Generate Knowledge

COVID-19: Pruebas de coagulación especial

Dra. Ana Marco Rico
Hematología y Hemoterapia
Hospital General Universitario de Alicante

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Introducción (1)

- La COVID19 es una infección vírica asociada a un síndrome de fallo respiratorio con neumonía
- Mayor riesgo de trombosis arterial y venosa
- Estudios necrópsicos
  - Microtrombos en la vasculatura pulmonar
  - Lesión endotelial
  - Componente inflamatorio

Wu C et al. JAMA 2020; 180:934-943

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Introducción (2)

- Los pacientes con evolución más grave
  - Perfil de activación de generación de trombina elevado
  - Aumento de dímero D e hiperfibrinogenemia
- Estado protrombótico global
  - Estado proinflamatorio
  - Lesión vascular y endotelial

Rodelo JR et al. Am J Emerg Med 2020; 30:1991-1999

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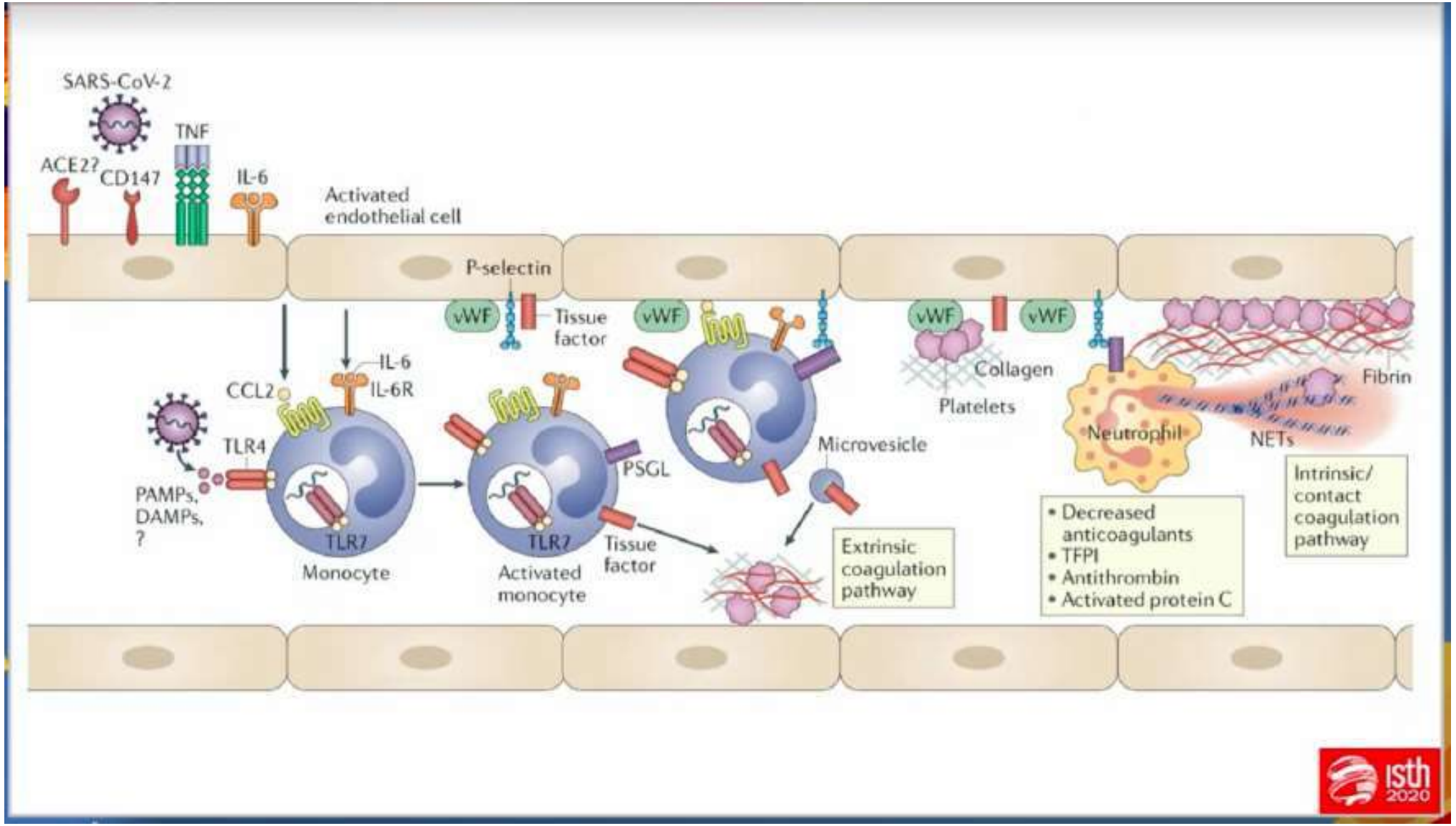

Zuo et al. JCI Insight 2020; 5; e140971

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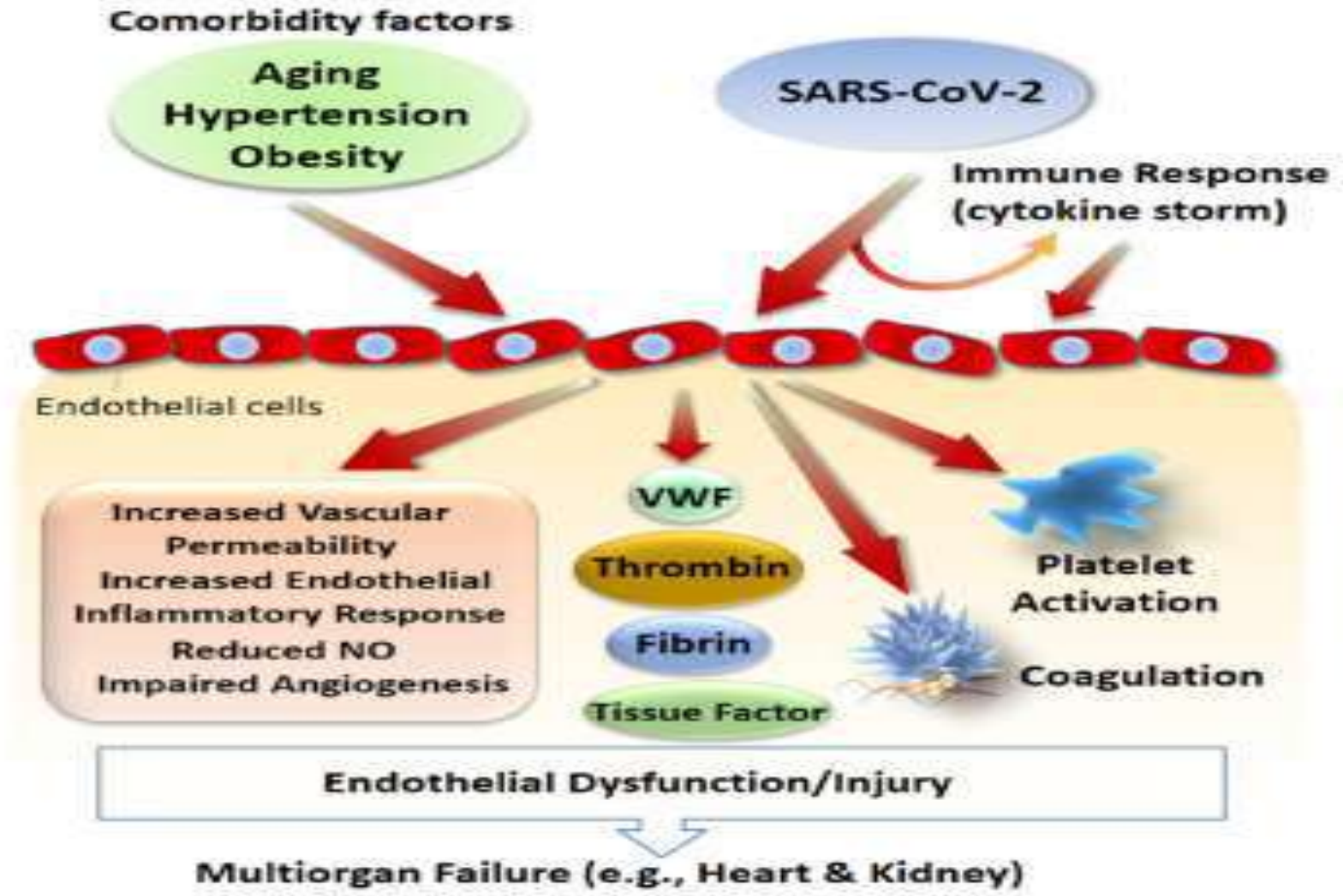

Amraei R et al. Cells 2020; DOI:10.3390/cells.9071652

Severe COVID-19 resembles...

- Disseminated intravascular coagulation (DIC)
- Thrombotic mycroangiopathy (TMA)
- Secondary haemophagocytic lymphohistiocytosis (sHLH)
- Sepsis
- Cytokine storm

Peyvandi et al, submitted

Estudios que evalúan ADAMTS13 y FVW en COVID-19

Study	Patient group (number of patients) comparison	Findings/Significance
Bazzan et al[104]	Nonsurvivor (9) vs. survivor (79)	Lower ADAMTS-13 and elevated VWF levels in nonsurvivors compared with survivors. After survival analysis, lower than 30% ADAMTS-13 levels were significantly associated with higher mortality
Huisman et al[105]	Relative to reference range (12)	Lower ADAMTS-13 and elevated VWF levels
Adam et al[106]	Relative to reference range (4)	Lower ADAMTS-13 and elevated VWF levels
Latimer et al[107]	Relative to reference range (1 pediatric patient)	Lower ADAMTS-13 and elevated VWF levels
Escher et al[108,109]	Case study, 1 patient and 3 more in the follow-up publication	Massive elevation of VWF and normal to lower-normal ADAMTS-13 activity. COVID-19 coagulopathy may be a distinct entity of highly prothrombotic alterations most probably an endothelial disease
Helms et al[7]	Relative to reference range (150)	Elevated VWF levels

Mancini et al.	Cross-sectional study. 3 different intensity of care units (50).	Lower ADAMTS-13 and elevated VWF levels

Katneni UK et al. TH 2020; 120:1668-1679

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Estos marcadores de hemostasia

ADAMTS13

- Síntesis endotelial y hepática
- Descenso leve/moderado en COVID-19
- Descenso severo en PTT
- A más descenso: mayor respuesta inflamatoria y daño endotelial
- ADAMTS13<30%: predice mortalidad en COVID-19 (Bazzan et al)

FACTOR VON WILLEBRAND

- Reactante de fase aguda
- Síntesis endotelial y plaquetar
- Aumento significativo en COVID-19
- Aumento IL-6 impide rotura de multímeros de alto peso molecular por ADAMTS13
- Plaquetas normales o leve descenso en COVID-19

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TABLE 1 Haemostatic investigations in the study with a brief overview and rationale

Measurand	Role	Rationale
Tissue factor pathway inhibitor	Inhibits factor VIIa and factor Xa, usually found on endothelial surface and platelets	To investigate for endothelial abnormality and abnormal physiological coagulation inhibition
Plasminogen activator inhibitor-1	Inhibits tissue plasminogen activator	To investigate for disturbed fibrinolysis
Tissue plasminogen activator	Activates plasminogen to plasmin	To investigate for disturbed fibrinolysis
Vascular endothelial growth factor	Angiogenic factor	To investigate for endothelial abnormality
Thrombin-antithrombin complexes	Formed in response to increased thrombin levels	To investigate for increased thrombin in plasma indicating activation of coagulation
Soluble thrombomodulin	Found on endothelium and a key part of the protein C pathway	To investigate for endothelial abnormality and abnormal physiological coagulation inhibition
Prothrombin fragment 1 + 2	Produced by activation of prothrombin to thrombin	A marker of thrombin generation
Protein C activity	Usually acts to inactivate factor V and factor VIII, after activation by the thrombin-thrombomodulin complex	Levels may be decreased in sepsis
Free protein S antigen	Co-factor for protein C	Levels may be decreased in sepsis

White D et al. Int J Lab Hematol 2020; 00:1-8

Evaluation of COVID-19 coagulopathy; laboratory characterization using thrombin generation and nonconventional haemostasis assays

Danielle White | Stephen MacDonald | Tara Edwards | Chris Bridgeman | Megan Hayman | Megan Sharp | Sally Cox-Morton | Emily Duff | Swati Mahajan | Chloe Moore | Melissa Kirk | Richard Williams | Martin Besser | Will Thomas

n=34 n=75

Measurand	Noncritical	Critical	P
Tissue factor pathway inhibitor, ng/mL, median (IQR)	1.50 (1.13-1.85)	1.95 (1.45-3.10)	<.05
Plasminogen activator inhibitor-1, ng/mL, median (IQR)	14.11 (9.64-21.40)	14.48 (7.44-15.04)	.99
Tissue plasminogen activator, ng/mL, median (IQR)	7.60 (4.71-12.10)	11.36 (7.44-15.04)	<.05
Vascular endothelial growth factor, ng/mL, median (IQR)	0.101 (0.063-0.166)	0.15 (0.098-0.221)	<.05
Thrombin-antithrombin complexes, ng/mL, median (IQR)	9.20 (6.70-11.99)	7.52 (5.57-9.75)	.13
Thrombomodulin, ng/mL, median (IQR)	3.01 (1.64-5.53)	3.13 (2.09-5.64)	.34
Prothrombin fragment 1 + 2, ng/mL, median (IQR)	1.55 (1.33-2.23)	1.53 (0.91-2.53)	.86
Protein C activity, IU/dL, median (IQR)	95 (87-114)	100 (86-120)	.90
Free protein S antigen, IU/dL, median (IQR)	52.1 (42.4-61.8)	54.5 (45.3-69.6)	.29

Abbreviation: IQR, inter-quartile range.

Platelets, x10x9/L, mean (SD)	221 (87)	288 (138)	<.05
PT, seconds, median (IQR)	13.3 (12.53-14.45)	13.6 (12.75-15.00)[a]	.25
APTT, seconds, mean (SD)	32.6 (3.8)	33.7 (5.0)[b]	.50
Fibrinogen, g/L, mean (SD)	4.33 (1.28)	6.42 (2.01)	<.05
D-dimer, ng/mL, median (IQR) Missing data, n	323 (158-600) 0	555 (297-2197) 1	<.05
Antithrombin, IU/dL, mean (SD)	94.6 (21.2)	94.5 (21.2)	.76
C-Reactive Protein, mg/L (CI)	60.6 (18)	177.3 (23.8)	<.05
Interleukin-6, pg/mL (CI)	18.3 (9.7)	74.2 (42.5)	<.05

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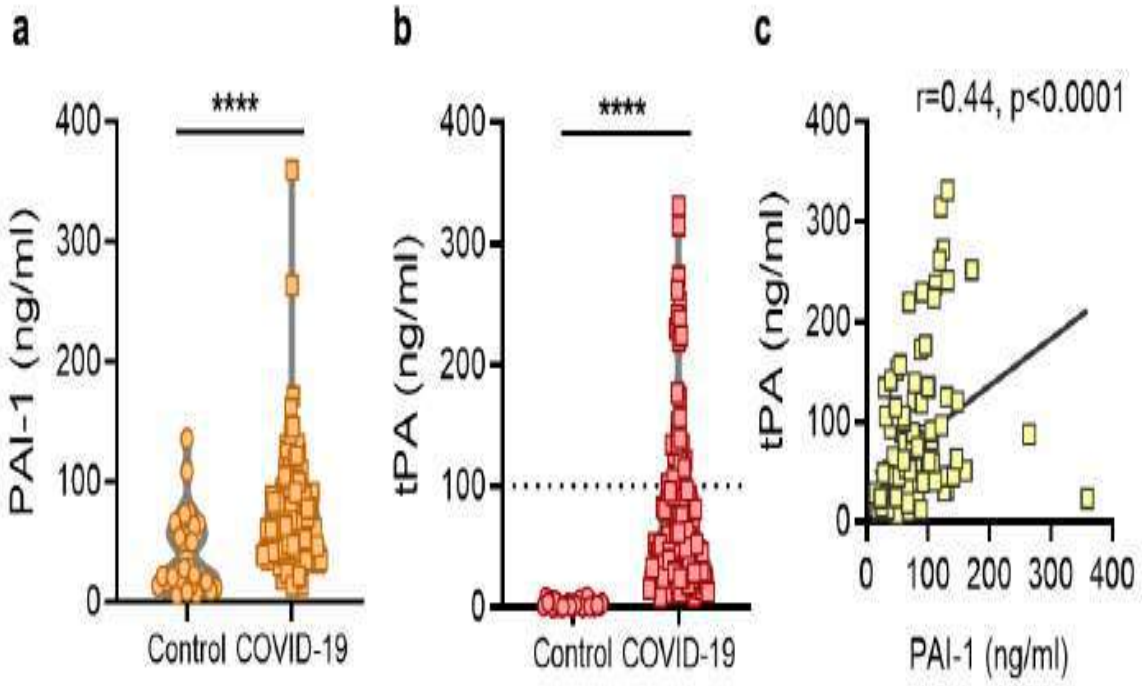
White D et al. IJLH 2020; 00:1-8

Conclusion: These results confirm increased fibrinogen and D-dimer in critical COVID-19-infected patients. Importantly, disease severity did not increase thrombin generation (including thrombin-antithrombin complexes and prothrombin fragment 1 + 2) when comparing both cohorts; counter-intuitively critical patients were hypo-coaguable. tPA, TFPI and VEGF were increased in critical patients, which are hypothesized to reflect endothelial dysfunction and/or contribution of heparin (which may cause endothelial TFPI/tPA release).

White D et al. IJLH 2020; 00:1-8

Plasma tissue plasminogen activator and plasminogen activator inhibitor-1 in hospitalized COVID-19 patients



- 118 pacientes ingresados por COVID-19 y 30 controles sanos
- Aumento de tPA y PAI-1
  - -Disfunción endotelial
  - -Asociados a peor estatus respiratorio
- Aumento de tPA: mayor mortalidad
- Fibrinolisis espontánea

Zuo et al. MedRxiv 2020; DOI: 10.1101/2020.08.29.20184358

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COVID-19 y pruebas globales de la hemostasia

Hypercoagulability of COVID-19 patients in intensive care unit: A report of thromboelastography findings and other parameters of hemostasis

Mauro Panigada[1] | Nicola Bottino[1] | Paola Tagliabue[1] | Giacomo Grasselli[2] | Cristina Novembrino[3] | Veena Chantarangkul[3] | Antonio Pesenti[1,2] | Flora Peyvandi[2,3] | Armando Tripodi[3]

JTH 2020; 18: 1738-1742

24 pacientes de UCI. TEG y TGT
⇨hipercoagulabilidad

In vitro hypercoagulability and ongoing *in vivo* activation of coagulation and fibrinolysis in COVID-19 patients on anticoagulation

Annabel Blasi*, Fien A. von Meijenfeldt†, Jelle Adelmeijer†, Andrea Calvo*, Cristina Ibañez*, Juan Perdomo*, Juan Carlos Reverter‡, Ton Lisman†

JTH 2020; 18:2646-2653

23 pacientes COVID-19.

Ongoing in vivo activation of coagulation and fibrinolysis despite low-therapeutic anticoagulation in COVID-19 patients

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Hipótesis

- Estado protrombótico en pacientes con COVID19 a pesar de tromboprofilaxis
- Coagulopatía con datos sugerentes de PTT
- Evaluar papel del complejo FVW antigénico/ADAMTS13

Objetivos

- **Principal**
  - Estudiar el complejo FvW antigénico/ADAMTS13 como marcador de lesión endotelial y de hipercoagulabilidad en pacientes con COVID19
- **Secundario**
  - Valoración de gravedad clínica y control evolutivo del complejo FvW antigénico/ADAMTS13 en pacientes con infección por COVID19

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Material y Métodos (1)

- Estudio prospectivo
- Pacientes diagnosticados por PCR de COVID19 en el HGUA
- Meses de Abril y Mayo de 2020
- 2 grupos de pacientes según precisasen o no ingreso hospitalario
- Marcadores de hemostasia:
  - Estudio básico de hemostasia
  - Dímero D
  - Fibrinógeno
  - Actividad de ADAMTS13
  - FvW antigénico

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Material y Métodos (2)

- Análisis estadístico
- Variables cuantitativas se expresaron como mediana (p25-p75)
- Variables cualitativas en %
- T de student para comparación de medias de los parámetros de hemostasia en ambos grupos
- $p<0,05$ se consideró estadísticamente significativo

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Características de los pacientes en la inclusión

	Inpatients (n=50)	Outpatients (n=102)	p-value
Edad (años)	68.39 (61.43–79.05)	60.95 (48.30–69.03)	<0.05
Hombres n (%)	34 (68)	60 (59)	0.12
Hipertension	31 (62)	36 (35)	<0.05
Diabetes mellitus	16 (32)	17 (16)	<0.05

Profilaxis con HBPM

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Parámetros de hemostasia INGRESADOS/AMBULANTES

Parámetro	Ingresados (n=50)	Ambulantes (n=102)	P valor
Dímero D (µg/mL)	2.48 (0.88–6.86)	0.4 (0.27–0.56)	<0.05
Fibrinógeno (mg/dL)	511 (395–568)	346.5 (291–374)	<0.05
ADAMTS13 actividad (%)	44.4 (32.5–60.8)	59.9 (43.4–78.75)	<0.05
VWF antigénico (%)	337.8 (270.0–394.9)	121.6 (95.75–151.95)	<0.05
APTT ratio	1.01 (0.95–1.11)	0.95 (0.87–1.02)	0.06
INR	1.13 (1.08–1.29)	1.01 (1.0–1.06)	<0.05
Plaquetas (10^9/L)	220 (160–262)	215 (178–262)	0.52

Parámetros de hemostasia PLANTA/UCI

Parámetro	Planta (n=28)	UCI (n=22)	P valor
Dímero D (µg/mL)	0.89 (0.73–2.31)	4.64 (2.66–11.04)	<0.05
Fibrinógeno (mg/dL)	511 (383–561)	505 (400–576)	0.94
ADAMTS13 actividad(%)	46.5 (40.4–60.9)	38.85 (26–60)	<0.05
VWF antigénico (%)	279.95 (217.15–345.15)	368.6 (336.3–400)	<0.05
APTT ratio	1.07 (0.97–1.11)	0.98 (0.93–1.12)	0.14
INR	1.16 (1.08–1.32)	1.1 (1.06–1.26)	0.29
Plaquetas (10^9/L)	204 (132–274)	228 (166–250)	0.87

Correlación y significación estadística de los parámetros hemostáticos

	D-dimer (µg/mL)	Fibrinogen (mg/dL)	ADAMTS13 activity (%)	VWF antigen (%)	Platelets	APTT R
Fibrinogen (mg/dl)	r=0.14 p<0.05					
ADAMTS13 activity (%)	r=-0.19 p<0.05	r=-0.28 p<0.05				
VWF antigen (%)	r=0.55 p<0.05	r=0.54 p<0.05	r=-0.32 p<0.05			
Platelets	r=-0.01 p=0.945	r=0.01 p=0.875	r=0.20 p<0.05	r=-0.25 p<0.05		
APTT R	r=0.01 p=0.95	r=0.35 p<0.05	r= -0.11 p=0.17	r=0.16 p=0.06	r=-0.05 p=0.49	
INR	r=-0.20	r=-0.23	r=0.14	r=-0.35	r=0.04	r=-0.39

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Conclusiones del estudio

- Disbalance entre FVW antigénico/ADAMTS13 actividad en COVID-19
- La disfunción endotelial y la hipercoagulabilidad persisten a pesar de la tromboprofilaxis
- A mayor ratio de FVW antigénico/ADAMTS13 actividad: peor pronóstico

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Proyectos futuros en nuestro laboratorio

- Valoración del estado de hipercoagulabilidad con test globales de hemostasia: test de generación de trombina y tromboelastograma
- Cómo se modifican otros marcadores de lesión endotelial y de fibrinolisis
- Colaboración con distintos centros
- Contacto: marco_anaric@gva.es

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Take-home messages

- **Respuesta inflamatoria**
- **Lesión endotelial**
  - Aumento de multímeros de alto peso molecular de FVW
  - Inhibición de actividad de ADAMTS13
  - Aumento de PAI, tPA, TFPI
  - Gravedad de respuesta inflamatoria y lesión endotelial
  - Pacientes UCI>Pacientes de planta>Pacientes ambulantes
- **Estado de hipercoagulabilidad y lesión endotelial existen a pesar de tromboprofilaxis**

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