Differences in the platelet proteome between patients with cancer and a high risk of thrombosis and healthy controls

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SPEZIALFORSCHUNGSBEREICH SFB-54: „INTHRO"
Cellular Mediatiors Linking Inflammation and Thrombosis

Disclosures for Lisa-Marie Mauracher

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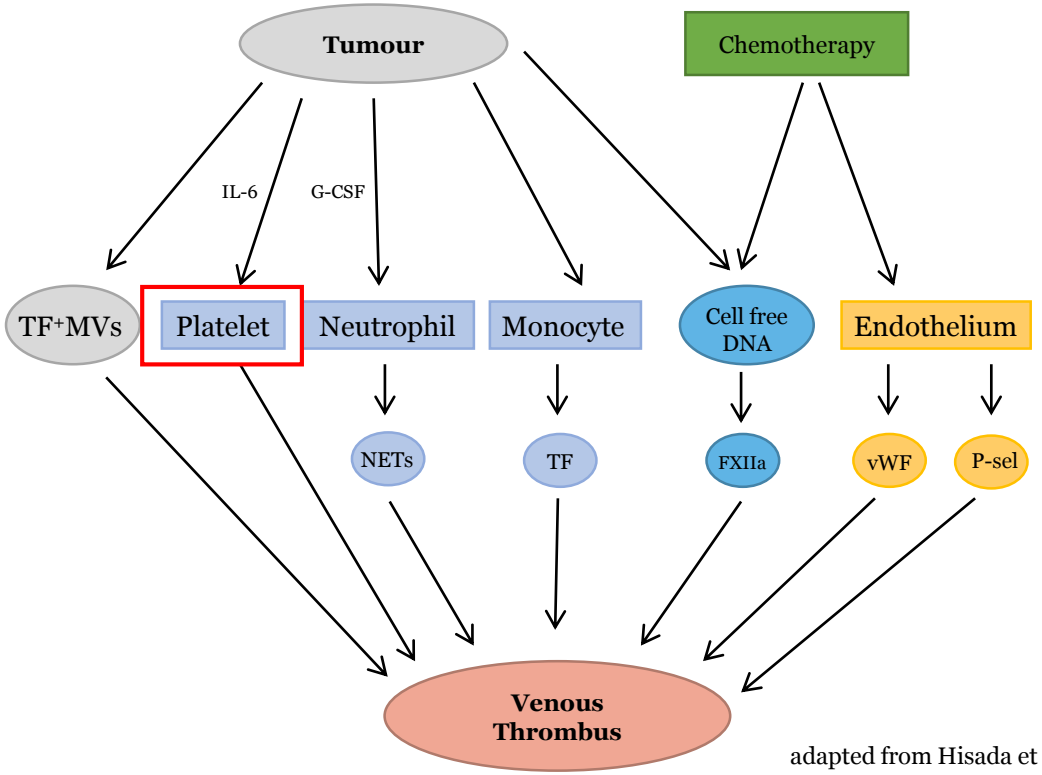
Employee	No relevant conflicts of interest to declare
Consultant	No relevant conflicts of interest to declare
Major Stockholder	No relevant conflicts of interest to declare
Speakers Bureau	No relevant conflicts of interest to declare
Honoraria	No relevant conflicts of interest to declare
Scientific Advisory Board	No relevant conflicts of interest to declare

Background

- Venous thromboembolism is a multifactorial disease. Different alterations of the haemostatic system contribute to the propensity to develop VTE
- Especially patients with cancer have an increased risk to develop thrombosis but this risk is not equally distributed



adapted from Hisada et al., 2015, J Thromb Haemost

Cancer and Thrombosis Study (CATS) Study Design

- Ongoing prospective, observational cohort study
- Patients with newly diagnosed cancer or progression of disease after complete or partial remission
- Written informed consent
- Exclusion if:
  - Chemotherapy (3M)
  - Radiotherapy/surgery (2W)
- 2 year observation period until
  - Occurrence of VTE
  - Death
  - Loss of follow-up
  - Withdrawal of consent

Aim

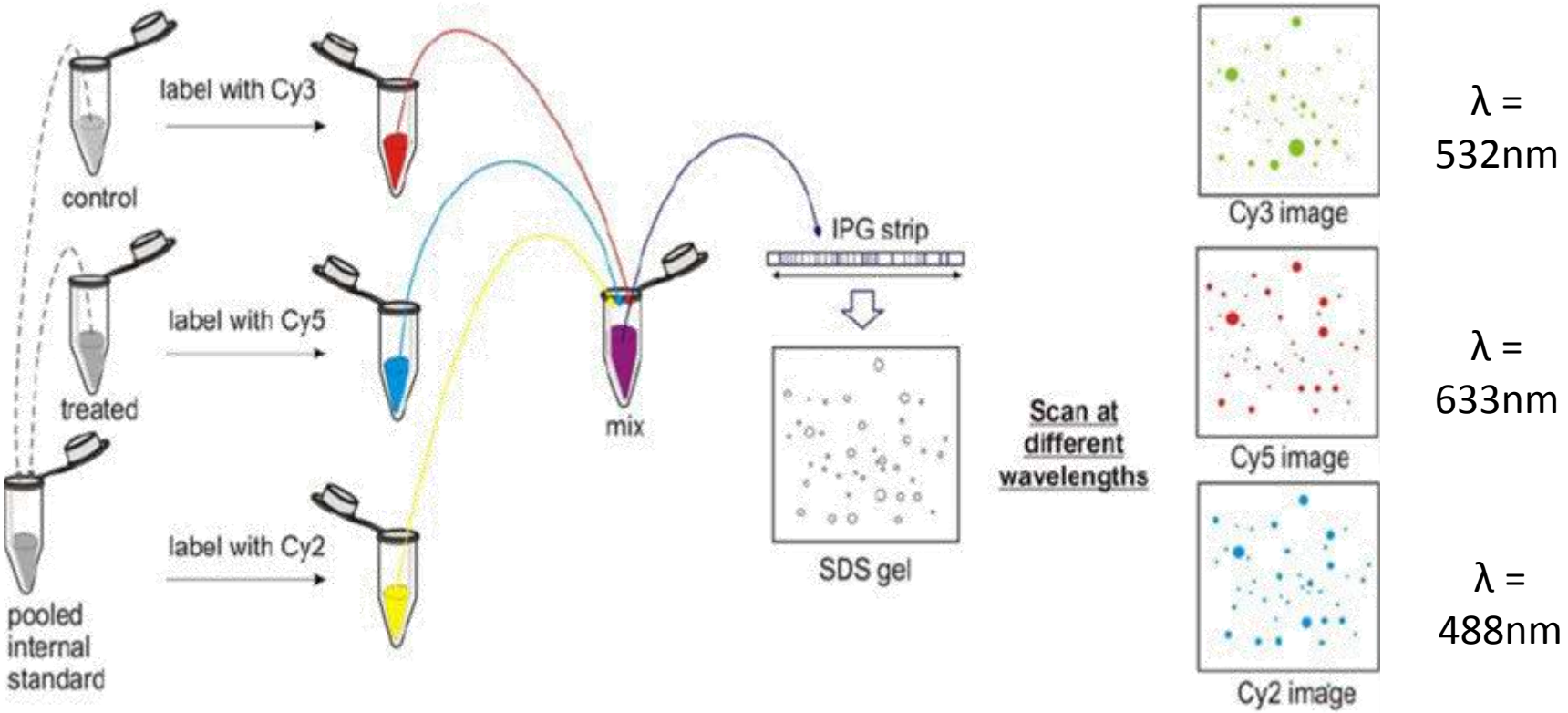
The aim of this project is to investigate, if there are differences in the platelet proteome of patients with cancer vs. matched healthy controls

Population Characteristics

Characteristics median	**Patients with cancer n=45**	**Healthy control n=45**	**P-value**
Age (IQR)	59 years (52 – 65)	57 years (50 – 62)	0.872
Female	42.2% (n=19)	42.2 % (n=19)	
Laboratory values			
Platelet count [G/L] (IQR)	273 (214 – 301)	234 (215 – 286)	0.61
MPV [fl] (IQR)	10.1 (9.7 – 10.8)	10.9 (10.1 – 11.4)	0.959

Characteristics	**Brain n=20**	**Lung n=19**	**Pancreas n=6**
Age	54 (45 – 61)	61 (57 – 65)	69 (61 – 74)
Female	45% (n=9)	36,8 % (n=7)	50 % (n=3)
Deaths (after 1 year)	n=4	n=8	n=4
Events (after 1 year)	n=1	n=3	n=1

Two-dimensional differential in gel electrophoresis (2D DIGE)



http://www.fitgene.com/fuwu/danbaizhizuxue/DIGE.html

4310 **matched protein species**

↓

Wilcoxon-Mann Whitney- Test → p-value smaller than 0.05 in all CATS vs HD

113

↓

More than 80% matched

108

↓

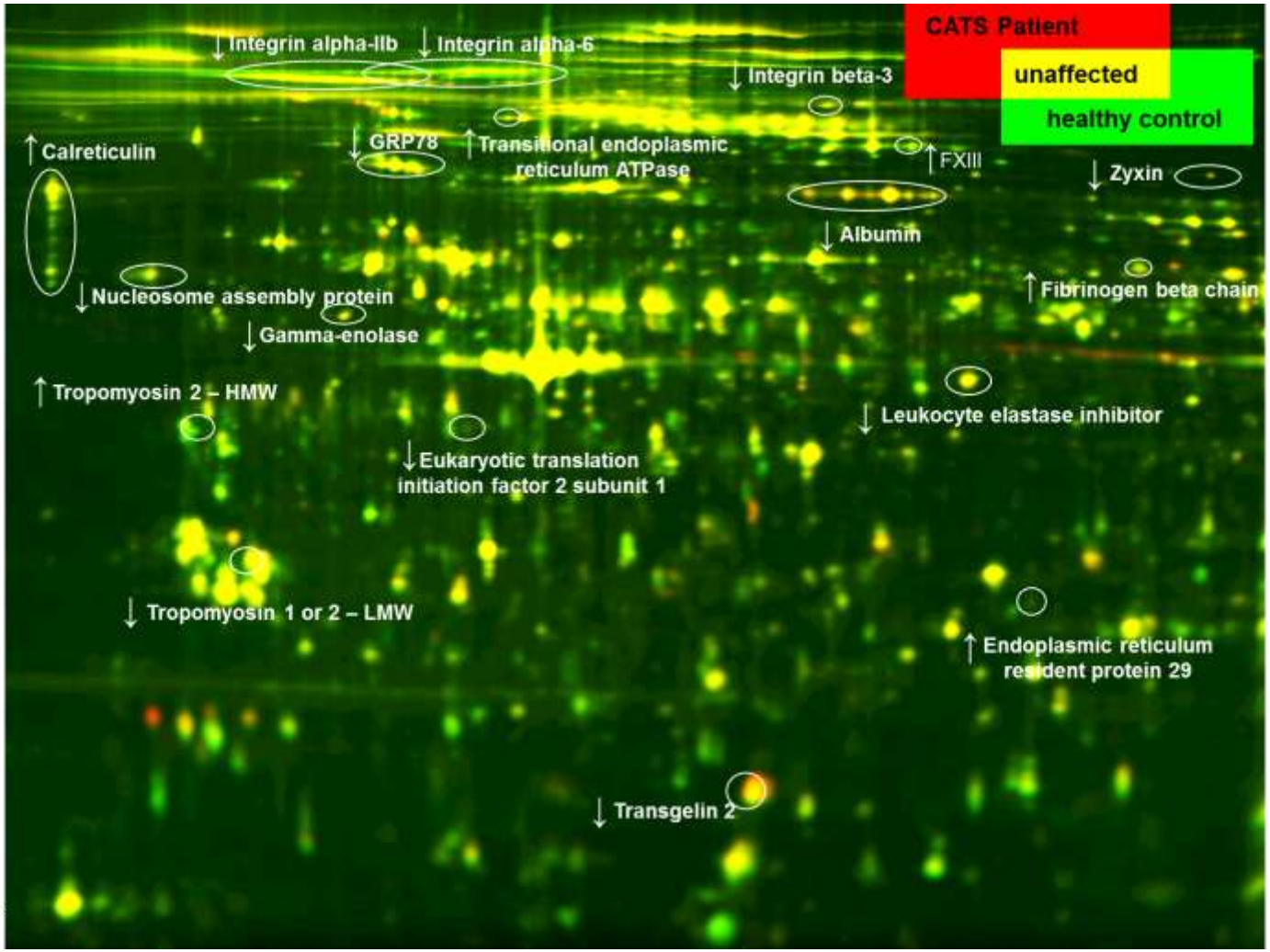
At least 10% change between CATS vs HD (in either a cancer type or all)

89

→ 38 unknown

→ 51 protein species = 18 different proteins

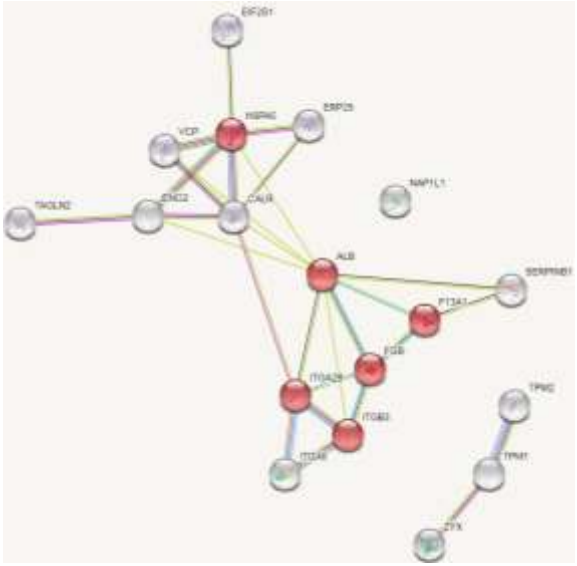
2D DIGE gel showing differentially expressed platelet proteins



Differentially expressed platelet proteins (statistical design → explorative)

	Protein Name (Uniprot)	CATS (n=45) vs HD (n=45)	
		median	p-value
Upregulated in cancer	FXIII (P00488)	1.24	0.007
	Tropomyosin 2 – HMW (P07951)	1.15	0.013
	Endoplasmic reticulum resident protein 29 (P30040)	1.14	0.008
	Fibrinogen beta chain (P02675)	1.14	0.021
	Calreticulin (P27797)	1.10	0.001
	Transitional endoplasmic reticulum ATPase (P55072)	1.09	0.039
	GRP78 (P11021)	1.06	0.023
Downregulated in cancer	Leukocyte elastase inhibitor (P30740)	0.95	0.014
	Eukaryotic translation initiation factor 2 subunit 1 (P05198)	0.94	0.027
	Transgelin-2 (P37802)	0.93	0.046
	Integrin alpha-6 (P23229)	0.92	0.033
	Integrin alpha-IIb (P08514)	0.92	0.001
	Tropomyosin 1 oder 2 – LMW (P09493)	0.92	0.035
	Zyxin (Q15942)	0.91	0.010
	Nucleosome assembly protein 1 (P55209)	0.89	0.002
	Albumin (P02768)	0.88	0.006
	Gamma-enolase (P09104)	0.85	0.010
	Integrin beta-3 (P05106)	0.85	0.008

Pathway analysis of significant proteins



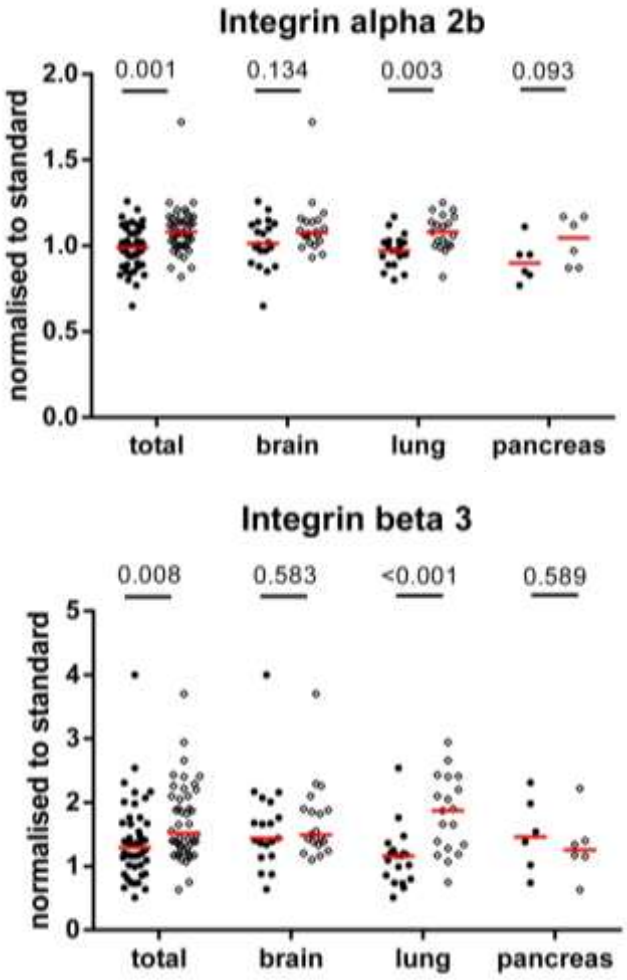
Biological Process (GO)			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0002576	platelet degranulation	6	1.06e-06
GO:0007160	cell-matrix adhesion	5	0.000262
GO:0032940	secretion by cell	7	0.000278
GO:0007229	integrin-mediated signaling pathway	4	0.00119
GO:0042060	wound healing	7	0.00132
			(more ...)

Molecular Function (GO)			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0051087	chaperone binding	5	5.26e-06

Cellular Component (GO)			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0031091	platelet alpha granule	5	2.49e-06
GO:0044421	extracellular region part	14	1.97e-05
GO:0005576	extracellular region	14	3.73e-05
GO:0030141	secretory granule	6	3.73e-05
GO:0031988	membrane-bounded vesicle	13	3.73e-05

String analysis, String-db.org

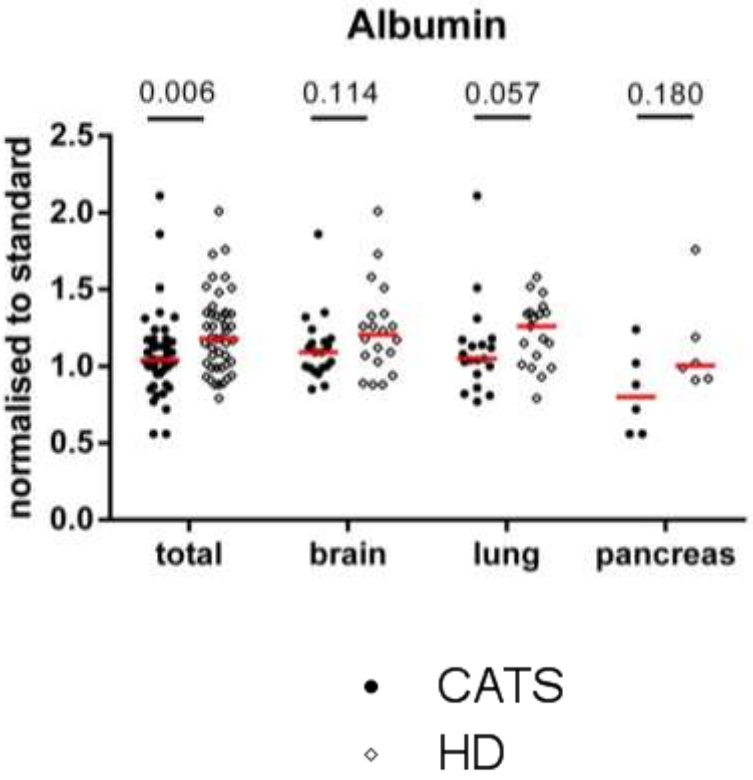
Integrin alpha 2b (p=0.001, 8% decreased)
Integrin beta 3 (p=0.008, 15% decreased)



- Integrin alpha 2b (CD41) associates through disulfide bonds with integrin beta 3 (CD61)
- Interact with fibrinogen, von Willebrand factor, fibronectin, and thrombospondin
- Mediates platelet aggregation
- Crucial for blood coagulation
- Therefore it is centrally involved in thrombosis
- Platelets with reduced Integrin alpha 2b/beta 3 expression are less reactive → poor prognosis (Riedl et al, *Thromb Haemost*, 2017)

Zotz et al, *JTH* 2005; Ma et al, *JTH* 2007

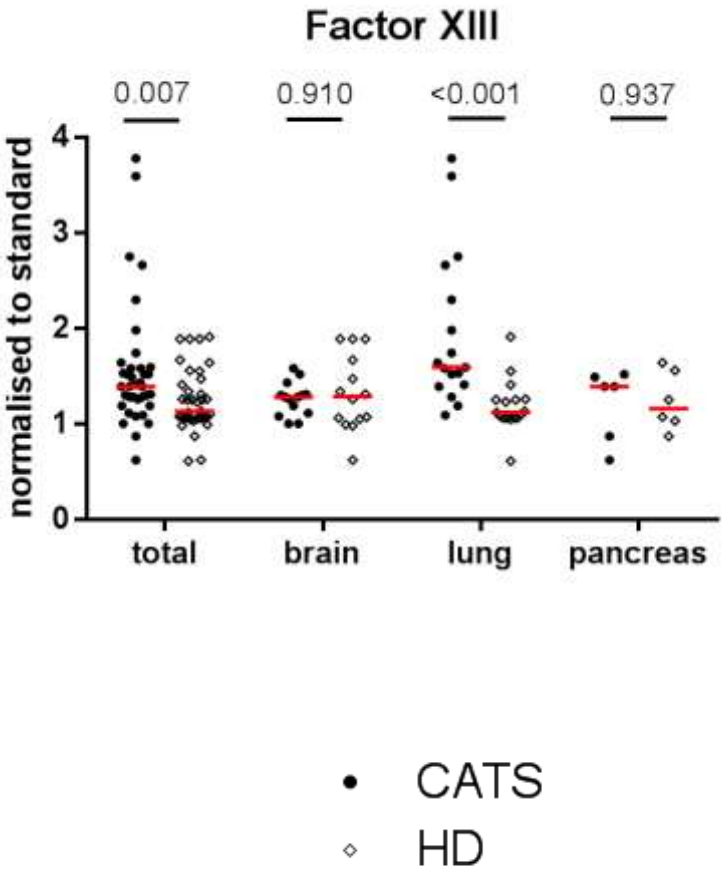
Albumin (p=0.006, 12% decreased in cancer)



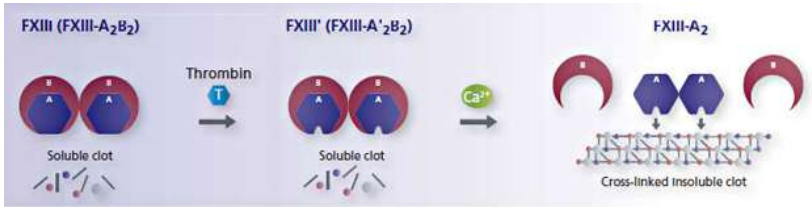
- Decreased serum albumin levels
  - significantly associated with increased risk of VTE
  - associated with mortality
  - is a marker for poor prognosis in patients with cancer

Königsbrügge et al, *Oncologist*, 2016

Coagulation factor XIII (p=0.007, 24% increased in cancer)



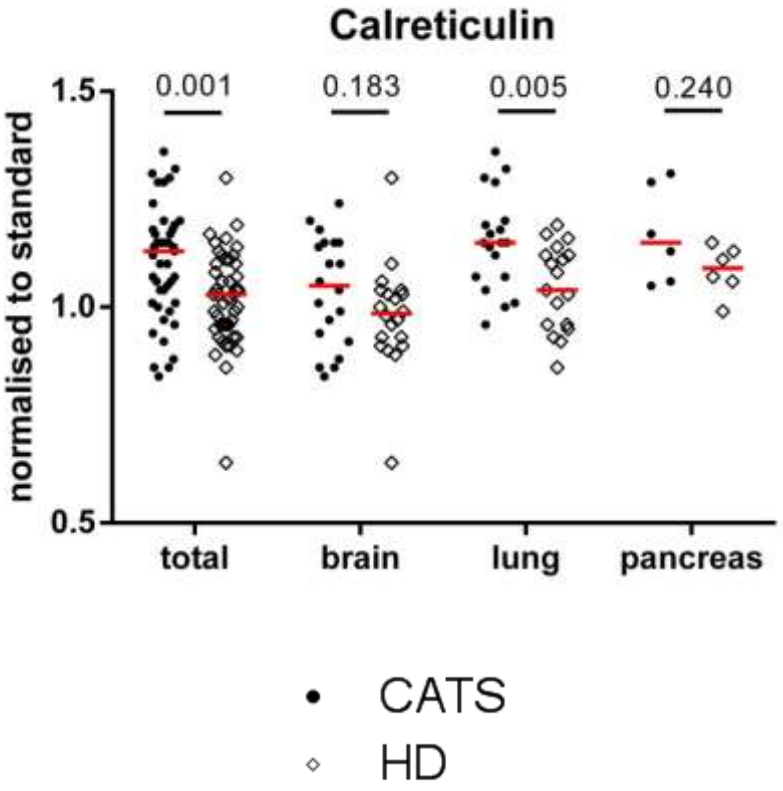
- Coagulation factor XIII is stored in the alpha granules of platelets
- FXIII + Thrombin + Ca^{2+} → activated FXIII → cross-linked fibrin clot



- FXIII deficiency can cause haemorrhagic diathesis

Schwartz Robert, Factor XIII Deficiency, Medscape 2017; www.corifact.com

Calreticulin (p=0.001, 10 % increased in cancer)



- Calcium-binding chaperone that promotes folding and quality control in ER
- C1q binding (complement), DNA binding, integrin binding
- Stored in granulomere of platelets
- High CALR expression in lung cancer was associated with poor overall survival
- Mutation in the CALR gene was detected in 88% of patients with primary myelofibrosis and in 67% of thrombocythemia

Turon et al, *J Hepatol* 2015;Wang et al, *Oncotarget* 2017

Protein expression pattern
Brain differs from lung/pancreas

Upregulated in cancer

Downregulated in cancer

Protein Name (Uniprot)	CATS (n=45) vs matched HD (n=45)		Brain Cancer (n=20) vs matched HD (n=20)		Lung Cancer (n=19) vs matched HD (n=19)		Pancreas Cancer (n=6) vs matched HD (n=6)	
	median	p-value	median	p-value	median	p-value	median	p-value
FXIII (P00488)	1.24	0.007	0.99	0.910	1.41	<0.001	1.2	0.937
Tropomyosin 2 – HMW (P07951)	1.15	0.013	0.93	0.602	1.16	0.012	1.12	0.699
Endoplasmic reticulum resident protein 29 (P30040)	1.14	0.008	1.03	0.988	1.21	<0.001	1.14	1.000
Fibrinogen beta chain (P02675)	1.14	0.021	1.13	0.091	1.19	0.172	1.16	0.485
Calreticulin (P27797)	1.10	0.001	1.07	0.183	1.11	0.005	1.05	0.24
Transitional endoplasmic reticulum ATPase (P55072)	1.09	0.039	1.15	0.201	1.14	0.201	0.93	0.937
GRP78 (P11021)	1.06	0.023	1.01	0.799	1.13	0.006	1.21	0.093
Leukocyte elastase inhibitor (P30740)	0.95	0.014	0.94	0.289	0.88	0.014	0.97	0.937
Eukaryotic translation initiation factor 2 subunit 1 (P05198)	0.94	0.027	0.96	0.383	0.87	0.008	1.02	0.699
Transgelin-2 (P37802)	0.93	0.046	1.00	0.547	0.77	0.014	0.99	0.699
Integrin alph-6 (P23229)	0.92	0.033	0.99	0.402	0.87	0.030	0.92	0.818
Integrin alpha-IIb (P08514)	0.92	0.001	0.95	0.134	0.90	0.003	0.86	0.093
Tropomyosin 1 oder 2 – LMW (P09493)	0.92	0.035	0.97	0.795	0.86	0.008	0.93	0.310
Zyxin (Q15942)	0.91	0.010	1.02	0.989	0.80	0.013	0.66	0.065
Nucleosome assembly protein 1 (P55209)	0.89	0.002	0.90	0.063	0.89	0.043	0.88	0.065
Albumin (P02768)	0.88	0.006	0.90	0.114	0.83	0.057	0.80	0.180
Gamma-enolase (P09104)	0.85	0.010	0.90	0.142	0.90	0.085	0.81	0.180
Integrin beta-3 (P05106)	0.85	0.008	0.97	0.583	0.62	<0.001	1.17	0.589

More than 12 %

Summary

- Patients with cancer have a different platelet proteome compared to sex and age matched healthy controls
- In total, 18 proteins were identified as significantly different
- Very interesting proteins
  - Albumin
  - Integrin alpha 2b
  - Integrin beta 3
  - Calreticulin
  - Coagulation factor XIII
- Different proteins are significant in lung/pancreas compared to brain
- Especially data on integrins, FXIII and Calreticulin match with data on mRNA-seq data from tumour-educated platelets (Best et al, *Cancer Cell*, 2015)

Outlook

- Validation with Western Blot
- Plasma FXIII measurement
- Interaction analysis including data from other cohorts and studies with chronic inflammation and platelet activation from collaborators (SFB 54 in-thro)
- Longitudinal study → change of platelet proteome over a time course of 6 months after enrolment
- NET parameters (H3Cit, nucleosomes and cell free DNA) will be measured

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- Ella Grilz
- Lena Hell
- Christina Reumiller
- Georg Schmidt
- Cihan Ay
- And the whole group

Funding:

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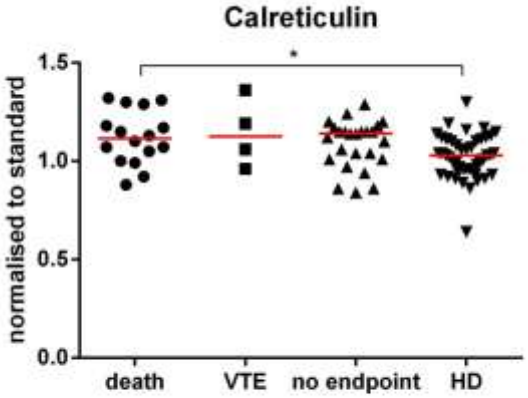
Cellular Mediators Linking Inflammation and Thrombosis

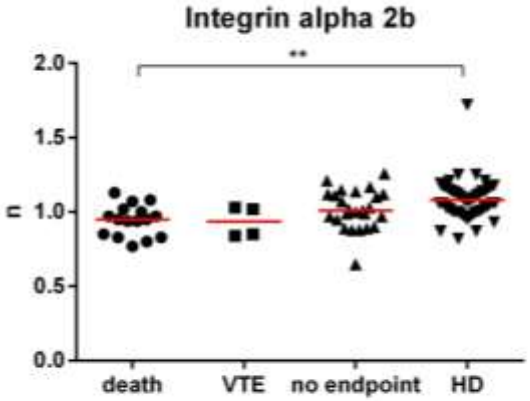
FWF
Der Wissenschaftsfonds.

Non parametric test

Characteristics Median (IQR)	cancer	HD	p-value
Prothrombin F1,2 [pmol/l]	196 (139 - 346)	180 (134 - 249)	0,322
D-Dimer [µg(ml]	0,93 (0,58 - 1,8)	0,31 (0,27 - 0,46)	<0,001
sPTT [s]	34,1 (30,9 - 36,8)	35,1 (32,3 - 37,4)	0,03
Antithrombin III aktivität [%]	111 (100 - 127)	103 (96 - 110)	0,001
basophils [%]	0,1 (0-0,65)	0 (0 - 1)	0,643
CRP [mg/dl]	0,435 (0,1 - 1,13)	0,15 (0,07 - 0,26)	0,002
eosinophils [%]	1 (0,1 - 2,5)	2 (1 - 4,3)	0,625
Erythrocytes [T/l]	4,7 (4,2 - 4,9)	4,8 (4,5 - 5,1)	0,17
Fibrinogen [mg/dl]	380 (316 - 484)	303 (258 - 350)	<0,001
FVIII %	220 (166 - 276)	147 (114 - 187)	0,001
hb [g/dl]	13,4 (12,4 - 14,65)	14,4 (13 - 15,3)	0,262
leukocytes [G/l]	8,27 (6,89 - 11,57)	5,83 (5,2 - 6,94)	<0,001
lymphocytes [%]	16 (9 - 23)	31 (25 - 37)	0,143
MCH [pg]	29,6 (27,65 - 31,3)	30,2 (29,2 - 31)	0,012
MCV [fl]	86,2 (83,7 - 90,4)	87,1 (85,2 - 89,4)	0,02
monocytes [%]	7 (4,85 - 8,75)	8 (6 - 10)	0,597
MTV [fl]	10,1 (9,65 - 10,8)	10,9 (10,1 - 11,4)	0,959
neutrophils [%]	75 63,05 - 83,05)	57,7 (51 - 65)	0,38
platelets [G/l]	273 (214 - 301)	215 (215 - 286)	0,61
thromboplastinzeit [%]	98 (84,5 - 108)	92 (85,5 - 110)	0,617
triglyceride [mg/dl]	120 (89 - 161)	99 (69 - 130)	0,296
Age [years]	59,29 (51,82 - 65,34)	56,75 (49,95 - 61,68)	0,872



ANOVA
Bonferroni



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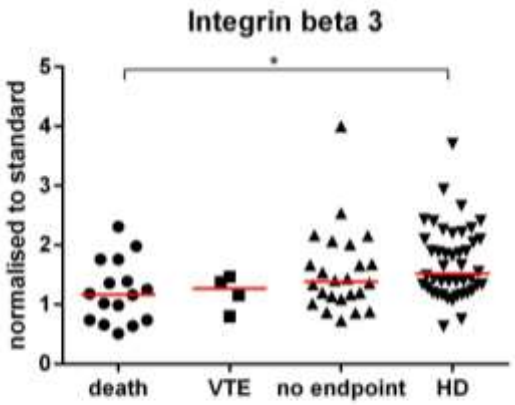

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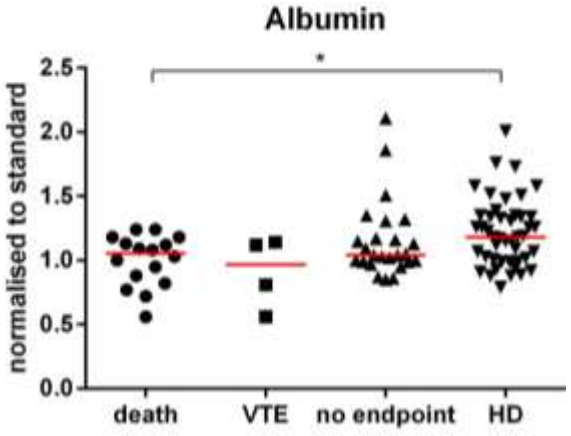

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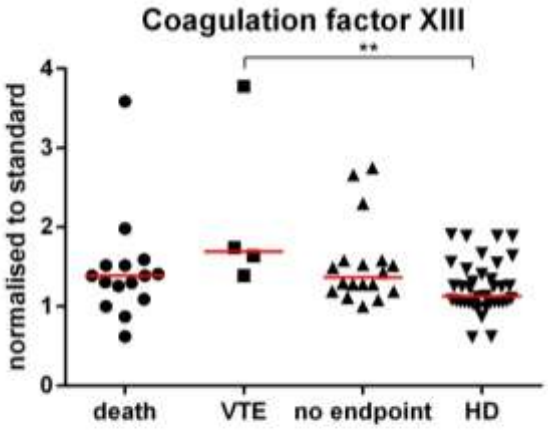

ANOVA
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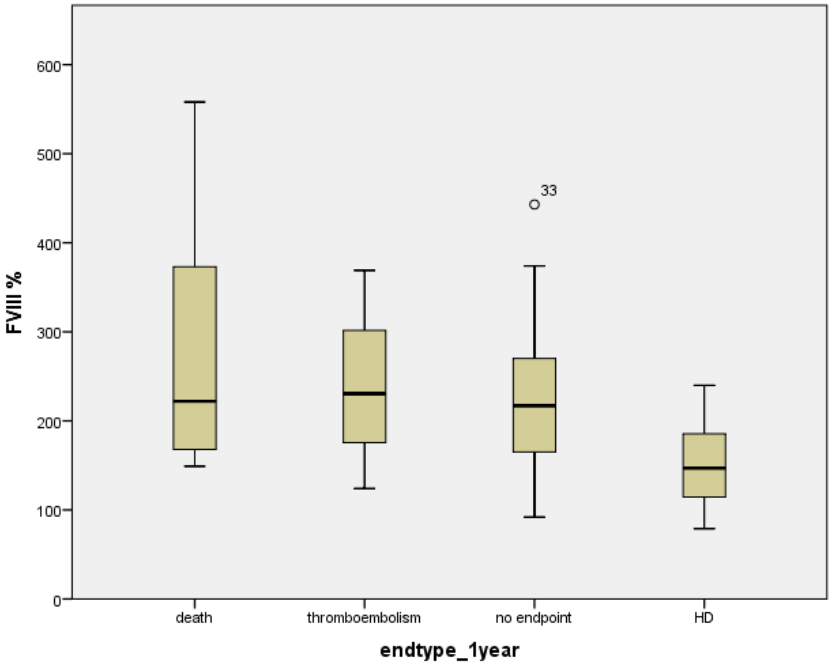
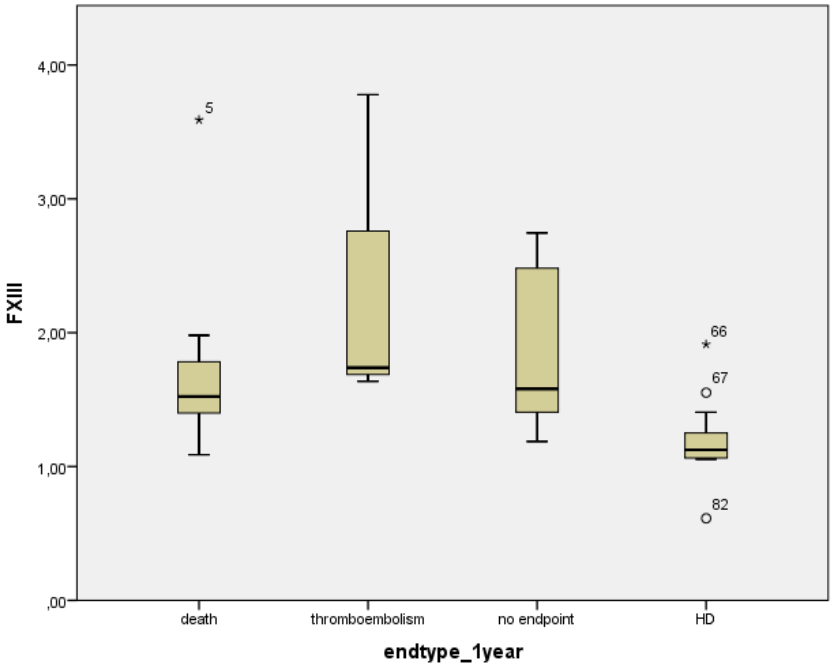
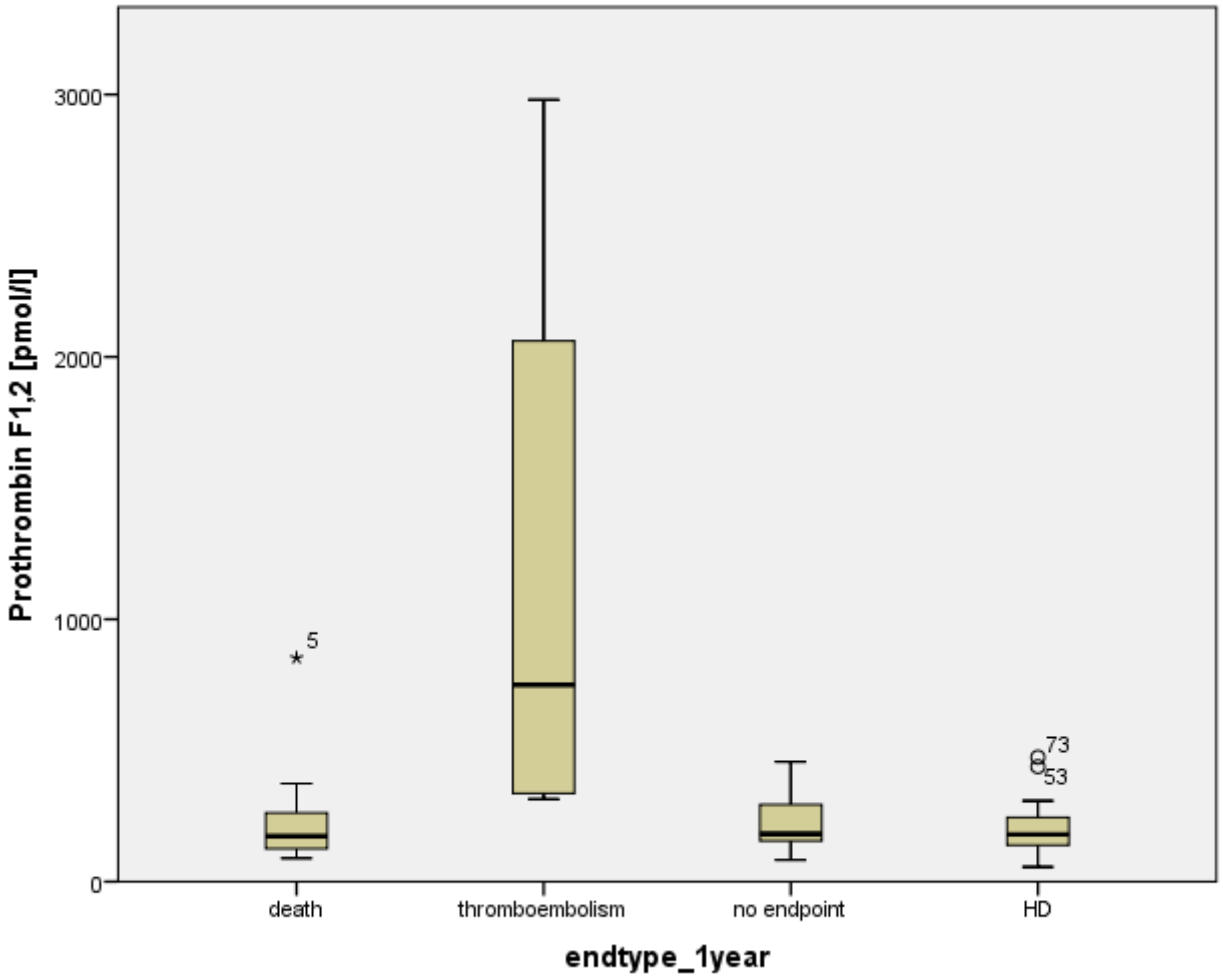
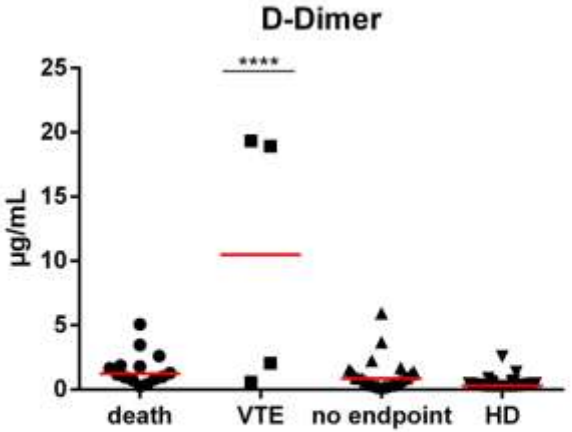
CATS vs HD



Lung only




Prothrombin F1,2 [pmol/l]
3000
2000
1000
0
5
73
53
death
thromboembolism
no endpoint
HD
endtype_1year



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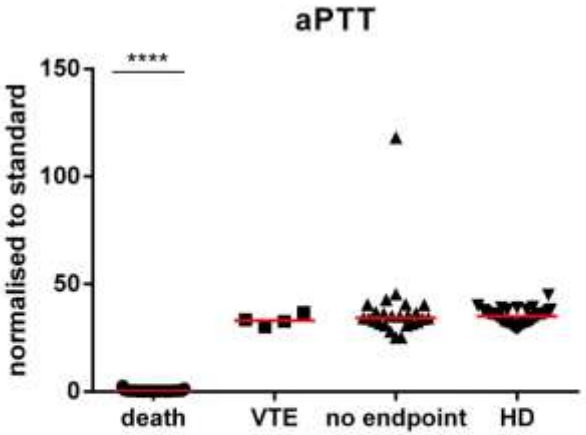

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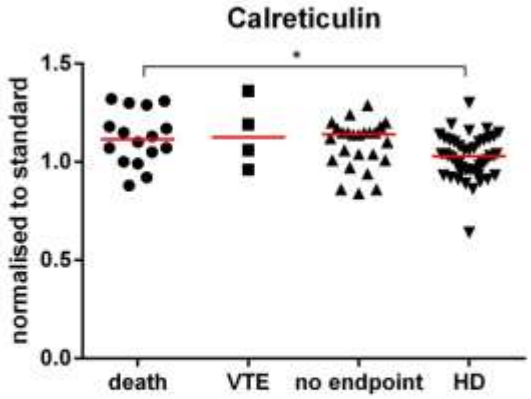

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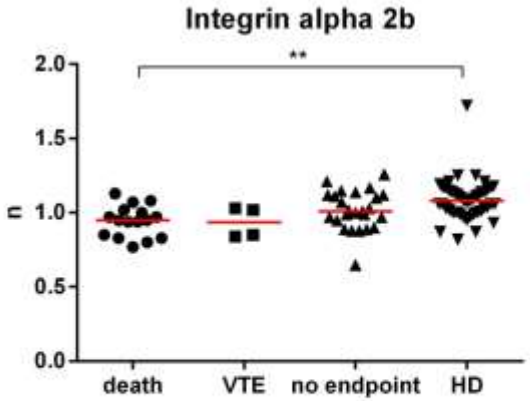

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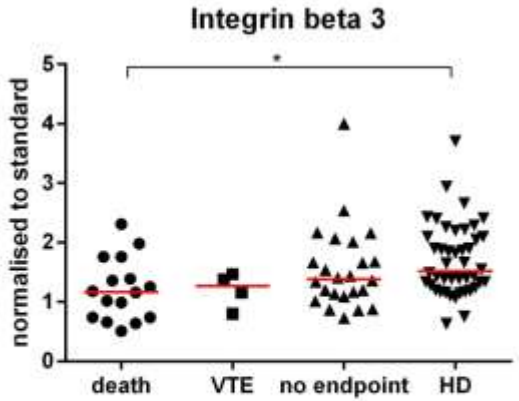

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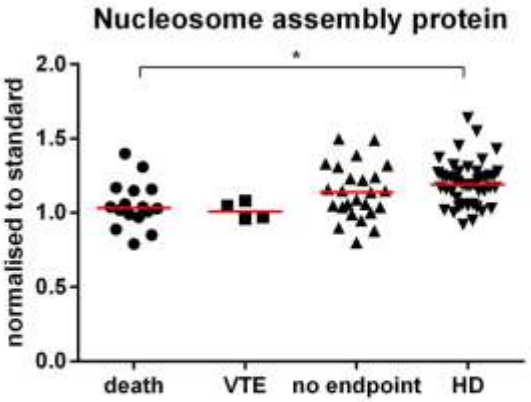

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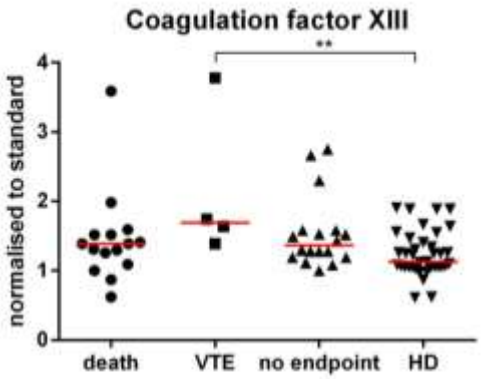

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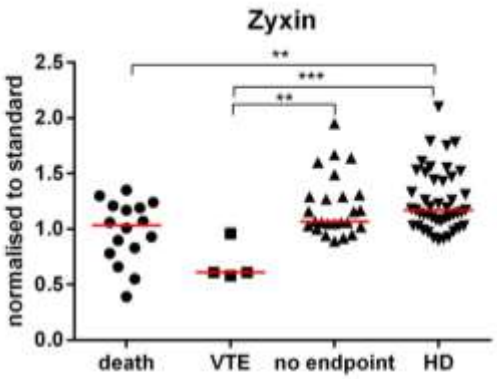

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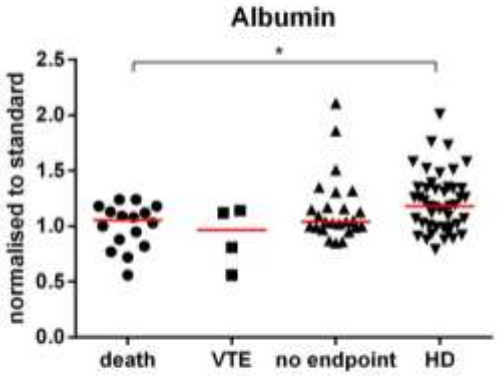

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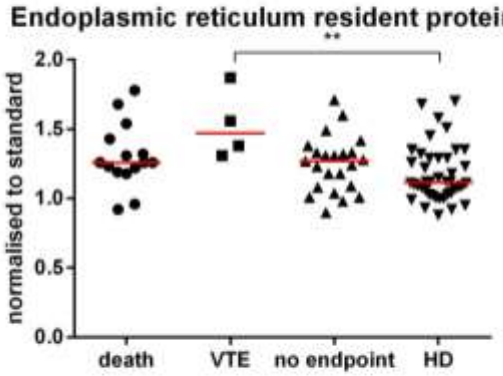

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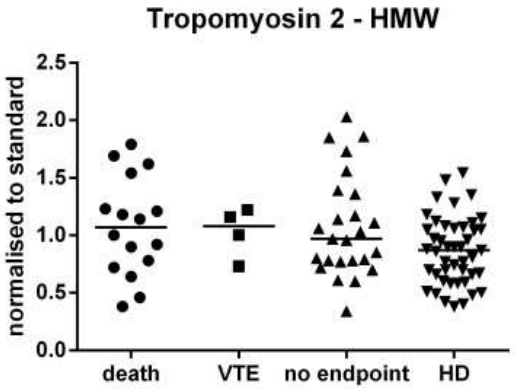

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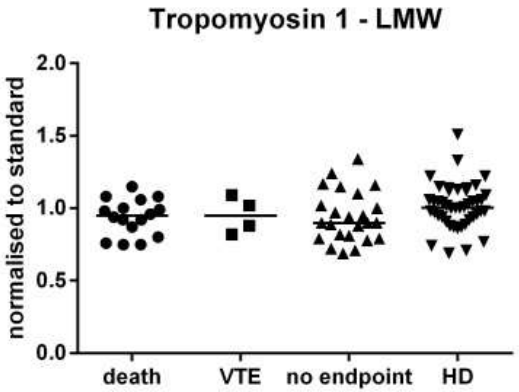
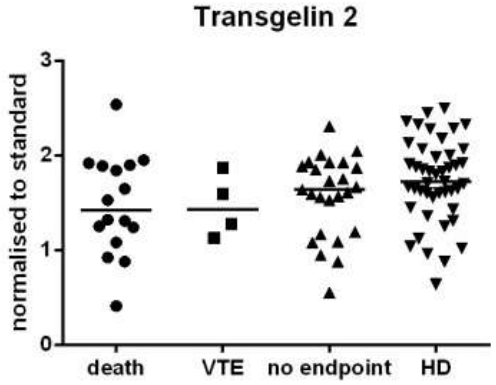
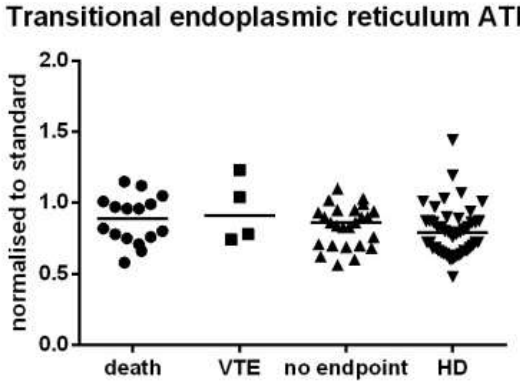


ANOVA
Bonferroni

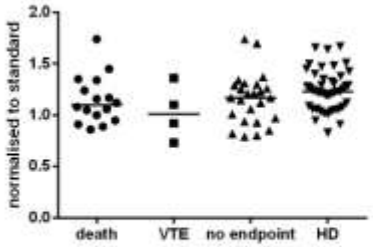


ANOVA
Bonferroni



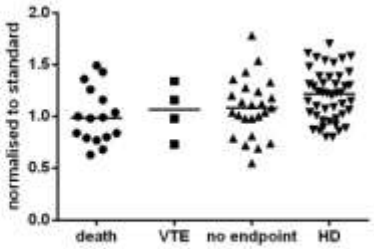
ANOVA
Bonferroni

Eukaryotic translation initiation factor 2 subunit 1



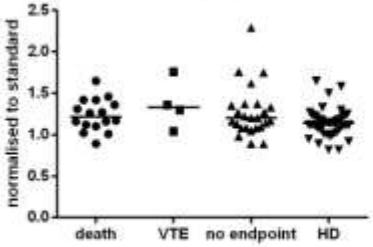
ANOVA
Bonferroni

Gamma enolase



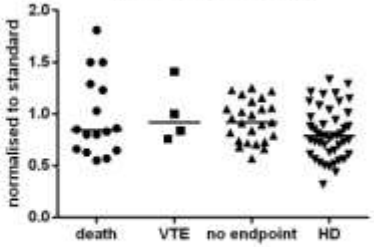
ANOVA
Bonferroni

GRP78



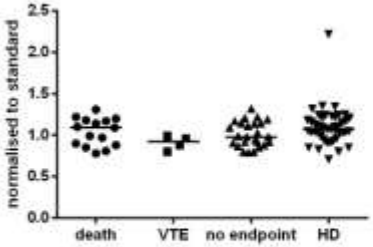
ANOVA
Bonferroni

Fibrinogen beta chain



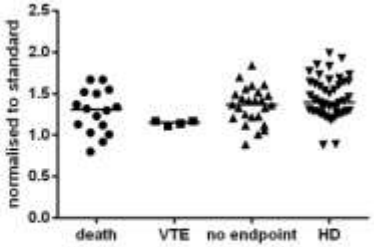
ANOVA
Bonferroni

Integrin alpha 6



ANOVA
Bonferroni

Leukocyte elastase inhibitor



ANOVA
Bonferroni

Master NR	name	uniprot	Median CATS vs HD	P value CATS vs HD	median brain	P value brain	Median lung	P value lung	Median pancreas	P value pancreas
	Integrin alpha-IIb		0,92	0,012	0,95	1	0,9	0,084	0,86	1
	Calreticulin	P27797	1,1	0,021	1,07	1	1,11	0,015	1,05	1
1798	Nucleosome assembly	P55209	0,89	0,032	0,9	0,063	0,89	0,015	0,88	0,975
1952	Fibrinogen beta chain	P02675	1,14	0,085	1,13	1	1,19	0,036	1,16	1
3906	Transgelin-2	P37802	0,93	0,085	1	1	0,77	0,015	0,99	1