, 1st DP and 2nd DP were highest in the patient with pancreatic cancer, followed by the patient with esophageal cancer. **Figure 2.** Shows the results of statistical analysis for the CWA-APTT in patients with cancer, patients without cancer and healthy volunteers (upper column), cancer patients with and without pancreatic cancer, and patients with benign pancreatic diseases (lower column). The peak times of the CWA-APTT in patients with cancer, (median, 2nd DPT 33.9 sec; 1st DPT 36.9 sec; FFC 39.5 sec) were significantly longer than those in patients without cancer (median, 2nd DPT 31.9 sec, p <0.01; 1st DPT 34.8 sec, p <0.001; FFC 36.4 sec, p <0.001) and healthy volunteers (median, 2nd DPT 31.7 $^{\$1}$, two with VTE and one with acute coronary syndrome; $^{\$3}$, two with VTE; $^{\$4}$, four with VTE, one with DIC and one with ACI; $^{\#1}$, two with VTE and one with ACI; $^{\#2}$, two with VTE and one with ACI; $^{\#3}$, two with VTE, one with DIC and one with ACI; $^{\#4}$, four with VTE, two with ACI, two with DIC and one with thrombotic microangiopathy; VTE, venous thromboembolism; DIC, disseminated intravascular coagulation; ACI, acute cerebral infarction.

Table 2. Stage and thrombosis in patients with pancreatic cancer and other cancers.

	Stage	n	Age (years)	Sex (F:M)	Thrombosis (n, %)
Pancreatic cancer	I	5	73.5±13.0	3:2	$3^{\$1}$, 60%
	II	4	80.7±5.7	1:3	0, 0%
	III	3	72.5±13.4	2:1	$2^{\$3}$, 66.7%
	IV	18	67.4±11.1	6:12	$6^{\$4}$, 33.3%
	Total	30	70.2±11.4	12:18	11, 36.7%
Other cancers	I	24	76.4±8.9	7:30	$3^{\#1}$, 12.5%
	II	19	71.6±9.0	4:14	$3^{\#2}$, 15.8%
	III	18	73.0±11.2	5:14	$4^{\#3}$, 22.2%
	IV	37	69.5±11.1	10:14	$9^{\#4}$, 24.3%
	Total	98	73.2±10.2	26:72	19, 19.4%

CWA, clot waveform analysis; APTT, activated partial thromboplastin time; navy line, fibrin formation curve; pink line, 1st derivative curve (velocity); light blue, 2nd derivative curve (acceleration); solid line, patient; dotted line, healthy volunteer

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 June 2023 doi:10.20944/preprints202306.0031.v1

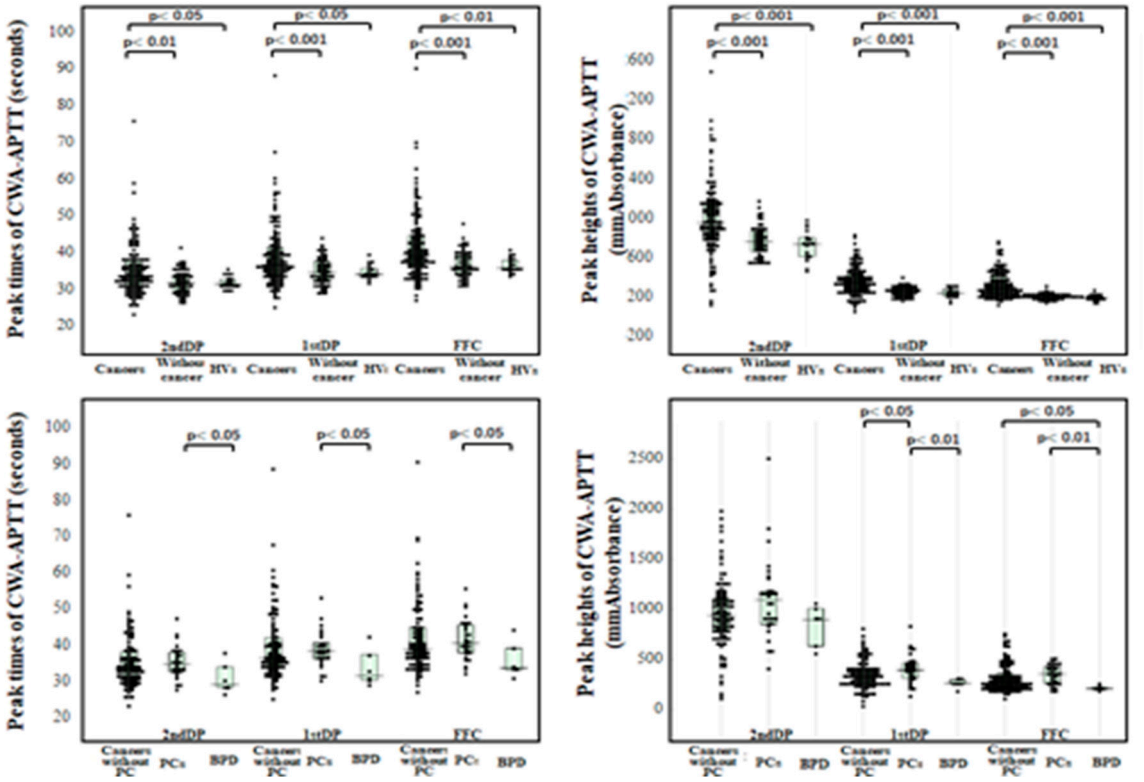

Figure 2. CWA-APTT in patients with cancer, patients without cancer and healthy volunteers (upper column), cancer patients with and without pancreatic cancer, and patients with benign pancreatic diseases. CWA, clot waveform analysis; APTT, activated partial thromboplastin time; HV, healthy volunteer; PC, pancreatic cancer; BPD, benign pancreatic disease; FFT, fibrin formation time; FFH, fibrin formation height; 1stDPT, first derivative peak time; 1stDPH, first derivative peak height; 2nd DPT, second derivative peak time; 2nd DPH, second derivative peak height; sec, $p < 0.05$; 1st DPT 34.3 sec, $p<0.05$; FFC 36.0 sec, $p < 0.01$).

	Cancers	Without cancer	PCs	Without PC	BPDs	HVs
2nd DPT (sec)	33.9 (31.1-38.1)	31.9 (29.7-34.4)	34.8 (32.6-38.0)	33.6 (30.8-38.1)	29.1 (28.3-33.9)	31.7 (31.1-32.9)
2nd DPH (mmAbsorbance)	953 (792-1143)	761 (661-866)	1094 (850-1181)	940 (786-1110)	898 (633-1007)	734 (609-800)
1st DPT (sec)	36.9 (34.0-41.7)	34.8 (32.5-37.8)	38.4 (36.2-40.6)	36.5 (33.6-42.0)	31.5 (30.3-37.4)	34.3 (33.8-35.9)
1st DPH (mmAbsorbance)	345 (258-434)	255 (212-292)	353 (264-436)	331 (257-406)	325 (263-459)	235 (209-270)
FFC (sec)	39.5 (36.4-44.8)	36.4 (34.5-39.7)	38.9 (36.1-44.8)	40.6 (37.9-45.6)	33.7 (33.0-39.1)	36.0 (35.6-38.2)
FFH (mmAbsorbance)	285 (225-411)	201 (185-220)	355 (263-426)	274 (221-361)	211 (202-225)	187 (170-206)

The peak heights in patients with cancer (median, 2nd DPH 953 mm absorbance; 1st DPH 345 mm absorbance; FFC 285 mm absorbance) were also significantly higher than those in patients without cancer (median, 2nd DPH 761 mm absorbance, $p < 0.001$; 1st DPH 255 mm absorbance, $p < 0.001$; FFC 201 mm absorbance, $p < 0.001$) and healthy volunteers (median, 2nd DPH 734 mm absorbance, $p < 0.001$; 1st DPH 235 mm absorbance, $p < 0.001$; FFC 187 mm absorbance, $p < 0.001$). There were no significant differences in the peak times or heights of the CWA-APTT between patients without cancer and healthy volunteers. The peak times of the CWA-APTT were significantly longer in patients with pancreatic cancer than patients with benign pancreatic diseases (median, 2nd DPT 34.8 sec vs. 29.1 sec,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 June 2023 doi:10.20944/preprints202306.0031.v1

p <0.05; 1st DPT 38.4 sec vs. 31.5 sec, p <0.05; FFC 38.9 sec vs. 33.7 sec, p <0.05). There were no significant differences in the peak times between patients with pancreatic cancer and those with other cancers. The 1st DPH of CWA-APTT was significantly higher in patients with pancreatic cancer (median, 353 mm absorbance) than in patients with other cancers (median, 331 mm absorbance, p <0.05) and patients with benign pancreatic diseases (median, 325 mm absorbance, p <0.01). The FFC was significantly higher in patients with pancreatic cancer (median, 355 mm absorbance) than patients with benign pancreatic diseases (median, 211 mm absorbance, p <0.01).

Regarding the CWA-sTF/FIXa in PRP without phospholipids (**Figure 3**), the peak times of the CWA-sTF/FIXa were shorter in patients without cancer than in patients with CWA, clot waveform analysis; sTF/FIXa, small amount of tissue factor induced FIX activation; navy line, fibrin formation curve; 1st derivative curve (velocity); light blue, 2nd derivative curve (acceleration); solid line, patient; dotted line, healthy volunteer cancers and healthy volunteers. The peak heights of FFC, 1st DP and 2nd DP were highest in patient with pancreatic cancer followed by patient with esophageal cancer. In the statistical analysis (**Figure 4**), the peak times of the CWA-sTF/FIXa were shorter in patients without cancer (median, 2nd DPT 70.2 sec; 1st DPT 85.0 sec; FFC 86.2 sec) than in patients with cancer (median, 2nd DPT 78.3 sec, p <0.001; 1st DPT 95.9 sec, p<0.001 and FFC 97.2 sec, p <0.001) and healthy volunteers (median, 2nd DPT 76.9 sec, p <0.01; 1st DPT 95.3 sec, p<0.001 and FFC 94.6 sec, p <0.01). The 2nd DPT values of patients with pancreatic cancer (median, 78.1 sec, p <0.05) and with other cancer (median, 78.3 sec, p <0.05) were significantly longer than those of patients with benign pancreatic diseases (median, 59.7 sec). There were no significant differences in peak times among patients with pancreatic cancer, other cancers and benign pancreatic diseases). The 2nd DPH (median, 47.1 mm absorbance vs. 36.9 mm absorbance, p <0.01), 1st DPH (median 95.9 mm absorbance vs. 85.0 mm absorbance, p <0.001) and FFH (median, 384 mm absorbance vs. 324 mm absorbance, p <0.001) were significantly higher in patients with cancer than in those without cancer. The 1st DPH was significantly higher in patients with pancreatic cancer (median, 127 mm absorbance) than in patients with other cancers (median, 96.1 mm absorbance, p <0.05) and patients with benign pancreatic diseases (median, 74.7 mm absorbance, p < 0.01). The FFH (median, 463 mm absorbance vs. 268 mm absorbance, p < 0.01) was significantly higher in patients with pancreatic cancer than patients with benign pancreatic diseases. There was no significant difference in the 2nd DPH or FFH between patients with pancreatic cancer and patients with other cancers.

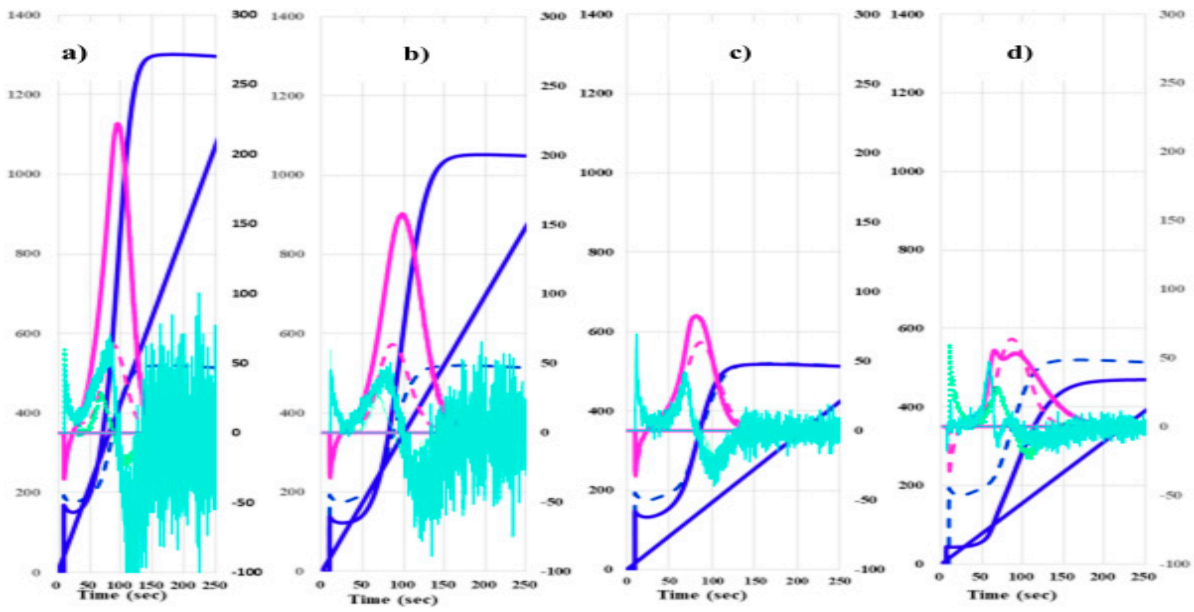

Figure 3. CWA-sTF/FIXa in patient with pancreatic cancer a), esophageal cancer b), chronic hepatitis c) or pancreatic cyst d).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 June 2023 doi:10.20944/preprints202306.0031.v1

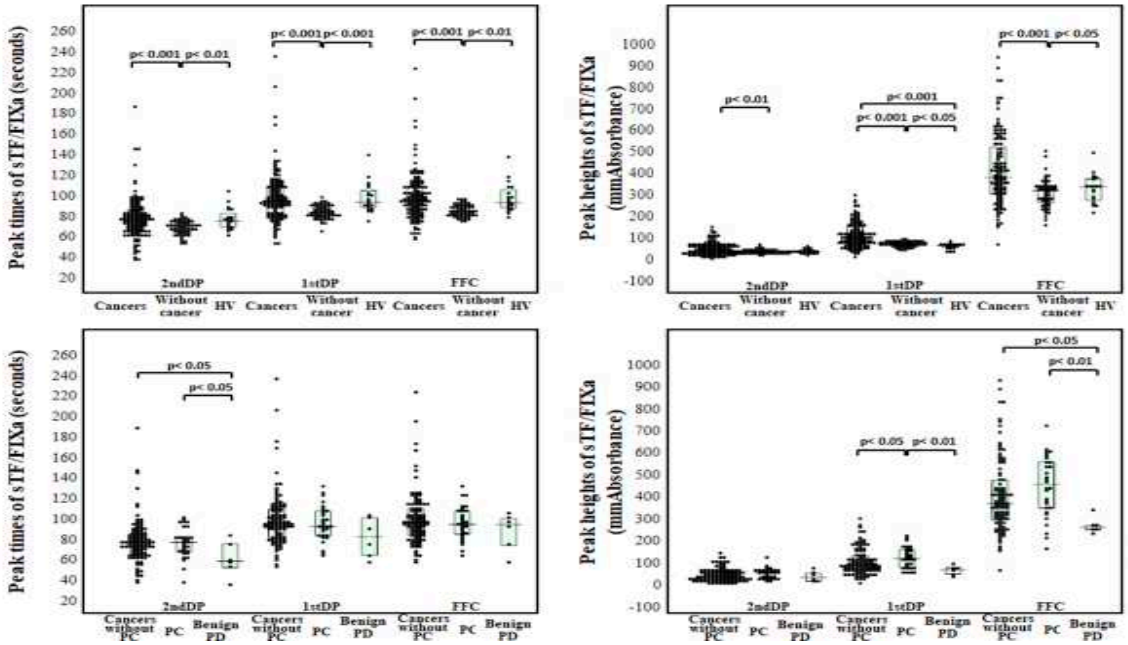

Figure 4. CWA-sTF/FIXa in patients with cancer, without cancer and healthy volunteers (upper column), cancer patients with and without pancreatic cancer, and patients with benign pancreatic diseases. CWA, clot wave form; sTF/FIXa, small amount of tissue factor induced FIX activation; HV, healthy volunteer; PC, pancreas cancer; BPD, benign pancreatic disease; FFT, fibrin formation time; FFH, fibrin formation height; 1stDPT, first derivative peak time; 1stDPH, first derivative peak height; 2nd DPT, second derivative peak time; 2nd DPH, second derivative peak height.

	Cancers	Without cancer	PCs	Without PC	BPDs	HVs
2ndDPT (sec)	78.3 (67.5-87.0)	70.2 (66.1-75.4)	78.1 (68.9-83.6)	78.3 (67.1-86.9)	59.7 (53.5-76.8)	76.9 (70.1-84.0)
2ndDPH (mmAbsorbance)	47.1 (29.4-68.2)	36.9 (31.5-43.1)	61.5 (36.3-70.3)	44.8 (28.3-67.5)	40.3 (22.7-58.8)	37.4 (31.0-48.1)
1stDPT (sec)	95.9 (84.0-110.3)	85.0 (81.2-90.9)	93.8 (84.5-108.7)	96.0 (82.8-110.7)	83.6 (65.5-102.3)	95.3 (89.8-106.4)
1stDPH (mmAbsorbance)	104 (74.9-155)	75.6 (65.8-83.7)	127 (84.8—165.0)	96.1 (72.1-139.6)	74.7 (55.5-84.7)	67.8 (58.2-74.5)
FFC (sec)	97.2 (86.0-110.4)	86.2 (82.2-91.6)	95.7 (86.1-109.1)	97.4 (84.8-111.2)	95.4 (75.2-101.4)	94.6 (89.4-106.9)
FFH (mmAbsorbance)	384 (306-526)	324 (267-344)	463 (354-565)	374 (301-482)	268 (256-275)	341 (278-378)

Regarding CWA-TT, the peak heights were significantly higher in PRP than in PPP. The second 1st DPH was extremely high in patients with pancreatic cancer (**Figure 5**). In the statistical analysis, the 2nd DPT, 1st DPT-1 and FFC were significantly shorter in cancer patients (median, 34.6 sec, $p < 0.001$; 40.5 sec, $p < 0.001$; and 108 sec, $p < 0.001$, respectively) and patients without cancers (median, 34.7 sec, $p < 0.001$; 41.0 sec, $p < 0.001$ and 129 sec, $p < 0.001$, respectively) than in healthy volunteers (median, 42.7 sec; 49.9 sec and 138 sec, respectively). Only the 1st DPT-2 was significantly shorter in

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 June 2023 doi:10.20944/preprints202306.0031.v1

patients with cancer (median, 116 sec) than in patients without cancer (median, 145 sec, p <0.001) and healthy volunteers (median, 133 sec, p < 0.01) (**Figure 6**). There were no significant differences in peak times among patients with pancreatic cancer, other cancers or benign pancreatic diseases. The 2nd DPH (median, 241 mm absorbance vs. 102 mm absorbance, p < 0.001), 1st DPH-1 (median, 146 mm absorbance vs. 82.5 mm absorbance, p <0.001), 1st DPH-2 (median, 82.6 mm absorbance vs. 27.9 mm absorbance, p <0.001) and FFH (median, 922 mm absorbance vs. 695 mm absorbance, p <0.001) were significantly higher in patients with cancer than in healthy volunteers. The 2nd DPH, 1st DPH-2 and FFH were significantly higher in patients with cancer than in patients without cancer (median, 141 mm absorbance, p < 0.001; 51.8 mm absorbance, p <0.001 and 911 mm absorbance, p <0.01). The 2nd DPH and 1st DPH-1 were significantly lower in patients with benign pancreatic diseases than in patients with pancreatic cancer (median, 157 mm absorbance vs. 261 mm absorbance, p <0.05 and 98.5 mm absorbance vs. 176 mm absorbance, p <0.05, respectively) or other cancers (median, 237 mm absorbance, p <0.05 and 141 mm absorbance, p <0.05, respectively). There were no significant differences in the 1st DPT-2 or FFH among patients with pancreatic cancer, other cancers or benign pancreatic diseases.

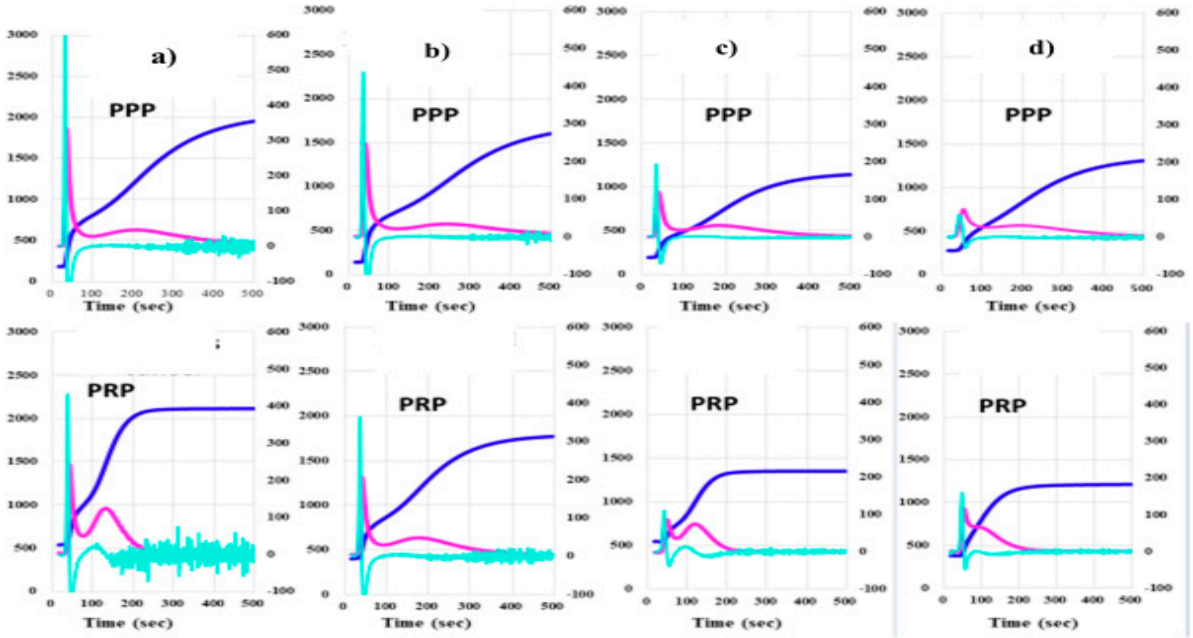

Figure 5. CWA-TT in patient with pancreatic cancer a), esophageal cancer b), chronic hepatitis c) or pancreatic cyst d). CWA, clot waveform; analysis TT, thrombin time; navy line, fibrin formation curve; pink line, 1st derivative curve (velocity); light blue, 2nd derivative curve (acceleration); solid line, patient; dotted line, healthy volunteer.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 June 2023 doi:10.20944/preprints202306.0031.v1

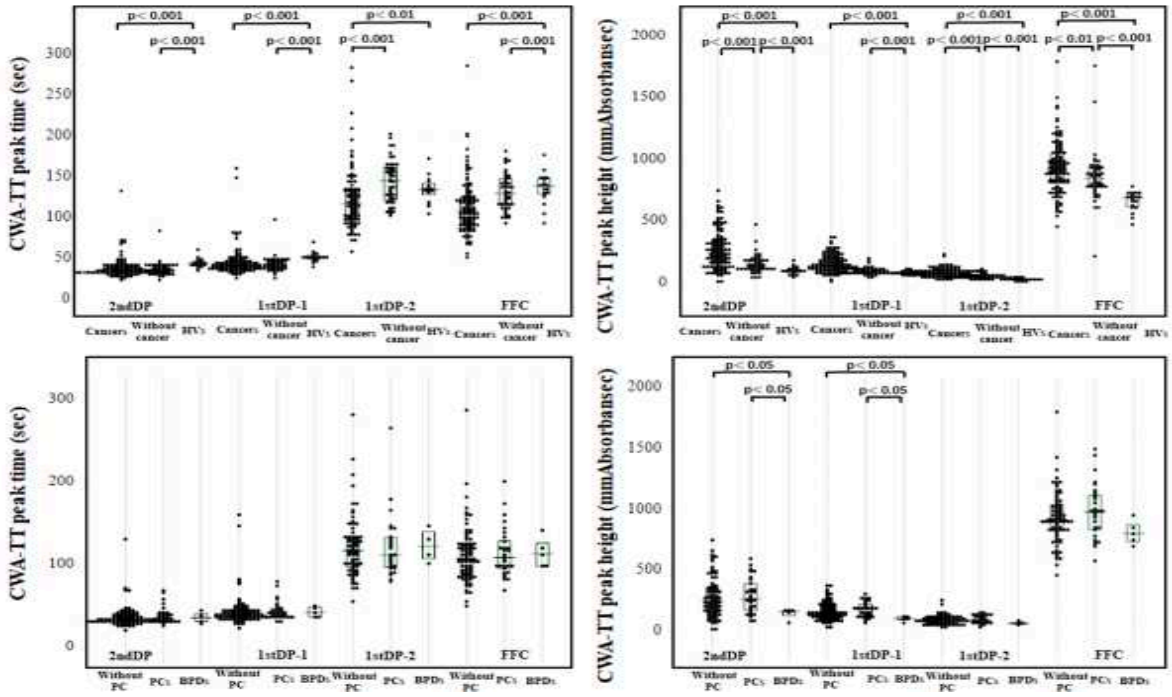

Figure 6. CWA-TT in patients with cancer, patients without cancer and healthy volunteers (upper column), cancer patients with and without pancreatic cancer, and patients with benign pancreatic diseases. CWA, clot waveform analysis; TT, thrombin time; HV, healthy volunteer; PC, pancreatic cancer; BPD, benign pancreatic disease; FFT, fibrin formation time; FFH, fibrin formation height; 1stDPT, first derivative peak time; 1stDPH, first derivative peak height; 2nd DPT, second derivative peak time; 2nd DPH, second derivative peak height.

4. Discussion

CAT is a condition whose relevance has been recognized frequently both among physicians who deal with VTE and oncologists [29]. The annual incidence of VTE is 0.5% in cancer patients compared to <0.1% in the general population and active cancer accounts for 20% of the overall incidence of VTE [29]. Several mechanisms underlying the prothrombotic state in these patients have been proposed. Increased levels of clotting factors, leukocytes, platelets, and TF-positive microparticles are all potential factors that alone or in combination increase CAT [30]. After activation of coagulation system, fibrin- related markers, such as D-dimer, SF and FDP are increased, and these biomarkers are used for the diagnosis and exclusion of VTE [31,32]. The platelet activation receptor C-type lectin-like receptor 2 (CLEC-2), and its endogenous ligand podoplanin have attracted the attention of many researchers [33].

In this study, 24.6% of patients with thrombotic diseases had malignant neoplasms, indicating that these cancer patients were in a hypercoagulable state. In particular, 36.7% of pancreatic cancer patients were associated with thrombosis, suggesting that pancreatic cancer patients are markedly hypercoagulable and have a high risk for thrombosis. Patients with pancreatic cancer have a very high risk of both venous and arterial thrombosis compared to those with other cancers, caused by their tumor-driven hypercoagulable state [34]. The present study also showed the significant prolongation of peak times of CWA-APTT which is equal to routine APTT, in cancer patients including those with pancreatic cancer. Although these findings may prompt the misunderstanding that hypocoagulability exists in cancer patients, the markedly increased peak heights of the CWA-APTT and CWA- sTF/FIXa actually suggest a hypercoagulable state in patients with cancer including those with pancreatic cancer. The APTT using massive phospholipids and substances that activate the contact system does not reflect physiological coagulation, whereas the sTF/FIXa using PRP instead of massive phospholipids, does reflect physiological coagulation.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 June 2023 doi:10.20944/preprints202306.0031.v1

	Cancers	Without cancer	PCs	Without PC	BPDs	HVs
2ndDPT (sec)	34.6 (31.6-39.8)	34.7 (31.3-39.9)	35.8 (32.0-41.1)	34.0 (30.9-39.6)	35.9 (31.2-41.1)	42.7 (40.3-45.8)
2ndDPH (mmAbsorbance)	241 (148-352)	141 (112-185)	261 (167-387)	237 (150-351)	157 (122-160)	102 (83.7-113.4)
1stDPT-1 (sec)	40.5 (36.3-46.0)	41.0 (36.9-46.3)	41.7 (37.1-47.0)	39.9 (35.6-46.0)	42.6 (36.5-49.3)	49.9 (47.9-52.9)
1stDPH-1 (mmAbsorbance)	146 (109-205)	92.4 (81.0-110)	176 (118-218)	141 (106-204)	98.5 (89.2-117)	82.5 (74.8-94.9)
1stDPT-2 (sec)	116 (97.3-133.7)	145 (121-161)	112 (97.3-133.2)	117 (97.4-133.6)	122 (NC)	133 (127-141)
1stDPH-2 (mmAbsorbance)	82.6 (60.3-106.3)	51.8 (42.6-68.0)	81.4 (64.5-122.2)	83.6 (58.4-104.8)	56.5 (NC)	27.9 (23.3-32.9)
FFC (sec)	108 (90.7-124.5)	129 (114-147)	109 (95.1-128.8)	107 (88.9-124.3)	113 (98.1-126.6)	138 (128-147)
FFH (mmAbsorbance)	922 (822-1055)	850 (779-933)	975 (827-1113)	911 (821-1032)	796 (729-869)	695 (618-729)

The mechanism underlying hypercoagulability in pancreatic cancer [35–37] involves increased procoagulant factors, including fibrinogen [38], FVIII [39] and TF [40,41] and reduced anticoagulant factors [36,42]. As fibrinogen is measured by clotting assay, the fibrinogen activity may be higher than the fibrinogen antigen in the hypercoagulable state. Markedly high peak heights of the sTF/FIXa using small amounts of TF suggests that intrinsic TF released cancer cells exist in plasma. Thrombin causes an elevation in FVIII activity and the activation cycle from thrombin to FXIa, called the thrombin burst [22]. Elevated peak heights, especially the second peak of the 1st DPH (1st DPH-2) of CWA-TT suggest that the thrombin burst worsens hypercoagulability in cancer patients including those with pancreatic cancer. Platelet aggregation induced by tumor cells is a key contributor to the prothrombotic state in pancreatic cancer. Pancreatic cancer cell lines induce platelet aggregation in vitro via a thrombin-dependent mechanism [43]. In the present study, the CWA-sTF/FIXa assay in PRP showed hypercoagulability and the CWA-TT showed that the peak heights were significantly higher in PRP than in PPP, suggesting that platelets may play an important role in hypercoagulability in cancer patients. Human pancreatic tumors and cell lines have been shown to express plasminogen activator inhibitor type 1 (PAI-1), a key inhibitor of fibrinolysis [44], suggesting that the evaluation of fibrinolysis is important. However, CAT with hyperfibrinolysis may cause DIC in patients with advanced stomach cancer [45], whereas DIC is rare in patients with pancreatic cancer. TF borne by circulating microparticles released from TF-expressing pancreatic cancer cells might contribute to thrombosis formation [46]. Elevated cytokines [47] and neutrophil extracellular traps [48] may cause inflammatory thrombosis. These reports suggest that an evaluation of white blood cells including neutrophils and monocytes might be important.

5. Conclusions

Elevated peak heights of CWA-APTT, CWA-sTF/FIXa and CWA-TT indicated marked hypercoagulability induced by TF released from cancer cells, platelet activation and thrombin burst in patients with malignant neoplasms including pancreatic cancer.

Author Contributions: Conceptualization, H.W. and S.K.; methodology, Y.I.; software, H.W.; validation, H.I. and S.K.; formal analysis, H.W.; investigation, I.M.; data curation, S.F., Y.S., Y.N., H.M., Y.N. and Y.Y.; writing—original draft preparation, M.K.; writing—review and editing, H.W.; visualization, H.M.; supervision, H.S.; project administration, M.S.; funding acquisition, H.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by a Grant-in-Aid from the Ministry of Health, Labour and Welfare of Japan.

Institutional Review Board Statement: The study protocol (2019-K9) was approved by the Human Ethics Review committees of Mie Prefectural General Medical Center, and informed consent was obtained from each patient.

Informed Consent Statement: Informed consent was obtained from all subjects using opt out method involved in the study.

Data Availability Statement: The data presented in this study are available on request to the corre sponding author. The data are not publicly available due to privacy restrictions.

Acknowledgments: The authors thank Mrs Nisii H and Sakano Y for their kind support in performing the assay for the CWA. The authors also thank the many technicians at Central Laboratory for measuring the laboratory data and the many physicians who took care of the patients.

Conflicts of Interest: The measurements of CWA were partially supported by Instrumentation La boratory Japan. In the other points, the authors declare no conflict of interest.

References

1. Hisada Y, Mackman N.: Cancer-associated pathways and biomarkers of venous thrombosis. Blood. 2017; 130: 1499-506
2. Mahajan A, Brunson A, White R, Wun T.: The Epidemiology of Cancer-Associated Venous Thromboembolism: An Update. Semin Thromb Hemost. 2019; 45: 321-5
3. Cohen O, Caiano LM, Tufano A, Ageno W.: Cancer-Associated Splanchnic Vein Thrombosis. Semin Thromb Hemost. 2021 Nov;47(8):931-941.
4. Fernandes CJ, Morinaga LTK, Alves JL Jr, Castro MA, Calderaro D, Jardim CVP, Souza R.: Cancer-associated thrombosis: the when, how and why. Eur Respir Rev. 2019; 28: 180119.
5. Fernandes CJ, Morinaga LTK, Alves JL Jr, Castro MA, Calderaro D, Jardim CVP, Souza R.: Cancer-associated thrombosis: the when, how and why. Eur Respir Rev. 2019; 28: 180119.
6. Kim AS, Khorana AA, McCrae KR.: Mechanisms and biomarkers of cancer- associated thrombosis. Transl Res. 2020; 225: 33-53.
7. Timp JF, Braekkan SK, Versteeg HH, Cannegieter SC. Epidemiology of cancer- associated venous thrombosis. Blood. 2013; 122: 1712-1723.
8. Zeller JA, Tschoepe D, Kessler C.: Circulating platelets show increased activation in patients with acute cerebral ischemia. Thromb Haemost. 1999; 81: 373-377.
9. Ferroni P, Riondino S, Vazzana N, Santoro N, Guadagni F, Davì G.: Biomarkers of platelet activation in acute coronary syndromes. Thromb Haemost. 2012; 108: 1109-1123.
10. Varki A: Trousseau's syndrome: multiple definitions and multiple mechanisms. Blood. 2007; 110: 1723-1729.
11. Bao L, Zhang S, Gong X, Cui G.: Trousseau Syndrome Related Cerebral Infarction: Clinical Manifestations, Laboratory Findings and Radiological Features. J Stroke Cerebrovasc Dis. 2020; 29: 104891
12. Sharma BK, Flick MJ, Palumbo JS. Cancer-Associated Thrombosis: A Two-Way Street. Semin Thromb Hemost. 2019; 45: 559-568.
13. Suzuki-Inoue K.: Platelets and cancer-associated thrombosis: focusing on the platelet activation receptor CLEC-2 and podoplanin. Blood. 2019; 134: 1912-8.
14. Maestre A, Trujillo-Santos J, Visoná A, Lobo JL, Grau E, Malý R, Duce R, Monreal M; RIETE Investigators. D-dimer levels and 90-day outcome in patients with acute pulmonary embolism with or without cancer. Thromb Res. 2014; 133: 384-9.
15. Gerotziafas GT, Mahé I, Lefkou E, AboElnazar E, Abdel-Razeq H, Taher A, Antic D, Elalamy I, Syrigos K, Van Dreden P.: Overview of risk assessment models for venous thromboembolism in ambulatory patients with cancer.Thromb Res. 2020; 191: S50-S57.
16. Litvinov RI, Pieters M, de Lange-Loots Z, Weisel JW.: Fibrinogen and Fibrin. Subcell Biochem. 2021; 96: 471-501.
17. Wada H, Matsumoto T, Ohishi K, Shiraki K, Shimaoka M: Update on the Clot Waveform Analysis. Clin Appl Thromb Hemost. 2020; 26:1076029620912027
18. Matsumoto T, Nogami K, Shima M. A combined approach using global coagulation assays quickly differentiates coagulation disorders with prolonged aPTT and low levels of FVIII activity. Int J Hematol 2017; 105: 174-83
19. Nogami K. Clot Waveform Analysis for Monitoring Hemostasis. Semin Thromb Hemost 2022; (Online ahead of print)
20. Konstantinidi A, Sokou R, Parastatidou S, Lampropoulou K, Katsaras G, Boutsikou T, Gounaris AK, Tsantes AE, Iacovidou N: Clinical Application of Thromboelastography/Thromboelastometry (TEG/TEM) in the Neonatal Population: A Narrative Review. Semin Thromb Hemost. 2019 ; 45: 449-457
21. Tripodi A: Thrombin Generation Assay and Its Application in the Clinical Laboratory. Clin Chem. 2016; 62: 699-707.
22. Wada H, Ichikawa Y, Ezaki E, Matsumoto T, Yamashita Y, Shiraki K, Shimaoka M, Shimpo H: The reevaluation of thrombin time using a clot waveform analysis. J. Clin. Med. 2021, 10, 4840.

23. Kobayashi N, Maegawa T, Takada M, Tanaka H, Gonmori H: Criteria for diagnosis of DIC based on the analysis of clinical and laboratory findings in 345 DIC patients collected by the Research Committee on DIC in Japan. Bibl Haemotol, 1983; 49: 265-275
24. Ezaki M, Wada H, Ichikawa Y, Ikeda N, Shiraki K, Yamamoto A, Moritani I, Shimaoka M, Shimpo H.: Plasma Soluble Fibrin Is Useful for the Diagnosis of Thrombotic Diseases. J Clin Med. 2023; 12: 2597.
25. Mohamad Dbouk, Bryson W. Katona, Randall E. Brand, Amitabh Chak, Sapna Syngal, James J. Farrell, Fay Kastrinos, Elena M. Stoffel, Amanda L. Blackford, Anil K. Rustgi, Beth Dudley, Linda S. Lee, Ankit Chhoda, Richard Kwon, Gregory G. Ginsberg, Alison P. Klein, Ihab Kamel, Ralph H. Hruban, Jin He, Eun Ji Shin, Anne Marie Lennon, Marcia Irene Canto, Michael Goggins: The Multicenter **Cancer** of **Pancreas** Screening Study: Impact on **Stage** and Survival. J Clin Oncol. **2022;** 40: 3257–3266.
26. Kobayashi M, Wada H, Fukui S, Mizutani H, Ichikawa Y, Shiraki K, Moritani I, Inoue H, Shimaoka M, Shimpo H.: A Clot Waveform Analysis Showing a Hypercoagulable State in Patients with Malignant Neoplasms. J Clin Med. 2021; 10: 5352.
27. Matsumoto T, Wada H, Toyoda H, Hirayama M, Yamashita Y, Katayama N.: Modified clot waveform analysis to measure plasma coagulation potential in the presence of the anti-factor IXa/factor X bispecific antibody emicizumab: comment. J Thromb Haemost. 2018; 16: 1665-1666
28. Wada H, Shiraki K, Matsumoto T, Ohishi K, Shimpo H, Shimaoka M: Effects of platelet and phospholipids on clot formation activated by a small amount of tissue factor. Thromb Res 2020; 193: 146-153
29. Fernandes CJ, Morinaga LTK, Alves JL Jr, Castro MA, Calderaro D, Jardim CVP, Souza R. Cancer-associated thrombosis: the when, how and why. Eur Respir Rev. 2019; 28: 180119.
30. Hisada Y, Mackman N.: Cancer-associated pathways and biomarkers of venous thrombosis. Blood. 2017; 130: 1499-1506.
31. Falanga A, Marchetti M, Russo L.: Hemostatic Biomarkers and cancer prognosis: Where Do We Stand? Semin Thromb Hemost. 2021; Online ahead of print.
32. Johnson ED, Schell JC, Rodgers GM.: The D-dimer assay. Am J Hematol. 2019; 94: 833-9.
33. Suzuki-Inoue K.: Platelets and **cancer**-associated **thrombosis**: focusing on the platelet activation receptor CLEC-2 and podoplanin. Blood. 2019; 134: 1912-1918.
34. Campello E, Bosch F, Simion C, Spiezia L, Simioni P.: Mechanisms of thrombosis in pancreatic ductal adenocarcinoma. Best Pract Res Clin Haematol. 2022; 35: 101346.
35. Campello E, Ilich A, Simioni P, Key NS: The relationship between pancreatic cancer and hypercoagulability: a comprehensive review on epidemiological and biological issues. Br J Cancer, 2019; 121; 359-371
36. Sun W, Ren H, Gao CT, W.D. Ma WD, Luo L, Liu Y, *et al.:* Clinical and prognostic significance of coagulation assays in pancreatic cancer patients with absence of venous thromboembolism. Am J Clin Oncol, 2015; 38: 550-556
37. Zalatnai A, Perjesi E, Galambos E: Much more than trousseau syndrome. The broad spectrum of the pancreatic paraneoplastic syndromes. Pathol Oncol Res, 2018; 24: 1-10
38. Falanga A: Thrombophilia in cancer. Semin Thromb Hemost,2005; 31: 104-110
39. Vormittag R, Simanek R, Ay C, Dunkler D, Quehenberger P, Marosi C, *et al.:* High factor VIII levels independently predict venous thromboembolism in cancer patients: the cancer and thrombosis study. Arterioscler Thromb Vasc Biol, 2009; 29: 2176-2181
40. Khorana AA, Fine RL: Pancreatic cancer and thromboembolic disease. Lancet Oncol, 2004; 5: 655-663
41. van den Berg YW, Osanto S, P.H. Reitsma PH, Versteeg HH: The relationship between tissue factor and cancer progression: insights from bench and bedside. Blood, 2012; 119: 924-932
42. Unsal E, Atalay F, Atikcan S, Yilmaz A: Prognostic significance of hemostatic parameters in patients with lung cancer. Respir Med, 2004; 98: 93-98
43. Heinmoller E, Schropp T, Kisker O, Simon B, Seitz R, Weinel RJ. Tumor cell-induced platelet aggregation in vitro by human pancreatic cancer cell lines. *Scand. J. Gastroenterol.* 1995; **30**: 1008–1016.
44. Sawai H, Liu J, Reber HA, Hines OJ, Eibl G. Activation of peroxisome proliferator-activated receptor-gamma decreases pancreatic cancer cell invasion through modulation of the plasminogen activator system. Mol. Cancer Res. 2006; 4: 159–167.
45. Hwang IG, Choi JH, Park SH, Oh SY, Kwon HC, Lee SI, Lim DH, Lee GW, Kang JH.: Chemotherapy in advanced gastric cancer patients associated with disseminated intravascular coagulation. Cancer Res Treat. 2014; 46: 27-32.
46. Geddings JE, Mackman N. Tumor-derived tissue factor-positive microparticles and venous thrombosis in cancer patients. Blood. 2013; 122: 1873–1880.
47. Seruga B, Zhang H, Bernstein LJ, Tannock IF. Cytokines and their relationship to the symptoms and outcome of cancer. Nat. Rev. Cancer. 2008; 8: 887–899
48. Abdol Razak Norbaini: Elaskalani Omar, Metharom Pat. Pancreatic Cancer-Induced Neutrophil Extracellular Traps: A Potential Contributor to Cancer-Associated Thrombosis. International Journal of Molecular Sciences. 2017; 18: 487

 doi:10.20944/preprints202306.0031.v1