Thrombocytopenia and increased risk of adverse outcome in COVID-19 patients

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Abstract

Background. Thrombocytopenia was common in the coronavirus disease 2019 (COVID-19)

patients during the infection, while the role of thrombocytopenia in COVID-19 pathogenesis and

its relationship with systemic host response remained obscure. The study aimed to systematically

evaluate the relationship between thrombocytopenia in COVID-19 patients and clinical,

haematological and biochemical markers of the disease as well as adverse outcomes.

Methods. To assess the relationship between abnormal platelets level and disease progression, a

multi-center retrospective cohort study was conducted. COVID-19 patients with

thrombocytopenia and a sub-cohort of matched patients without thrombocytopenia were

compared for their clinical manifestations, haematological disorders, biochemical parameters,

inflammatory markers and clinical outcome.

Results. Thrombocytopenia was present in 127 of 2209 analyzed patients on admission.

Compared with the control group, thrombocytopenia patients developed significantly higher

frequency of respiratory failure (41.9% vs. 22.6%, P=0.020), intensive care unit entrance (25.6%

vs. 11.5%, P=0.012), disseminated intravascular coagulation (45.2% vs. 10.6%, P<0.001), more

altered platelet morphology indexes and coagulation perturbation, higher levels of inflammatory

markers. In addition, a significantly increased all-cause mortality (hazard ratio 3.08, 95%

confidence interval 2.26-4.18, P<0.001) was also observed in the patients with

thrombocytopenia. Late development of thrombocytopenia beyond 14 days post-symptom was

observed in 61 patients, from whom a comparable mortality rate yet longer duration to death was

observed in comparison with those having early thrombocytopenia.

Conclusions. Our finding from this study adds to previous evidence that thrombocytopenia is

associated with adverse outcome of the disease and recommend that platelet count and indices to

55   be included alongside other haematological, biochemical and inflammatory markers in COVID-

56   19 patients' assessment during the hospital stay.

57

Introduction

Coronavirus disease 2019 (COVID-19) caused by a novel RNA virus (SARS-CoV-2) has widely

spread to become the greatest public health challenge to date. Up to 5 August 2021, it had

affected over 200,000,000 individuals in more than 200 countries and territories worldwide

causing over 4,300,000 deaths , severely stressed health systems and the global economy. In the

majority of cases, COVID-19 is a mild illness, while some develop severe disease and a minority

of patients developed critical disease with septic shock or respiratory and/or multi-organ failure

(Wu & McGoogan 2020). Laboratory tests have been assessed with regard to their value as

prognostic indicators. Among all the laboratory indicators, the hematologic parameters have

been intensively described, especially with lymphopenia commonly established with significance

for their prognostic capacity for severe or deadly outcome after SARS-COV-2 infection (Frater

et al. 2020). Both CD4 and CD8 counts decreased during the early course and were associated

with adverse outcomes. Leukocytosis appears to herald bacterial infection or superinfection

(Lippi & Plebani 2020) and neutrophilia is an expression of the cytokine storm and

hyperinflammatory state (Chen et al. 2020; Huang et al. 2020; Mehta et al. 2020; Qin et al.

2020), both acting as important pathogenetic markers in COVID-19. Thrombocytopenia as a

common feature in viral hemorrhagic fever virus, had also been reported in respiratory virus

infection and several well-studied viruses, such as human immunodeficiency virus, influenza

virus, cytomegalovirus, Ebola virus and rhinovirus (Assinger 2014). Thrombocytopenia has been

described in other two known human coronaviruses, with a proportion of 55% in the SARS

(Frater et al. 2020), and 36% in the middle east respiratory syndrome (Assiri et al. 2013), and

recorded in the episode of emerging SARS-COV-2 infection. Although previous evidence

indicating that platelets (PLT) can influence disease outcome is abundant, clinical studies

addressing the key host responses in COVID-19 related thrombocytopenia are limited. The

82 detailed information on the relationship between PLT and other clinical outcomes/presentations

83 were absence. Here we investigated a large cohort of COVID-19 patients who were

84 consecutively observed, with the aim to systematically evaluate the relationship between

85 thrombocytopenia in COVID-19 patients and clinical, haematological and biochemical markers

86 of the disease as well as adverse outcomes.

87

88
89 **Materials & Methods**
90 **Study design and Patient enrollment**

91 A retrospective cohort study was conducted in four designated hospitals for COVID-19 in Hubei

92 province in China, including Wuhan First Hospital, Renmin Hospital of Wuhan University, and

93 Tongji Hospital, Tongji Medical College in Wuhan city, and Huangmei hospital in Huanggang

94 city. All the patients were confirmed by positive SARS-COV-2 RNA detection on

95 nasopharyngeal swab samples that were collected for real-time reverse transcriptase polymerase

96 chain reaction (RT-PCR) assay .

97 **Sample collection and Data extraction**

98 All of the electronic medical records of the hospitals were reviewed by trained medical staff,

99 from which demographic information, epidemiological history, underlying comorbidities,

100 clinical manifestations, laboratory test, CT scan, treatment history and outcome were extracted

101 and summarized by standardized EpiData data collection form. The outcome included respiratory

102 failure, intensive care unit (ICU) entrance, acute heart injury, acute kidney injury, septic shock

103 and disseminated intravascular coagulation (DIC). The plasma with 0.109 mol/L sodium citrate

104 was collected for the detection of coagulation parameters. The serum was collected for the

105 detection of cytokines. The study was approved by the Research Ethics Commission of Tongji

106   Hospital, Tongji Medical College (TJ-IRB20200102), and the requirement for informed consent

107   was waived by the Ethics Commission.

108   Definition

109   Thrombocytopenia was defined as PLT $<100\times10^9$/L and non-thrombocytopenia was defined

110   as the PLT $\geq100\times10^9$/L which were evaluated on admission. Respiratory failure was defined as

111   arterial oxygen partial pressure < 60 mmHg with or without carbon dioxide partial pressure $>$

112   50mmhg under the condition of breathing air at rest. According to the KDIGO Clinical Practice

113   Guideline, Acute kidney injury was defined as any of the following: an increase in serum

114   creatinine levels by ≥0.3 mg/dl (≥26.5 μmol/l) within 48 hours; increase in serum creatinine to

115   ≥1.5 times baseline, which was known or presumed to have occurred within the prior 7 days;

116   urine volume <0.5 ml/kg/h for 6 hours. Acute heart failure was defined as a clinical syndrome of

117   current or previous symptoms and/or signs caused by structural and/or functional abnormalities

118   of the heart, combined with elevated levels of natriuretic peptides or objective evidence of

119   cardiogenic pulmonary or systemic congestion (Bauersachs et al. 2022). Septic shock was

120   defined as persistent hypotension requiring vasopressors to maintain MAP $\geq$ 65 mm Hg and

121   serum lactate levels > 2 mmol/L (18 mg/dL) on the basis of adequate volume resuscitation

122   (Singer et al. 2016). The International Society on Thrombosis and Haemostasis (ISTH) DIC 8-

123   point score was applied for DIC calculation (Taylor et al. 2001). A platelet count $>100\times10^9$/L

124   accounts for 0 points, a score between $50\text{-}100\times10^9$/L for 1 point and a platelet count $<50\times10^9$/L

125   for 2 points. Prolonged prothrombin time, results in 0 points when <3 seconds, 1 point when 3-6

126   seconds and 2 points when ≥6 seconds. Fibrinogen levels ≥1 g/L result in 0 points, <1 g/L in 1

127   point. D-dimer levels of <0.4 μg/mL account for 0 points, levels between 0.4 and 4 μg/mL

128 account for 2 points and D-dimer levels >4 µg/mL account for 3 points. A score ≥5 is compatible

129 with overt DIC.

130 **Statistical analysis**

131 Propensity score matching (PSM) was performed (1:6 for thrombocytopenia versus non-

132 thrombocytopenia patients), with age, sex and days from symptom onset to admission used for

133 propensity score by the nearest-neighbor method with a caliper of 0.2 standard deviation. The

134 standardized mean difference (SMD) was measured to determine the balance between the two

135 groups before and after PSM, with its cut-off value of <0.2 regarded as indicating an adequate

136 balance between two groups. The inter-group comparison of patients' characteristics was made

137 for the matched patients to ensure the two groups were well balanced.

138   Descriptive statistics were performed for categorical variables as frequencies and proportions

139 or rates, as well as for continuous variables were expressed as medians and interquartile ranges.

140 A Mann-Whitney U test or a Kruskal-Wallis test was used to compare the differences of

141 continuous nonparametric variables between different groups. Pearson chi-square test or Fisher

142 exact test was performed to compare the differences of categorical variables. Kaplan-Meier

143 method was used to assess the survival rates, and the differences between groups with different

144 platelet counts were analyzed by the log-rank test. Hazard ratio (HR) with 95% confidence

145 interval (CI) were estimated using the Cox regression model, adjusting for baseline variables.

146 Cox regression model was used to perform multivariable analysis between patients with early

147 and late development of thrombocytopenia at 1-28 days and 28-56 days, respectively. A

148 generalized estimating equation (GEE) was used to perform the comparisons on the levels of the

149 parameters among two groups due to the repeated measurements in the same patient. Odds ratio

150 (OR) with 95% CI were estimated by multivariable logistic regression model, to investigate the

151 association between groups with different platelet counts and fatal outcome. Statistical analysis

152 was performed using R software (version 3.6.3), and a two-sided P value of less than 0.05 was

153 considered statistically significant.

154

155 **Results**

156 **Patient recruiting and demographic information**

157 From 3 January to 10 March 2020, 2346 hospitalized COVID-19 patients were enrolled, among

158 whom 137 patients were excluded because of incomplete or missing data on the platelet count or

159 other important parameters during hospitalization. Among the remaining 2209 COVID-19

160 patients available, 127 (5.7%) were recorded with thrombocytopenia, including 34 females and

161 93 males, with median age of 67 (IQR 56-75) years old. Compared with 2082 (94.3%) patients

162 with PLT $\geq 100 \times 10^9$/L on admission, the thrombocytopenia patients were older, more likely to be

163 male gender, and with higher presence of hypertension, clinical manifestation of fever, fatigue

164 and anorexia. (**S1 Table**).

165   For the thrombocytopenia group, the median PLT counts were kept below the normal range

166 throughout the observation, with the nadir level observed at three weeks after disease and

167 increased thereafter until attaining near 100×10^9/L at four weeks after disease. For the non-

168 thrombocytopenia patients, the median PLT counts were kept within normal range during the

169 whole observation, and with the peaking counts observed at 15 and 18 days after disease,

170 respectively (**Fig 1A**).

171   The PLT counts were higher in survival patients than in fatal patients by GEE model, after

172 adjusting the variables of age, sex, delay from symptom onset to admission and comorbidities

173 (P<0.001, **S1 Fig A**). However, the patients with comorbidities had the comparable levels of PLT

174 counts at the different stages to those without (**S1 Fig B-E**).

The comparison of clinical features based on the PSM cohort

175

176 We used PSM based on age, sex and days from symptom onset to admission to create a matched

177 group with PLT within normal range on admission (729 patients) in **Table 1**. The age, sex, and

178 days from symptom onset to admission were comparable after matching (All SMD <0.2). Pre-

179 existing hypertension, diabetes and coronary heart disease were non-significantly different

180 between two groups. The major symptoms were fever, cough, fatigue, anhelation, anorexia, and

181 chest tightness, among which fever, fatigue and anorexia were reported with significantly higher

182 frequencies in the thrombocytopenia group than the non-thrombocytopenia group (P=0.019,

183 P=0.024 and P=0.007, respectively).

184 **Comparison of all-cause mortality and detrimental complications based on the PSM cohort**

185 Patients with thrombocytopenia developed a higher prevalence of respiratory failure (41.9% *vs.*

186 22.6%), ICU entrance (25.6% *vs.* 11.5%), acute heart injury (9.3% *vs.* 5.8%), acute kidney injury

187 (4.7% *vs.* 2.9%), septic shock (9.3% *vs.* 2.9%), and DIC (45.2% *vs.* 10.6%) compared to the

188 matched non-thrombocytopenia group. The difference remained significant for respiratory

189 failure, ICU entrance and DIC after adjusting the effect from age, sex, days from symptom onset

190 to admission and comorbidities (**Table 2**).However, there was no significant difference in the

191 risk of acute heart injury, acute kidney injury, and septic shock between thrombocytopenia group

192 and non-thrombocytopenia group. These results were in line with those obtained from the

193 database without PSM (**S2 Table**).

194 The mortality rate was increased from 18.4% (134/729) in the non-thrombocytopenia group to

195 47.2% (60/127) in the thrombocytopenia group. After adjusting for age, sex, days from symptom

196 onset to admission and comorbidities, the HR of the all-cause mortality in the thrombocytopenia

197   group was significantly increased (HR 3.08, 95% CI 2.26-4.18, P<0.001) compared with non-

198   thrombocytopenia (**Fig 1B**).

199   **Comparison of haematological disorders based on the PSM cohort**

200   The inter group comparison was made at three clinical stages separately, i.e., 1-10 days, 11-20

201   days and 21-30 days after symptom onset, revealing that monocyte counts, monocyte percentage

202   and basophil percentage, were decreased in thrombocytopenia *vs.* non-thrombocytopenia patients

203   at all three stages (**Fig 2E, F, I and S3 Table**). Moreover, significantly higher neutrophil counts

204   and lower levels of lymphocyte percentage were observed for thrombocytopenia over non-

205   thrombocytopenia group at both 11-20 days and 21-30 days after symptom onset (**Fig 2B and**

206   **C**). Eosinophil counts and percentage at both 1-10 days and 21-30 days after disease onset were

207   significantly lower in the thrombocytopenia group than the non-thrombocytopenia group (**Fig**

208   **2H and G**). The remaining indicators showed no inter-group difference, or only difference at the

209   last clinical stage, which was less likely to be related to thrombocytopenia (**Fig 2A, D and J**).

210   **Comparison of platelet morphology indexes and coagulation perturbation based on the**

211   **PSM cohort**

212   During the whole hospitalization, we evaluated platelet morphological indexes, which reflect the

213   etiology of [13] thrombocytopenia, and there were significant differences in two indexes

214   between the two groups (**Fig 3A-B**).. Mean platelet volume (MPV) increased, whereas the

215   thrombocytocrit decreased, which corresponded with the reduction of platelet count.

216   We also evaluated clotting profile and found a series of important coagulation factors with

217   significant differences, including prothrombin time (PT), activated partial thromboplastin time

218   (APTT), thrombin time (TT), fibrinogen (FIB), and fibrinogen degradation products (FDP) (**Fig**

219   **3C-G**). The thrombocytopenia group had higher levels of APTT and FDP at 1-10 days and 11-20

220 days after symptom onset, longer PT at 1-10 days, 11-20 days and 21-30 days, and higher level

221 of TT at 11-20 days when compared with the non-thrombocytopenia group. Lower level of FIB

222 was observed at 1-10 days and 11-20 days after symptom onset in the patients with

223 thrombocytopenia.

224 **Comparison of inflammatory markers and biochemical parameters based on the PSM**

225 **cohort**

226 Elevated levels of inflammation markers, including interleukin 6 (IL-6), tumor necrosis factor α

227 (TNF-α), C-reactive protein (CRP) and procalcitonin were observed at 1-10 days or 11-20 days

228 after symptom onset in the patients with thrombocytopenia (**Fig 4**). Especially for IL-6, the

229 patients with thrombocytopenia had persistently elevated levels at 1-10 days, 11-20 days and 21-

230 30 days after symptom onset, with their difference from the non-thrombocytopenia group further

231 enlarged, which were significant at 1-10 days and 11-20 days after symptom onset. Higher level

232 of CRP was demonstrated from the patients with thrombocytopenia at 1-10 days, 11-20 days and

233 21-30 days after symptom onset, and with significant difference at 11-20 days compared to the

234 non-thrombocytopenia group. The TNF-α and procalcitonin were elevated to higher levels in the

235 thrombocytopenia group, with the difference attaining significance at 1-10 days compared to the

236 non-thrombocytopenia group.

237 The levels of albumin (ALB) and total protein (TP) were reduced below normal range in the

238 thrombocytopenia patients at 11-20 days or 21-30 days after symptom onset. The lactate

239 dehydrogenase (LDH) was augmented above normal range for both groups, and to higher levels

240 in the thrombocytopenia patients at 1-10 days and 11-20 days after symptom onset. The urea was

241 exclusively elevated in the thrombocytopenia group, to a significantly higher level than the non-

242 thrombocytopenia group at 11-20 days and 21-30 days after symptom onset. Lower level of

243   creatine kinase-MB (CK-MB) was observed at 21-30 days after symptom onset in the patients

244   with thrombocytopenia (**Fig 5**).

245   **Demographic information, outcomes and laboratory findings of patients with late**

246   **development of thrombocytopenia**

247   Altogether 61 patients had late development of thrombocytopenia (defined as the first

248   observation of thrombocytopenia seen beyond 14 days post-symptom) (**S4 Table**). Altogether

249   184 developed early thrombocytopenia (defined as the first observation of thrombocytopenia

250   seen within 14 days post-symptom). These two groups added up to a total frequency of 11.1%

251   during the whole disease progress. Comparison between two groups revealed older age with non-

252   significant difference, significantly longer delay between disease and hospital admission, as well

253   as longer duration of hospitalization in the late-thrombocytopenia group. The patients with late

254   development of thrombocytopenia demonstrated higher levels of neutrophil counts at 21-30 days,

255   CRP at 11-20 days and 21-30 days after symptom onset compared with patients with early

256   development of thrombocytopenia (**S2 Fig A and E**). Lower levels of lymphocyte counts, INR

257   and procalcitonin were demonstrated from the patients with late development of

258   thrombocytopenia (**S2 Fig B, D and F**). Thrombocytocrit had a higher level in late development

259   of thrombocytopenia group at 1-10 days and 11-20 days after symptom onset (**S2 Fig C**).

260   The nadir of PLT counts were observed at three weeks after disease for early

261   thrombocytopenia and late thrombocytopenia (**S3 Fig A**). The all-cause mortality rate was

262   comparable between early thrombocytopenia and late thrombocytopenia; however, survival

263   analysis revealed a lower mortality rate at 21 days post symptom onset in the patients with late

264   thrombocytopenia than early thrombocytopenia (21.3% *vs.* 33.7%), which trend however, was

265   reversed from 28 to 56 days post symptom onset (30.8% and 5.4% for patients with late and

266   early thrombocytopenia, respectively) (**S3 Fig B**). This difference remained significant after

267   adjusting the effect from age, sex, days from symptom onset to admission and comorbidities at

268   1-28 days (HR 0.43, 95% CI 0.24-0.75, P<0.001) and 28-56 days (HR 4.89, 95% CI 1.91-12.55,

269   P=0.001).

270
271   **Discussion**

272   Platelets are the smallest enucleated blood cells at the center of coagulation and homeostasis,

273   acting as the first participants in primary coagulation and playing an important role in the

274   pathogenesis of thrombosis. Platelets are also part of innate immunity, which according to most

275   recent studies, are widely involved in inflammation and immune responses and may form a

276   bridge between the inflammatory and the coagulation system (Burzynski et al. 2019; Mancuso &

277   Santagostino 2017). Prior to this study, there is evidence suggesting thrombocytopenia to be a

278   potential risk factor for the mortality of COVID-19, nevertheless, the thrombocytopenia can be

279   used as a clinical biomarker for COVID-19 are still controversial (Amgalan & Othman 2020).

280   Moreover, the role of thrombocytopenia in COVID-19 pathogenesis and its relationship with

281   systemic host response remained obscure. Lippi et al. published a meta-analysis study including

282   nine studies with a total of 1779 COVID-19 patients. It revealed that platelet count was

283   significantly lower in patients with more severe COVID-19 and was deemed associated with an

284   increased risk of severe disease and mortality in patients with COVID-19 (Lippi et al. 2020). In

285   our study, based on a retrospective cohort study of COVID-19 patients, we comprehensively

286   evaluated the haematological indicators, platelet morphology indexes, coagulation factors,

287   inflammatory and biochemical markers in relations to thrombocytopenia at different stages of

288   COVID-19. We suggest that thrombocytopenia might play an important role in the COVID-19

289   clinical outcome through a complicated process that was driven by its close relation to the

290 haematological and coagulopathy progress, as well as inflammation response, which might be

291 inducible to late developed DIC and respiratory failure.

292   Based on previous studies and our study, the proportion of thrombocytopenia as a clinical

293 feature of COVID-19 was low, and bleeding is uncommon because the haemostatic balance is

294 shifted markedly towards thrombosis (Tang et al. 2020). The need for transfusion in COVID-19

295 patients is not high and the diagnosis of thrombocytopenia is often overlooked in COVID-19.

296   There appears to be some variability in the percentage of COVID-19 patients who present

297 with thrombocytopenia. For example, in a study analyzing data on 1,099 laboratory-confirmed

298 COVID-19 patients from 552 hospitals in China, the patients with severe infection had 57.7%

299 thrombocytopenia *vs*. 31.6% in less significant COVID-19 symptoms (Guan et al. 2020). Tang et

300 al.'s study reported thrombocytopenia with a similar percentage of 57.1% in the non-survivors

301 (Tang et al. 2020). In a series of COVID-19 patients from France, 24.9% of patients with

302 thrombocytopenia were identified (Maquet et al. 2020). Chen et al. analyzed 99 COVID-19

303 patients, showing a much lower percent of 12% with thrombocytopenia, and interestingly 4%

304 with thrombocytosis (Chen et al. 2020). A meta-analysis of pooling data from totally 31 studies

305 showed that thrombocytopenia has been reported in a higher proportion of severe patients *vs*.

306 non-severe COVID-19 patients (Danwang et al. 2020). The prevalence of thrombocytopenia in

307 the current case series was lower than most of the previous studies. According to our study, the

308 frequency of thrombocytopenia differed according to the clinical stages, when only 8.3%

309 developed early thrombocytopenia, while 2.8% developed late thrombocytopenia, which might

310 partially explain the variance among studies. In addition, our definition of thrombocytopenia is

311 different from other studies. Other studies defined thrombocytopenia as PLT less than 125 or 150

312 $\times 10^9$/L, while our definition of thrombocytopenia was less than 100×10^9/L. This more stringent

313 definition may lead to fewer patients being included in thrombocytopenia group.

314  The reduced PLT counts act as an important indicator of adverse disease. At least three-fold

315 increased risk of death and an increased risk of respiratory failure, ICU entrance and DIC were

316 associated with lower PLT counts when the effects from age, sex and other important risk factors

317 were accounted. There is an obvious dose effect of PLT on numerous important laboratory

318 indicators, such as lymphocyte, LDH, ALB, IL-6, thrombocytocrit, indicating the important role

319 of PLT participating in the anti-SARS-COV-2 host response. Moreover, the timing of

320 thrombocytopenia development and fatal was correlated, as those with late thrombocytopenia

321 tended to have late death than those with early thrombocytopenia.

322  To date, there are various mechanisms of viral induced thrombocytopenia which differed

323 dependent on the virus types. A study conducted by Xu et al. proposed the following possible

324 mechanisms: 1) direct infection of bone marrow cells by the virus and inhibition of platelet

325 synthesis. Following virus infection, cytokine storm destroys bone marrow progenitor cells

326 and leads to the decrease of platelet production. Lung injury indirectly results in reduction of

327 platelet synthesis; 2) platelet destruction by the immune system; 3) platelet aggregation in the

328 lungs, resulting in microthrombi and platelet consumption (Xu et al. 2020).In addition, there

329 might be potential platelet activation that were related to the thrombocytopenia, which is likely

330 contributory to the hypercoagulability but the extent to which it does and which signaling

331 pathways are involved warrant research. However, the exact mechanism of SARS-COV-2

332 related thrombocytopenia is far from clear. Further research is needed to clarify the mechanism

333  The current study was not designed to explore the pathophysiological mechanisms,

334 however, we indeed revealed significant association that was driven from decreased PLT on the

335   adverse COVID-19 outcome. In patients with thrombocytopenia, coagulation dysfunction

336   involving PT, APTT, TT, FIB and FDP have been determined in the current study and reported

337   in the previous studies (Guan et al. 2020). It's also notable that more deviation from the normal

338   value was observed for the coagulation factors, especially at 11-20 days after symptom onset.

339   This time corresponded with the deterioration phase of the detrimental cases, in whom DIC acted

340   as a hallmark manifestation, which might suggest a significant role of severity of

341   thrombocytopenia in the risk of extrinsic coagulation activation and DIC development. At the

342   same time, a dysregulated immune response caused by decreased PLT is likely also be

343   responsible for the increased disease severity of COVID-19, as a higher ratio of lymphopenia

344   and increased levels of neutrophils, serum CRP, and IL-6 were observed in the patients with

345   COVID-19 and thrombocytopenia observed at the early infection according to our study. The

346   cytokines, such as IL-6 etc., may have higher levels in the serum than in the plasma, which may

347   represent a higher expression state of systemic cytokine milieu. All these commonly seen

348   abnormalities may account for the propensity of poor outcomes in patients with COVID-19 and

349   early thrombocytopenia.

350   Disturbance of coagulation and fibrinolysis were also observed at acute COVID-19 infection

351   (Paessler & Walker 2013). Plasma PT and APTT are indexes of extrinsic and intrinsic

352   coagulation system during the coagulation cascade, respectively (Chua et al. 1993). In the current

353   research, we found a significant prolongation of both indicators occurring in the groups with

354   thrombocytopenia. All these findings, taken together, suggested a perturbed extrinsic pathway in

355   all COVID-19 patients and an even stronger activation of the intrinsic pathway in the severe

356   cases (Mairuhu et al. 2003). Increased concentration of D-dimer indicated the activation of

357   fibrinolysis, which is believed to be related to disseminated intravascular coagulation (Wada et

358   al. 1993), a feature commonly seen in the severest stage of SARS-COV-2 infection. Platelet,

359   although is the smallest of blood cells, have a central role in haemostasis regulation (George

360   2000). The morphology and size parameters (MPV, thrombocytocrit and PDW) were the

361   valuable reflections of platelet function (Thompson et al. 1982), both were also extensively

362   comprised in the process of thrombocytopenia and contributed to the fatal outcome and severity

363   of SARS-COV-2 infection.

364   These findings also offered alternative therapy choices of platelet transfusion for COVID-19

365   patients with thrombocytopenia, which was supported by the current finding that no detrimental

366   effect was seen from the patients with slightly higher PLT counts above the normal range. Still,

367   the usage of platelet transfusion for the SARS-COV-2 infection therapy should be cautiously

368   applied. On the other hand, considering that thrombocytopenia involved not only reduction in

369   PLT counts, but also platelet morphological changes, thus the mere platelet transfusion might not

370   be efficient in reducing the adverse outcome.

371   The study was subject to major limitations that although dose-effect relationship of platelet

372   counts and platelet hyperactivation, as well as the host immune response were observed, the

373   causal relationship cannot be inferred. Whether thrombocytopenia is one of the causations of

374   systematic host response, or only a representation of COVID-19 patients' condition requires

375   further clinical and experimental data to verify. Another limitation is that the drugs for the

376   therapy of COVID-19 during the hospitalization may have a bias on the results in this study.

377
378   **Conclusions**

379   In conclusion, thrombocytopenia is associated with an increased risk of adverse outcomes in

380   covid-19 patients, and platelet count and related indicators should be included in the evaluation

381   of covid-19 patients during hospitalization together with other hematological, biochemical, and

382   inflammatory indicators.

383

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387

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Basic information of patients throughout the course of the COVID-19 on the PSM database.

IQR, interquartile range. PSM, propensity score matching. COVID-19, coronavirus disease 2019. [*]P value was calculated by Mann-Whitney U test. [#]P value was calculated by Pearson chi-square test.

1   **Table 1. Basic information of patients throughout the course of the COVID-19 on the PSM**

2   **database.**

Characteristics	Total (N=856)	Thrombocytopenia (n=127)	Non-thrombocytopenia (n=729)	P value
Age, years (median, IQR)	66 (56-75)	67 (56-75)	66 (56-75)	0.459*
≤45	104 (12.1)	13 (10.2)	91 (12.5)	0.566#
45-60	170 (19.9)	29 (22.8)	141 (19.3)	
>60	582 (68.0)	85 (66.9)	497 (68.2)	
Sex, male, n (%)	610 (71.3)	93 (73.2)	517 (70.9)	0.596#
Days from disease onset to clinic visit, median (IQR)				
	9 (5-13)	8 (5-12)	9 (5-13)	0.441*
Length of stay, days, median (IQR)				
	18 (11-25)	14 (7-21)	18 (12-25)	<0.001*
Any comorbidity, n (%)	421 (49.2)	68 (53.5)	353 (48.4)	0.287#
Hypertension	332 (38.8)	55 (43.3)	277 (38)	0.257#
Diabetes	130 (15.2)	23 (18.1)	107 (14.7)	0.320 #
Coronary heart disease	87 (10.2)	14 (11.0)	73 (10.0)	0.728#
Cerebral infarction	61 (7.1)	8 (6.3)	53 (7.3)	0.695#
Symptoms, n (%)				
Fever	651 (76.1)	107 (84.3)	544 (74.6)	0.019#
Cough	543 (63.4)	80 (63.0)	463 (63.5)	0.911#
Fatigue	289 (33.8)	54 (42.5)	235 (32.2)	0.024#
Anhelation	228 (26.6)	37 (29.1)	191 (26.2)	0.490#
Anorexia	221 (25.8)	45 (35.4)	176 (24.1)	0.007#
Chest tightness	198 (23.1)	33 (26.0)	165 (22.6)	0.409#
Diarrhea	83 (9.7)	5 (3.9)	78 (10.7)	0.017#
Nausea	66 (7.7)	10 (7.9)	56 (7.7)	0.940#
Headache	14 (1.6)	3 (2.4)	11 (1.5)	0.749#

3   IQR, interquartile range. PSM, propensity score matching. COVID-19, coronavirus disease 2019.

4   *P value was calculated by Mann-Whitney U test. #P value was calculated by Pearson chi-square

5   test.

Multivariable analysis on the associations between thrombocytopenia and adverse outcome for COVID-19 patients using the PSM data.

The association between thrombocytopenia and adverse outcome for COVID-19 were calculated by the multivariable logistic regression models adjusted for age, sex, delay from symptom onset to admission and comorbidities. The number of patients included in multivariable analysis was inconsistent with that of the PSM database because the clinical outcome was not obtained from all the included patients. COVID-19, coronavirus disease 2019; PSM, propensity score matching; ARDS, acute respiratory distress syndrome; ICU, intensive care unit; DIC, disseminated intravascular coagulation.

1 **Table 2. Multivariable analysis on the associations between thrombocytopenia and adverse**

2 **outcome for COVID-19 patients using the PSM data.**

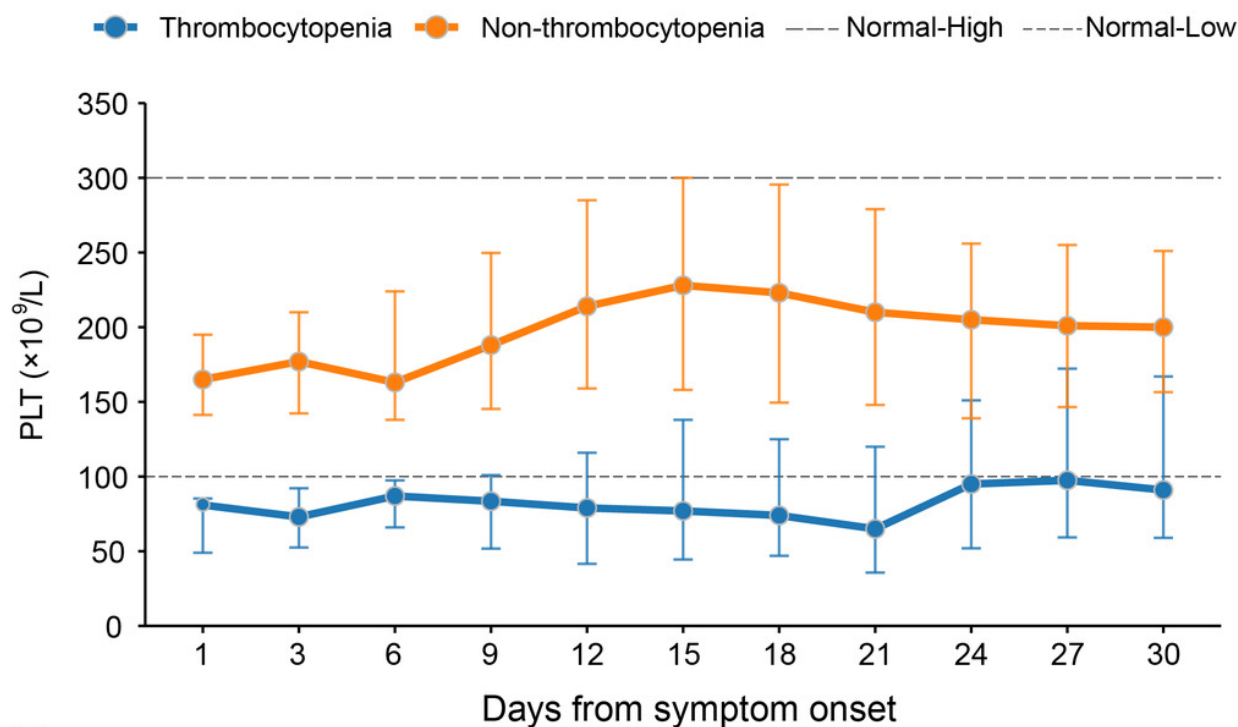
Parameter	Thrombocytopenia	Non-thrombocytopenia	Multivariable	
			OR (95% CI)	P value
~~ARDS~~	~~38~~	~~184~~		
~~Yes~~	~~4 (10.5)~~	~~7 (3.8)~~	~~2.94 (0.78-11.12)~~	~~0.112~~
~~No~~	~~34 (89.5)~~	~~177 (96.2)~~	~~Reference~~	
Respiratory failure	43	208		
Yes	18 (41.9)	47 (22.6)	2.52 (1.16-5.48)	0.020
No	25 (58.1)	161 (77.4)	Reference	
ICU entrance	43	208		
Yes	11 (25.6)	24 (11.5)	2.61 (1.24-5.49)	0.012
No	32 (74.4)	184 (88.5)	Reference	
Acute heart injury	43	208		
Yes	4 (9.3)	12 (5.8)	1.5 (0.44-5.1)	0.512
No	39 (90.7)	196 (94.2)	Reference	
Acute kidney injury	43	208		
Yes	2 (4.7)	6 (2.9)	1.33 (0.25-7.02)	0.739
No	41 (95.3)	202 (97.1)	Reference	
Septic shock	43	208		
Yes	4 (9.3)	6 (2.9)	3.31 (0.82-13.29)	0.091
No	39 (90.7)	202 (97.1)	Reference	
DIC	93	565		
Yes	42 (45.2)	60 (10.6)	7.16 (4.33-11.85)	<0.001
No	51 (54.8)	505 (89.4)	Reference	

3 The association between thrombocytopenia and adverse outcome for COVID-19 were calculated
4 by the multivariable logistic regression models adjusted for age, sex, delay from symptom onset
5 to admission and comorbidities. The number of patients included in multivariable analysis was
6 inconsistent with that of the PSM database because the clinical outcome was not obtained from
7 all the included patients. COVID-19, coronavirus disease 2019; PSM, propensity score matching;
8 ~~ARDS, acute respiratory distress syndrome;~~ ICU, intensive care unit; DIC, disseminated
9 intravascular coagulation.

Figure 1

The dynamic profile of platelet counts and 60-day Kaplan-Meier survival curves of COVID-19 patients stratified by platelet counts on admission.

A, dynamic profile of platelet count was plotted in the thrombocytopenia group and the non-thrombocytopenia group classified by the platelet count on admission. The median and interquartile range were shown. B, Survival curves on probability of survival from the thrombocytopenia group and the non-thrombocytopenia group and compared by log-rank test. The analysis was performed based on the PSM database.

A

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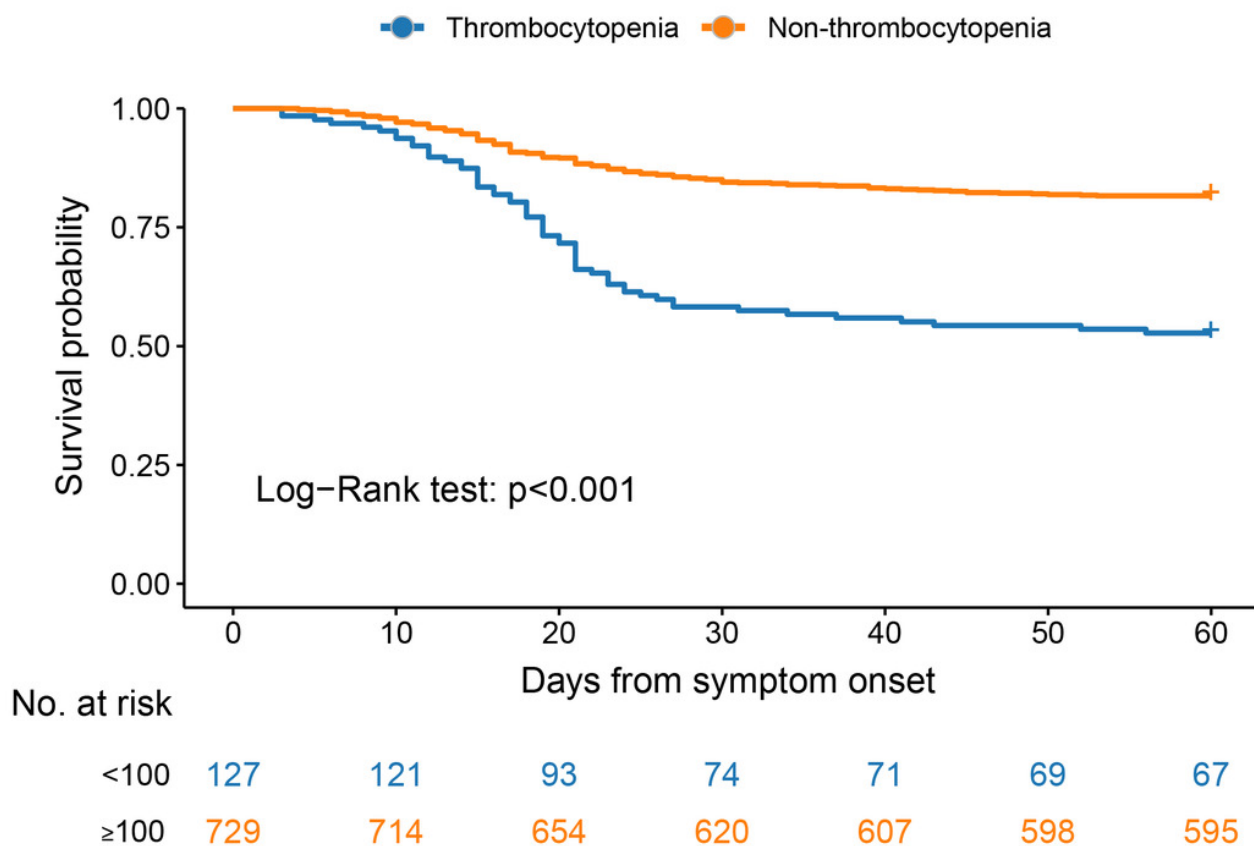
Figure 2

Dynamic profile of haematological indicators in groups of patients stratified by platelet counts on admission.

A, neutrophil percent; B, neutrophil count; C, lymphocyte percent; D, lymphocyte count; E, monocyte percent; F, monocyte count; G, eosinophil percent; H, eosinophil count; I, basophil percent; J, basophil count. Dots present the values for individual detection, and the lines and error bars indicate the median and interquartile range, respectively. Dotted line indicates the lower limit and upper limit of each laboratory indicator. *P <0.05 using a generalized estimating equation regression model adjusted for age, sex, delay from disease onset to hospital admission and comorbidities. The markers in comparisons without significant differences were not shown. The analysis was performed based on the PSM database.
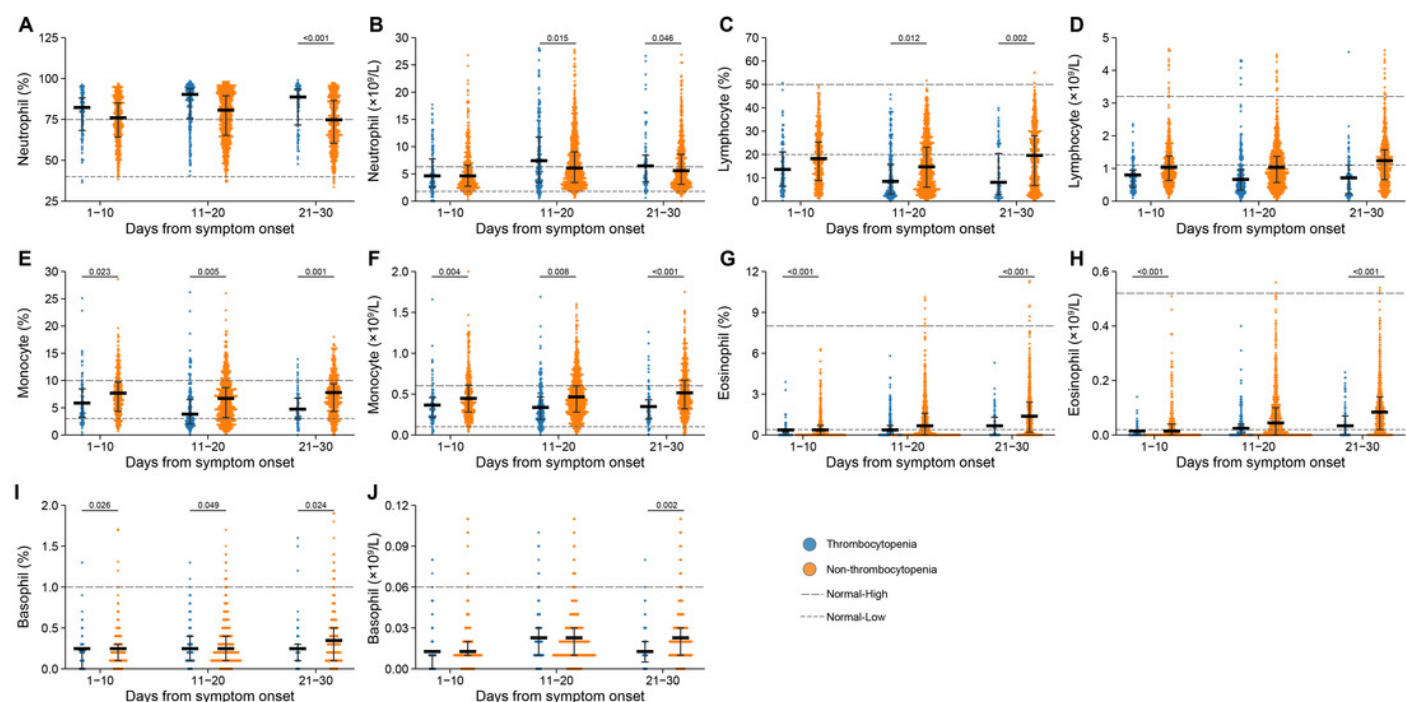
Figure 3

Dynamic profile of platelet morphology indexes and coagulation factors in groups of patients stratified by platelet counts on admission.

A, mean platelet volume (MPV); B, thrombocytocrit; C, prothrombin time (PT); D, activated partial thromboplastin time (APTT); E, thrombin time (TT); F, fibrinogen (FIB); G, fibrinogen degradation products (FDP). Dots present the values for individual detection, and the lines and error bars indicate the median and interquartile range, respectively. Dotted line indicates the lower limit and upper limit of each laboratory indicator. P-values were calculated using generalized estimating equation regression models adjusted for age, sex, delay from disease onset to admission, and comorbidities. The markers in comparisons without significant differences were not shown. The analysis was performed based on the PSM database.
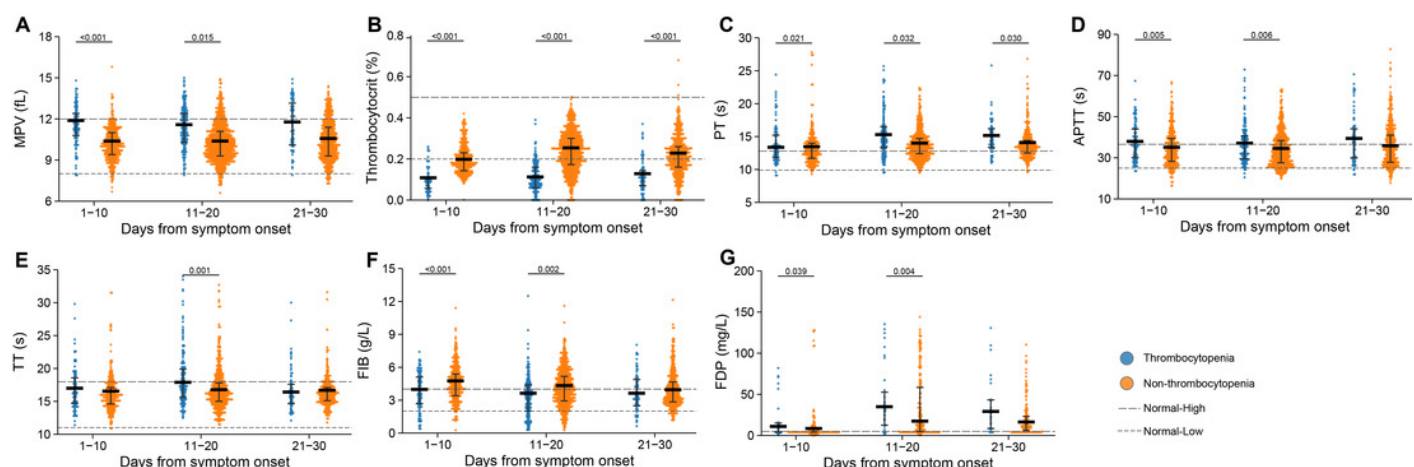
Figure 4

Dynamic profile of Inflammatory markers in groups of patients stratified by platelet counts on admission.

A, interleukin 6 (IL-6); B, tumor necrosis factor α (TNF-α); C, C reaction protein (CRP); D, procalcitonin. Dots present the values for individual detection, and the lines and error bars indicate the median and interquartile range, respectively. Dotted line indicates the lower limit and upper limit of each laboratory indicator. P-values were calculated using generalized estimating equation regression models adjusted for age, sex, delay from disease onset to admission, and comorbidities. The markers in comparisons without significant differences were not shown. The analysis was performed based on the PSM database.
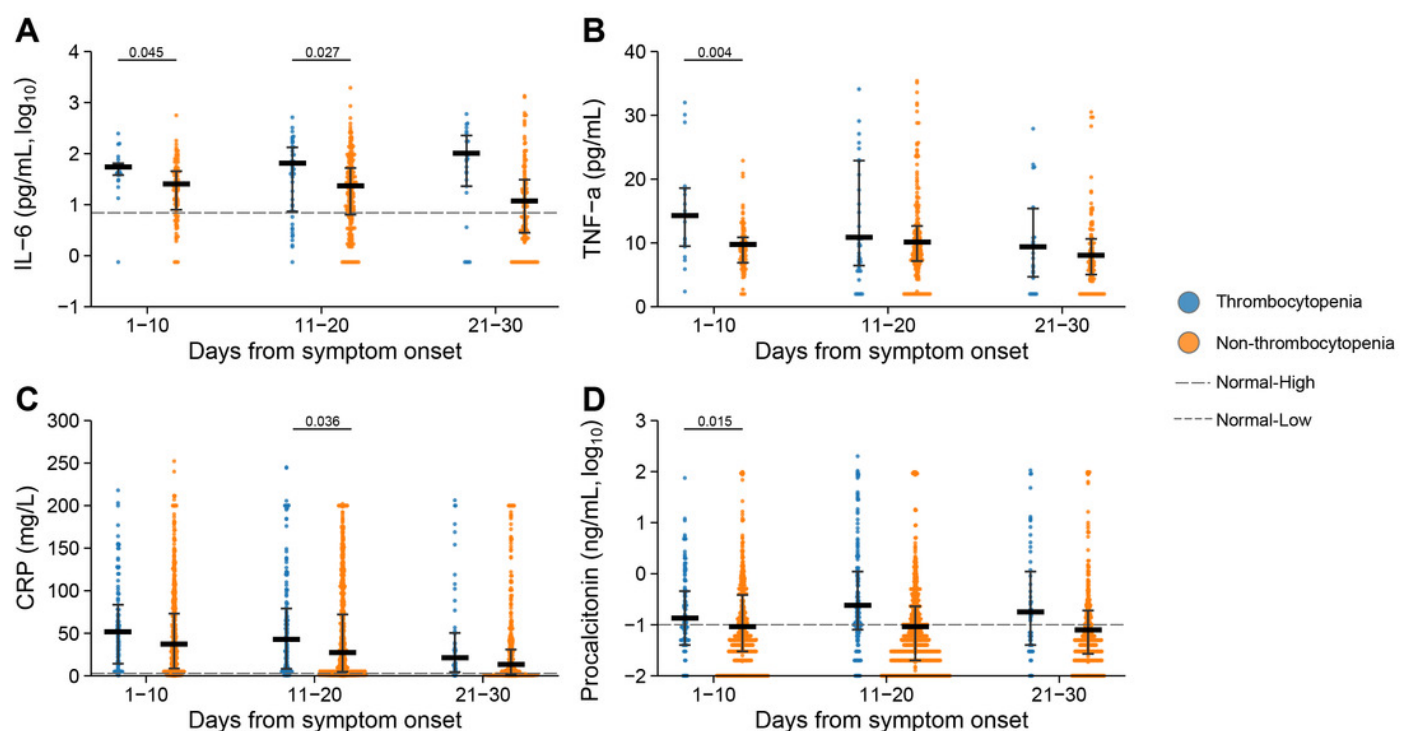
Figure 5

Dynamic profile of biochemical indicators in groups of patients stratified by platelet counts on admission.

A, total protein (TP); B, albumin (ALB); C, urea; D, lactate dehydrogenase (LDH); E, creatine kinase MB (CK-MB). Dots present the values for individual detection, and the lines and error bars indicate the median and interquartile range, respectively. Dotted line indicates the lower limit and upper limit of each laboratory indicator. P-values were calculated using generalized estimating equation regression models adjusted for age, sex, delay from disease onset to admission, and comorbidities. The markers in comparisons without significant differences were not shown. The analysis was performed based on the PSM database.