A validated clinical-genetic score for assessing the risk of thrombosis in patients with COVID-19 receiving thromboprophylaxis

Venous thromboembolic events (VTE) have emerged as a common complication among patients hospitalized for COVID-19 with an estimated incidence of 14-31%, increasing disease severity and mortality.[1] The incidence is even higher in critically ill patients admitted to intensive care units (ICU),[1,2] including those provided thromboprophylaxis at the moment of hospital admission.[2] Therefore, the effectiveness of anticoagulant prophylaxis is actually unclear due to no significant reduction in thrombotic complications despite prophylactic therapy.[2,3] These studies, however, face a major limitation: the lack of tools for assessing the risk of VTE. Many variables affect the appearance of a VTE, both clinical and genetic.[4] With this in mind, the present work examines whether the Thrombo inCode (TiC) score, which combines genetic and clinical risk variables and has shown the capacity to predict VTE in different populations,[5,6,7] is of use in predicting VTE in patients with COVID-19 who were administered prophylactic anticoagulation therapy at the time of hospital admission.

The PRECIS_COVID19 cohort consists of 734 patients; all aged over 18 years, with a confirmed diagnosis of COVID-19, all of whom were admitted to the *Hospital de la Santa Creu i Sant Pau* (Barcelona, Spain) between April and July 2020. COVID-19 was confirmed by real-time reverse-transcription polymerase chain reaction (PCR) assays using nasal and pharyngeal swabs. All patients were administered standard thromboprophylactic treatment at the time of their admission to hospital following international recommendations.[8]

A total of 279 patients had a D-Dimer value below 1,000 ng/mL (validated threshold to rule out VTE)[9] and were thus considered as non-VTE (control) patients. Of the remainder, 382 patients had D-Dimer levels of >1,000 ng/mL and were excluded from further analyses, and 73 suffered a VTE event during hospitalization (either a deep venous thrombosis or a pulmonary embolism). Diagnoses were confirmed using Doppler ultrasonography, magnetic resonance, arteriography, phlebography, pulmonary gammagraphy and computed tomography pulmonary angiography. The total number of subjects in the study was therefore n=352 (279 controls plus 73 cases). A total of five models were therefore compared, the details of which are described below:

1. Genetic risk score (GRS)

The twelve genetic variants reported by Soria et *al.*[5], including the variants rs6025, rs118203905, rs118203906, rs1799963, rs121909548 (chr1:173873176:C:A, *SERPINC1*), rs1801020 (chr5:176836532:A:G, *F12*), rs5985 (chr6:6318795:C:A, *F13*), rs2232698 (chr14:94756669:G:A, *SERPINE10*) and four variants providing the ABO:A1 haplotype (additive effect of A1 allele).

2. Clinical risk score (CRS)

Five clinical variables were assessed: age, sex, obesity, smoking habit, and diabetes. These variables have been shown to be associated with VTE and have been reported to be useful in estimating VTE risk.[7] Smoking habit was codified as a dichotomic variable (smoker/non-smoker); obesity was defined as body mass index >30.

3. Thrombo inCode model (TiC)

A combination of the variables in both the genetic risk score (GRS) and the clinical risk score (CRS) described in the models 1 and 2. The original Thrombo inCode (TiC) model also includes family history, oral contraceptive use and pregnancy, but were not evaluated, as they were difficult to obtain in the COVID19 context.

4. Factor V Leiden plus prothrombin score

The classic genetic thrombophilia model based on the Factor V Leiden (FVL) (rs6025; chr1:169519049:T:C, *F5*) and prothrombin (PT) G20210A (rs1799963; chr11:46761055:G:A, *F2*) mutations.

5. Factor V Leiden plus prothrombin plus clinical risk score

Combination of the variables in the FVL+PT and the CRS models explained before.

A descriptive analysis of both the genetic and clinical variables was performed, and the relationship with VTE assessed by the Chi-squared test for bivariate associations. The same method was used to evaluate the association between ICU admission and mortality rates (at 30 and 90 days after hospital admission), as well as the association between VTE and mortality rates and ICU admission. Significance was set at $P<0.05$.

All risk models were constructed by including the corresponding variables as additive linear predictors of VTE using logistic regression. The predictive capacity of the different models was examined using receiver operating curves (ROC), employing the optimal cut-off based on the Youden index.[11] The significance of the predictive capacity of each score was measured by comparing with a random model (area under the ROC curve [AUC] of 0.5), using the DeLong test.[13] In addition to determining test sensitivity

and specificity, the negative and positive predictive values (NPV and PPV) of each model were determined as well as the odds ratio (OR). All calculations were performed using SPSS v.26.0 software (Released 2019) (IBM Corp., Armonk, NY, USA).

Table 1 provides the descriptive analysis of the clinical and genetic variables of all 352 patients. The associations between VTE and four clinical variables (age, sex, obesity and smoking habit) and the genetic variants rs121909548 in *SERPINC1* and rs5985 in *F13* were significant ($P<0.05$). We found no carriers of the variants rs118203906 and rs118203905, corresponding to FV_Cambridge and FV_Hong Kong, respectively, in our cohort.

It is important to note that a significant association was detected between the suffering of a VTE and 30 and 90 days mortality (13.7% cases *vs.* 5% controls, $P=0.06$; 14% cases *vs.* 5.7% controls, $P<0.001$; respectively), as well as admission to an ICU (61.6% cases *vs.* 4.3% controls, $P<0.001$), highlighting the impact of VTE on the progression and severity of COVID-19. These results are in agreement with previous reports,[1,2] and reinforce the need to improve prophylactic strategies. In this context, some authors have examined predictive models that take into account clinical and laboratory variables related to VTE.[12] However, to our knowledge, and despite the strong genetic component of VTE,[4] no previous study has examined the genetic risk of COVID-19 patients suffering a VTE event.

Table 2 shows the predictive capacity of each of the five models tested. Only the FVL+PT model showed no predictive capacity at all (AUC 0.506, $P=0.86$). While these two genetic mutations imply a higher risk of VTE, their low frequency in the general population render them poor markers for predicting such events. Similar results have been reported previously.[5,7] The TiC score, in contrast, showed the best and an excellent predictive capacity, with an AUC of 0.78 (95% confidence interval [CI]: 0.72-0.84), a sensitivity of 68.5%, and a specificity of 76.7%. Patients identified as being at high risk of suffering a VTE by the TiC score had an associated OR of 7.16. The accuracy measures shown in Table 2 for this model reveal a number needed to treat (NNT) of 2.3.

It is important to note that the genetic risk variants included in the TiC score have known functional consequences on the coagulation pathway,[5] and the link between these variants and VTE has been established in different genome-wide association studies.[13] In addition, the useful predictive capacity of the GRS included in the TiC score has been validated in independent populations.[5,6,7] Furthermore, it should be noted that one of the

Table 1. Descriptive analysis of the clinical and genetic variables in the PRECIS_COVID19 cohort.

		PRECIS_COVID19 (N=352)	Patients with VTE (N=73)	Non-VTE Controls (N=279)	*P*-value
Age in years, mean (SD)		59.0 (15.0)	64.9 (11.5)	57.5 (15.4)	<0.001
Female, N (%)		158 (44.9)	25 (34.2)	133 (47.7)	0.040
Obesity, N (%)		38 (10.8)	21 (28.8)	17 (6.1)	<0.001
Smoking, N (%)		24 (6.8)	15 (20.5)	9 (3.2)	<0.001
Diabetes, N (%)		47 (13.4)	14 (19.2)	33 (11.8)	0.100
ABO:A1, N (%)	0	221 (62.8)	43 (58.9)	178 (63.8)	
	1/2	131 (37.2)	30 (41.1)	101 (36.2)	0.411
rs6025 *F5*, N (%)	0	344 (97.7)	71 (97.3)	273 (97.8)	
	1	8 (2.3)	2 (2.7)	6 (2.2)	1.000
rs1799963 *F2*, N (%)	0	349 (99.3)	72 (98.6)	277 (99.3)	
	1	3 (0.7)	1 (1.4)	2 (0.7)	1.000
rs121909548 *SERPINC1*, N (%)	0	344 (98)	68 (93.2)	276 (98.9)	
	1	8 (2.0)	5 (6.8)	3 (1.1)	0.011
rs1801020 *F12*, N (%)	0	204 (58.0)	46 (63.0)	158 (56.6)	
	1/2	148 (42.0)	27 (37.0)	121 (43.4)	0.325
rs5985 *F13*, N (%)	0	200 (56.8)	34 (46.6)	166 (59.5)	
	1/2	152 (43.2)	39 (53.4)	113 (40.5)	0.047
rs2232698 *SERPINE10*, N (%)	0	348 (98.9)	72 (98.6)	276 (98.9)	
	1	4 (1.1)	1 (1.4)	3 (1.1)	1.000

Frequency and distribution of clinical variables, and frequencies of both reference and risk alleles for the 12 genetic variants. The *P*-value (*P*) refers to the association with venous thromboembolic events (VTE) (bivariate analysis). SD: standard deviation.

Table 2. Capacity of the tested models to predict venous thromboembolic events.

	TiC	GRS	CRS	FVL+PT	FVL+PT+CRS
AUC	0.781 (0.72-0.84)	0.615 (0.54-0.69)	0.756 (0.70-0.82)	0.506 (0.43-0.58)	0.757 (0.70-0.82)
P	<0.001	0.002	<0.001	0.869	<0.001
Sensitivity	68.5 (56.6-78.9) 50/73	52.1 (40.0-63.9) 38/73	71.2 (59.4-81.2) 52/73	-	65.8 (53.7-76.5) 48/73
Specificity	76.7 (71.3-81.5) 214/279	72.0 (66.4-72.2) 201/279	62.7 (56.8-68.4) 175/279	-	74.9 (69.4-79.9) 209/279
PPV	43.5 (34.3-53.0) 50/115	32.8 (24.3-42.1) 38/116	33.3 (26.0-41.3) 52/156	-	40.7 (31.7-50.1) 48/118
NPV	90.3 (85.8-93.7) 214/237	85.2 (80.0-89.5) 201/236	89.3 (84.1-93.2) 175/196	-	89.3 (84.6-93.0) 209/234
LR+	2.94	1.87	1.91	-	2.63
LR-	0.41	0.67	0.46	-	0.46
OR	7.16 (4.06-12.61)	2.80 (1.65-4.75)	4.17 (2.38-7.31)	-	5.73 (3.29-9.79)

'*P*' refers to the significance against a random model of area under the ROC curve (AUC) of 0.5. Numbers (in parentheses) are 95% confidence intervals. TiC: thrombo inCode score; GRS: genetic risk score; CRS: clinical risk score; FVL+PT: Factor V Leiden and prothrombin mutations; FVL+PT +CRS: Factor V Leiden and prothrombin mutations plus clinical risk score; PPV: positive predictive value; NPV: negative predictive value; LR: likelihood ratio; OR: odds ratio.

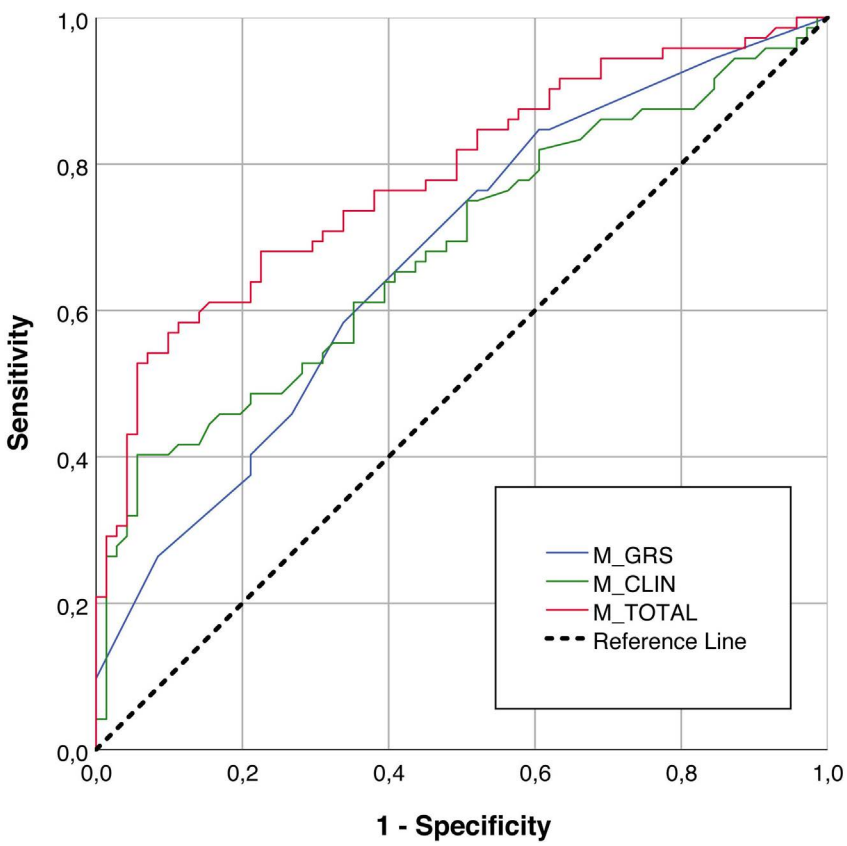

Figure 1. Predictive capacity of the different models. ROC curves are shown for each model: model with clinical variables only (M_CLIN); model with genetic variables only (M_GRS); and the combination of both models (M_TOTAL or TiC).

risk variants assessed is the A1 allele or haplotype for ABO blood type; the risk of VTE for A1 allele carriers is estimated to be increased by ~1.8 fold,[5] and has also been significantly associated with different indicators of COVID-19 severity.[14]

Certain clinical variables are associated with an increased risk of VTE, and different studies report a better capacity to predict VTE when genetic and clinical variables are combined.[8] In agreement with this, the present results show that the best predictive capacity was obtained when taking both into account - i.e., when using the TiC score (Figure 1). The results also show that the correlation between the risk estimates provided by the CRS and GRS models alone was not significant (β=0.039; *P*=0.6). These

models may, therefore, reflect different sources of risk.
The importance of thrombosis risk assessment in COVID-19 patients at hospital admission has been highlighted by a recent large observational study,[15] where they determine that there is a profoundly increased rate of VTE within the first week following positive COVID-19 testing. With an AUC of 0.78 and good predictive values, the TiC score is an excellent predictor of VTE risk at the time of hospital admission. In fact, although all patients included in this cohort were receiving prophylactic treatment, the TiC model showed that 68.5% of patients that ended up having a VTE event may have benefited from more intense prophylaxis than they received. In addition, it should also be underscored that the TiC model is easy to use; it takes into account only 12 genetic variants plus clinical variables usually included in patient's records.
The present work suffers from the limitation of a relatively small sample size (n=352). Also, the absence of VTE in the control patients was not confirmed diagnostically, although a validated D-Dimer threshold was used to identify them. Finally, all patients were admitted to the same hospital; the results need to be confirmed at other centers that treat other populations (although the TiC score has been validated for use in a number of other populations) .[5,6,7]
In conclusion, the present work shows that the TiC score is useful in identifying patients with COVID-19 at high risk of VTE. It could therefore guide clinical decisions regarding the optimal intensity of anticoagulation treatment at hospital admission. Such personalized therapy could have a substantial impact on the morbidity and mortality of patients with COVID-19.

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Contributions

JMS and JCS supervised the study, wrote the paper, obtained funds; Sergi M performed analyses; AMP performed analyses and cleaned the database; FRA analyzed and cleaned the database; SL analysed samples; Sara M and DM designed the study design and collected data; MAQ and AR collected data; AC analyzed samples and collected data; FV and MRA designed and supervised the study.

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Data-sharing statement

PRECIS_project data are deposited in a national repository and can be shared on demand and with the corresponding approval of the IIB Sant Pau Ethics Committee.

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