

Appendix

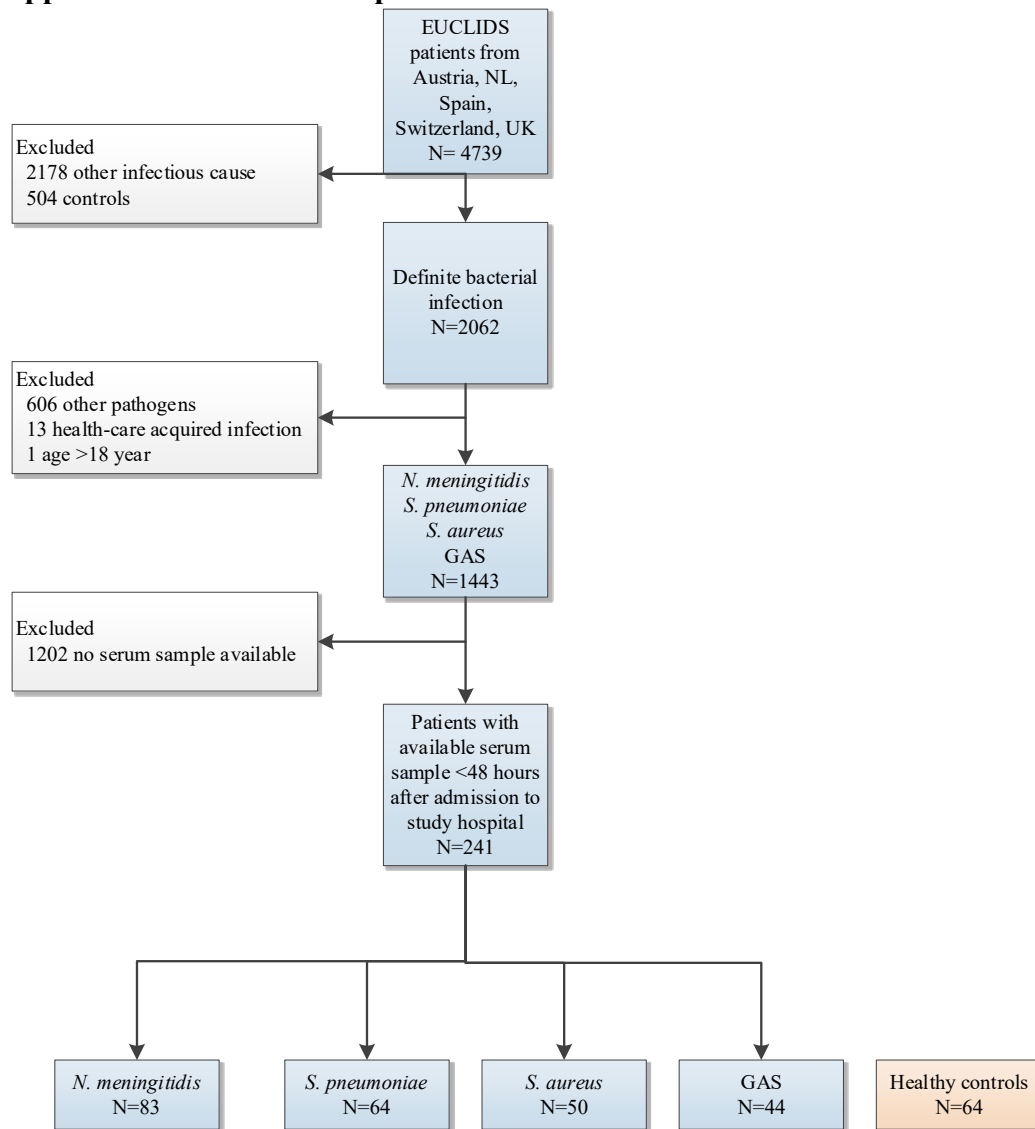
Appendix 1 - Overview of coagulation proteins and their relation with paediatric sepsis and childhood mortality in literature

	Paediatric sepsis*	Non-survivors^	References
Common pathway			
Fibronectin	↓	↓	[1, 2]
Contact activation (intrinsic) pathway			
Factor IX	No data [±]	No data	[3]
Protein C pathway			
Protein C	↓	↓	[4-6]
Protein S	↓	↓	[4, 5]
Thrombomodulin	↑	↑	[4, 7, 8]
Platelet adhesion			
ADAMTS-13	↓	↓	[9, 10]
vWF	↑	↑	[10]
Fibrinolysis			
PAI-1	↑	↑	[11-13]

*Compared to controls; ^Compared to survivors; [±] Only literature in adults

ADAMTS-13, a disintegrin and metalloprotease with thrombospondin type-1 motives; PAI-1, plasminogen activator inhibitor type 1; vWF, von Willebrand Factor

Appendix 2 – Flowchart of patient selection



EUCLIDS, European Union Childhood Life-threatening Infectious Disease study; GAS, group A streptococcus; NL, the Netherlands; UK, United Kingdom

Appendix 3 – Descriptive characteristics healthy controls vs invasive bacterial infections

	Invasive bacterial infections	Healthy controls	
	n=241	n=64	P-value
Female	110 (45.6)	30 (46.9)	0.97
Age in years, median [IQR]	3.3 (1.3-9.2)	5.4 (3.1-12.3)	<0.01
Ethnicity*			0.22
European	185 (76.8)	47 (73.4)	
Asian	13 (5.4)	4 (6.3)	
African	12 (4.9)	8 (12.5)	
Other	20 (8.3)	4 (1.7)	

*Available data: Invasive bacterial infections n=230, healthy controls n=63

IQR, interquartile range

Appendix 4 – Levels of haemostasis proteins

Table Appendix 4A – Invasive bacterial infections vs healthy controls

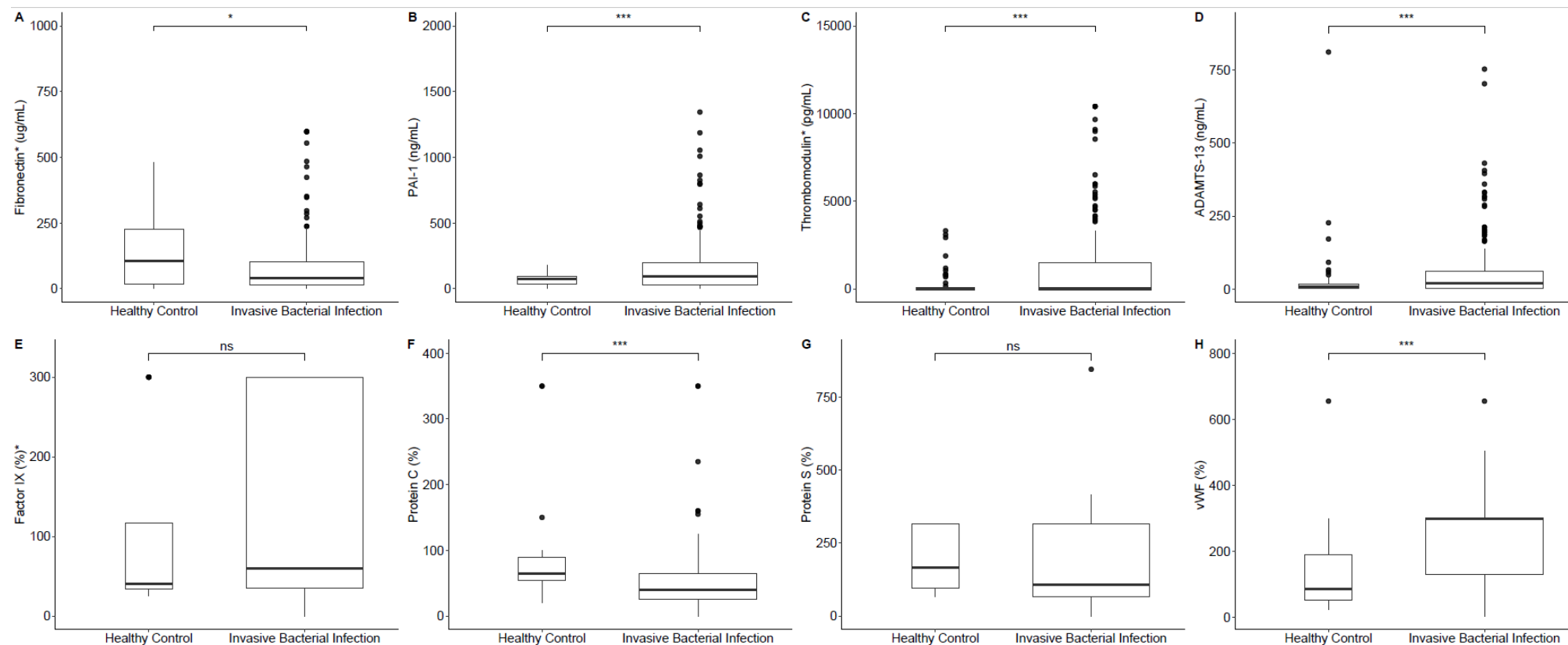
	Invasive bacterial infections	Healthy controls	P-value §
	n=241	n=64	
Serum	median (IQR)	median (IQR)	
Fibronectin (ug/ml)	41 (15 - 103)	106 (19-228)	0.02
PAI-1 (ng/ml)	96 (30 - 197)	77 (34-97)	0.00
Thrombomodulin (pg/ml)	10 (10 - 1490)*	10 (10-10)	0.00
ADAMTS13 (ng/ml)	21 (2-61)^	7 (2-17)	0.00
Citrate plasma	n=122	n=20	
Factor IX (%)	60 (35 - 300)	40 (34-116)	0.36
Protein C (%)	40 (25 -65)	65 (54-89)	0.00
Protein S (%)	108 (65-315)	165 (95-315)	0.08
vWF (%)	300 (131 - 300)	88 (54-190)	0.00

*n=239, ^n=240.

§ Adjusted for multiple testing.

ADAMTS-13, a disintegrin and metalloprotease with thrombospondin type-1 motives; IQR, interquartile range; PAI-1, plasminogen activator inhibitor type 1; vWF, von Willebrand Factor

Figure Appendix 4A – Haemostasis protein levels in controls and patients with invasive bacterial infections



A, Fibronectin; B, PAI-1; C, Thrombomodulin; D, ADAMTS-13; E, Factor IX; F, Protein C; G, Protein S; H, vWF

*Extreme values for Fibronectin, thrombomodulin and Factor IX were truncated to the 99th centile.

ns; not significant. **p-value \leq 0.05, ***p-value \leq 0.001

p-values are adjusted for multiple testing.

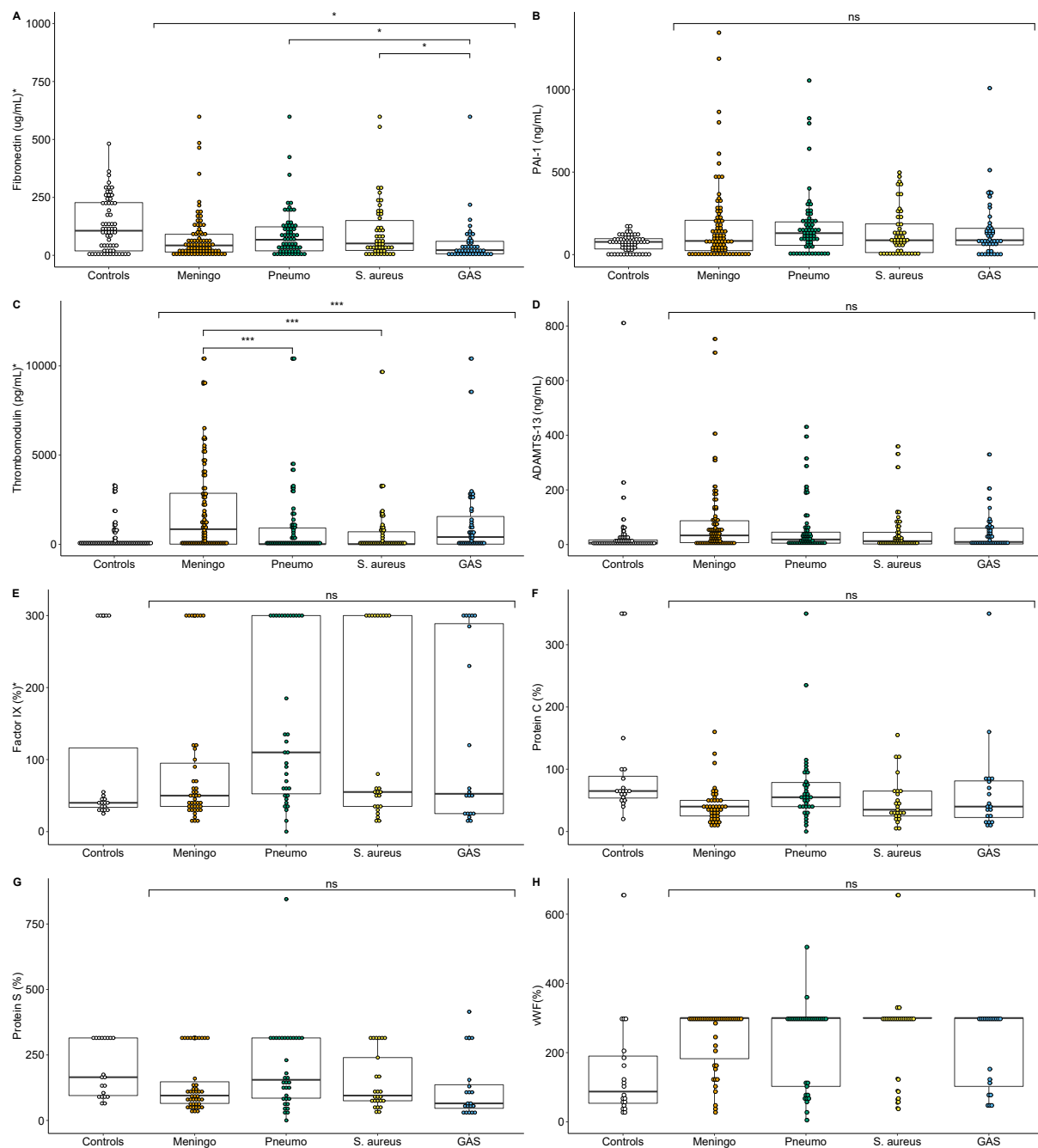
Table Appendix 4C – No invasive ventilation/inotropes vs need for invasive ventilation or inotropes

	No invasive ventilation/inotropes n=118	Need for invasive ventilation or inotropes n=123	P-value*
Serum	median (IQR)	median (IQR)	
Fibronectin (ug/ml)	59 (26-131)	28 (9-74)	0.00
PAI-1 (ng/ml)	110 (22-190)	86 (35-198)	0.65
Thrombomodulin (pg/ml)	10 (10-729)	640 (10-2641)	0.00
ADAMTS13 (ng/ml)	16 (2-60)	27 (4-64)	0.44
Citrate plasma	n=49	n=73	
Factor IX (%)	110 (50-300)	50 (30-115)	0.01
Protein C (%)	55 (35-80)	35 (25-55)	0.01
Protein S (%)	160 (75-315)	90 (60-135)	0.03
vWF (%)	300 (85-300)	300 (245-300)	0.06

*Adjusted for multiple testing

ADAMTS-13, a disintegrin and metalloprotease with thrombospondin type-1 motives; IQR, interquartile range; PAI-1, plasminogen activator inhibitor type 1; vWF, von Willebrand Factor

Appendix 5 - Haemostasis protein levels in controls and different pathogens



Legend:

A, Fibronectin; B, PAI-1; C, Thrombomodulin; D, ADAMTS-13; E, Factor IX; F, Protein C;

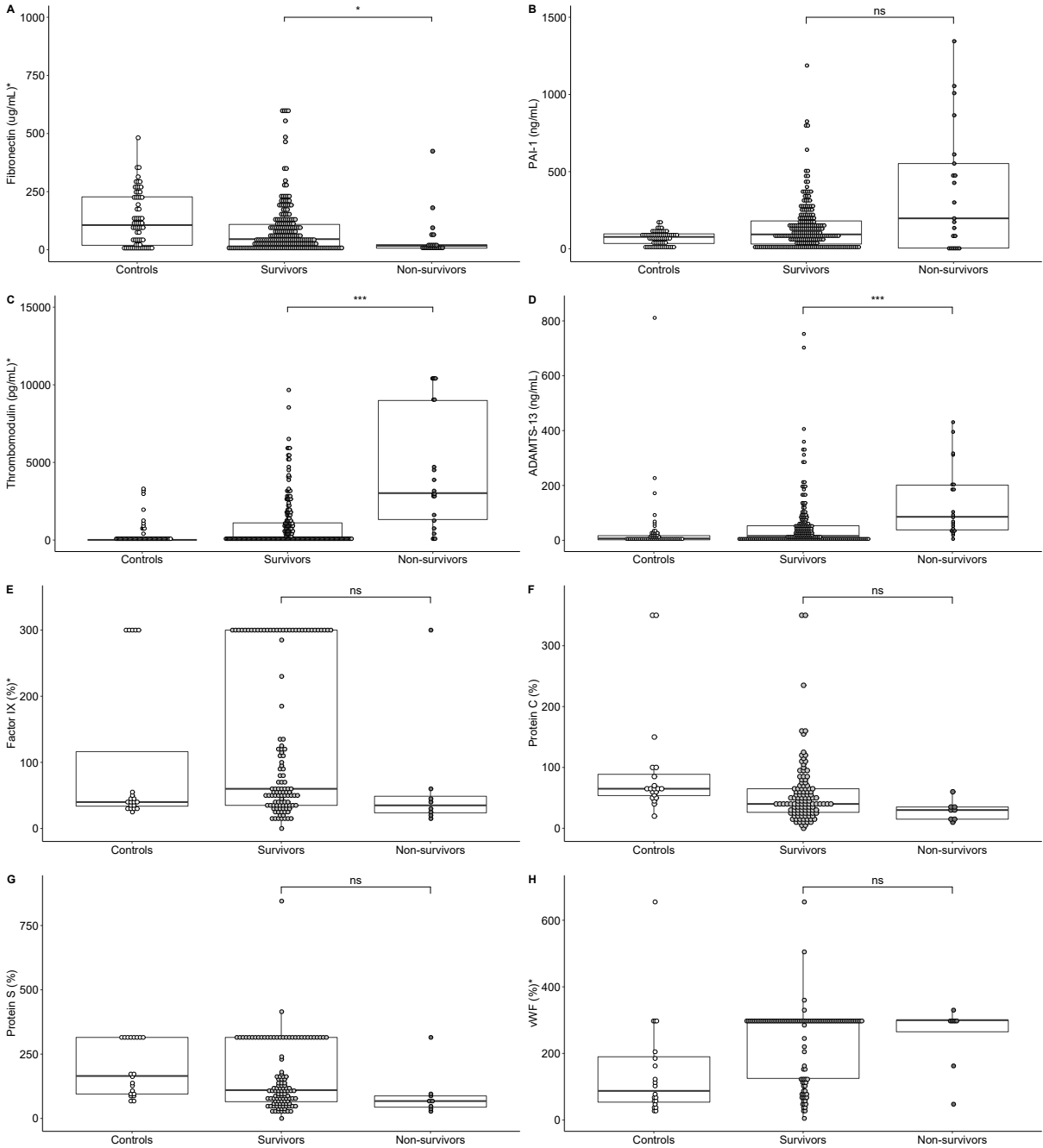
G, Protein S; H, vWF

*Extreme values for Fibronectin, thrombomodulin and Factor IX were truncated to the 99th

centile. ns, not significant, *p-value \leq 0.05, **p-value \leq 0.01, ***p-value \leq 0.001

p-values are adjusted for multiple testing.

Appendix 6 - Haemostasis protein levels in controls, survivors and non-survivors



A, Fibronectin; B, PAI-1; C, Thrombomodulin; D, ADAMTS-13; E, Factor IX; F, Protein C; G, Protein S; H, vWF

*Extreme values for Fibronectin, thrombomodulin and Factor IX were truncated to the 99th centile. ns, not significant, *p-value≤0.05, **p-value≤0.01, ***p-value≤0.001. p-values are adjusted for multiple testing.

Appendix 7 - Correlation of coagulation proteins with PIM2 score in all invasive bacterial infections (n=241)

	PIM2 score	
	Spearman's rho	P-value*
Fibronectin	-0.25	0.00
PAI-1	-0.01	0.94
Thrombomodulin	0.2	0.02
ADAMTS-13	0.13	0.15
Factor IX	-0.24	0.04
Protein C	-0.25	0.03
Protein S	-0.29	0.01
vWF	0.14	0.25

*P-values adjusted for multiple testing

ADAMTS-13, a disintegrin and metalloprotease with thrombospondin type-1 motives; IQR, interquartile range; PAI-1, plasminogen activator inhibitor type 1; vWF, von Willebrand Factor

Based on the Spearman's rho, significant correlations were classified as very weak (0-0.19), weak (0.2-0.39), moderate (0.4-0.59), strong (0.6-0.79) and very strong (0.8-1).[14]

Appendix 8 – Characteristics of patients with available citrate plasma (n=122) and patients without available citrate plasma (n=110)

	All invasive bacterial infections n=241	Citrate plasma available n=122	Citrate plasma not available n=110	P-value
Severity of illness				
Admitted to PICU	177 (73.4)	103 (84.8)	74 (62.2)	<0.001
Need for invasive ventilation or inotropes		73 (59.8)	50 (42.0)	<0.01
PIM2 score (%), median [IQR]		3.5 (1.0-10.9)	3.5 (0.8-11.7)	p=0.67
Death	21 (8.7)	8 (6.6)	13 (10.9)	0.33
Pathogen				
<i>Neisseria meningitidis</i>	83 (34.4)	43 (35.2)	40 (33.6)	p=0.88
<i>Streptococcus pneumoniae</i>	64 (26.6)	34 (27.9)	30 (25.2)	
<i>Staphylococcus aureus</i>	50 (20.7)	25 (20.5)	25 (21.0)	
<i>Group A streptococcus</i>	44 (18.3)	20 (16.4)	24 (20.2)	

IQR, interquartile range; PICU, paediatric intensive care unit; PIM, paediatric index of mortality

References

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