Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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HLA-C*17 in COVID-19 patients: Hints for associations with severe clinical outcome and cardiovascular risk

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Dear editor,

Specific HLA genotypes have been widely reported to correlate with protection or susceptibility to a range of infectious diseases, including COVID-19 [1–3]. More recently, association between HLA and KIR genotyping and the risk of developing a severe form of COVID-19 disease has also been observed [4].

Although age and the existence of comorbidities have clearly been recognized to be risk factors for developing a severe form of the disease [5], the impact of diverse genetic backgrounds in determining the heterogeneity of clinical outcomes remains a critical topic needing further exploration. This issue concerns also the immune response. Emerging data suggest that, in critical COVID-19 patients, an excessive uncoordinated cell-mediated response to the virus may be responsible for a detrimental over-inflammation [6–9]. Indeed, T cells of these patients showed no sign of exhaustion or augmented cell death, but they rather displayed activation markers, particularly in bronchoalveolar lavage fluid [7]. Together, these data indicate that, particularly for CD8+T cells, the observed lymphopenia might be the result of the recruitment of activated T cells in the inflammatory milieu [7–10] and, therefore, over-activation of these cells might represent a potential mechanism for immune-mediated tissue damage.

Considering the crucial role of human leucocyte antigen (HLA) class I molecules in triggering virus-specific $CD8^{+}$T cell activation, by presenting pathogen-derived peptides, it might be hypothesized that individual HLA class I polymorphisms might contribute to determine the strength of the immune response along the disease and, in turn, the severity of symptomatology and clinical outcome.

HLA class I typing (locus A, B and C) of our exploratory cohort of COVID-19 patients revealed HLA-C*17 significantly associated with the most severe form of the disease, requiring admission to Intensive Care Unit (ICU) (Fig. 1A and Table 1). More in detail, our analysis showed a protective (RR=0.16; $p = 0.014892$) and harmful (RR=3.08 $p = 0.000801$) roles of HLA-C*06 and HLA-C*17 respectively (Fig. 1A and Table 1). However, after the application of Q-test's correction for multiple tests, only HLA-C*17 remained as the only HLA class I allele that distinguished extremely severe from asymptomatic patients (Fig. 1A). Remarkably, by monitoring circulating lymphocytes in the ICU patient cohort, we observed that the level of HLA-DR expression on $CD8^{+}$T cells was significantly higher in HLA-C*17 patients (37.6 ± 12.6) than in patients not expressing the allele (18.0 ± 11.9) (Fig. 1B).

HLA class I molecules are also relevant for natural killer (NK) cell response, as they regulate NK cell activation by interacting with cognate activating or inhibitory killer immunoglobulin-like receptors (KIRs). Interestingly, it has been reported that HLA-C*17 represents the allotype with the strongest affinity for KIR2DS1, an activating receptor expressed on NK cells of some individuals [11]. Thus, we assessed whether the activating interaction between KIR2DS1 and cognate HLA-C*17 in COVID-19 patients could provide an alternative or additional explanation for the detrimental inflammation observed in this cohort of patients. However, whereas all HLA-C*17 patients carried the inhibitory KIR2DL1 gene, we found that only two out of the seven HLA-C*17 patients carried the KIR2DS1 gene (Fig. 1C), thus ruling out that the presence of HLA-C*17 might preferentially trigger tissue damage via NK cell cytotoxicity.

Next, we examined the correlations between the recurrence of this genotype and individual clinical features. Comorbidities such as hypertension, dyslipidemia and diabetes were present at similar frequency in ICU patients displaying or not HLA-C*17 (Table 1), suggesting a comparable cardiovascular risk during COVID-19 disease. Nevertheless, HLA-C*17 patients showed significantly higher level of troponin-T, one of the main clinical parameters associated with risk of cardiovascular complications, while other markers of inflammation and intravascular coagulation were higher in ICU patients but not significantly different in ICU patients expressing or not the HLA-C*17 allele (Fig. 1D and Table 1).

A possible mechanistic link for the presence of HLA-C*17 and high level of troponin-T may rely on the uncontrolled over-activation of $CD8^{+}$cytotoxic T cells and their segregation in infected tissues [6–8]. Cytotoxic CD8+ T cells might recognize dominant SARS-CoV-2-derived peptides in the context of HLA-C*17 and, in consequence, over-activated CD8+ T cells might effectively contribute to extended tissue damages responsible of vascular complications and multi-organ failure. In

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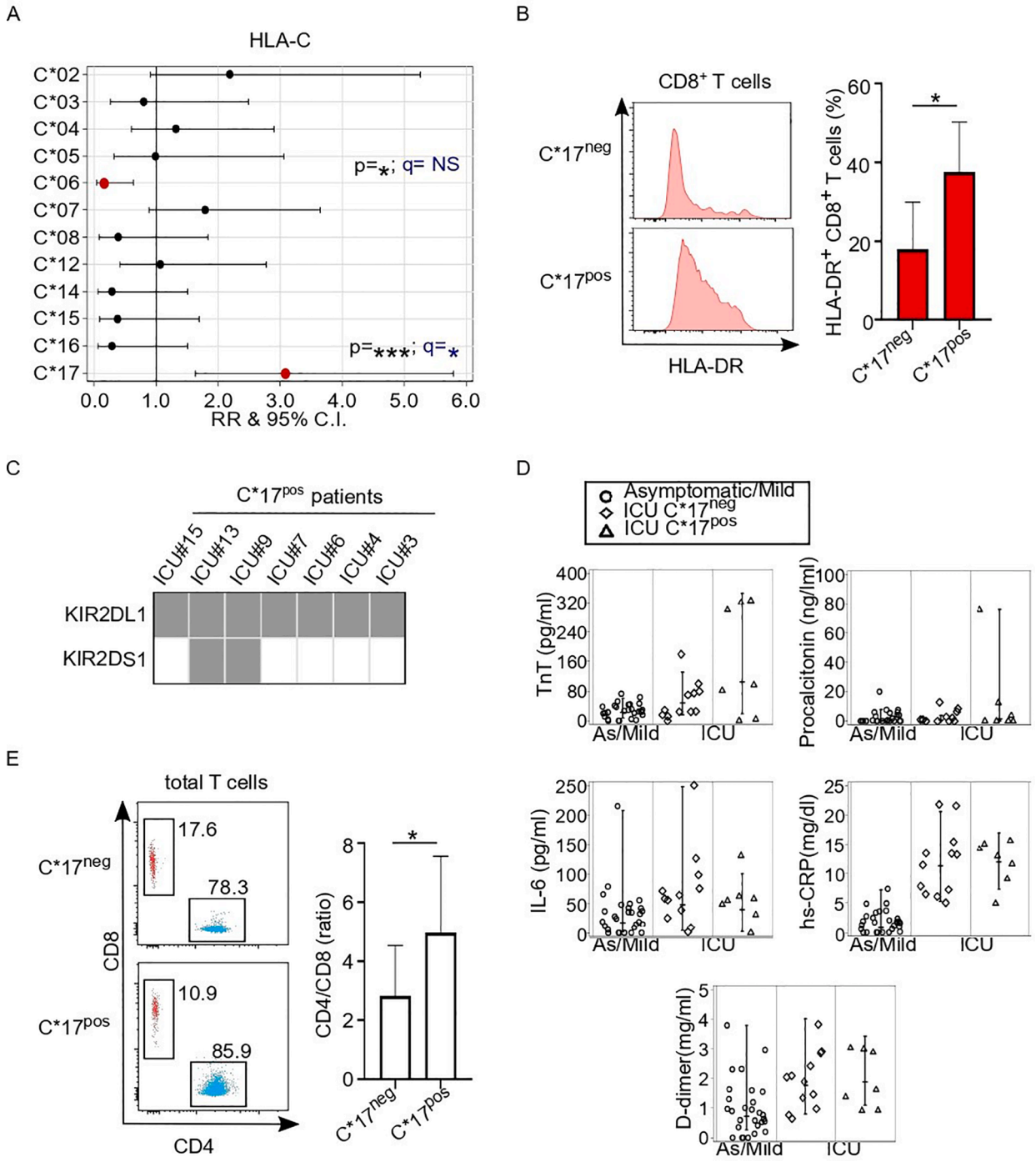

Fig. 1. (A) Caterpillar plot showing the impact of each HLA-C superfamily allele on disease state of SARS-CoV-2 infected patients. Risk ratios (RRs), and corresponding 95% confidence interval (95% CI), were derived from the analysis of 2×2 contingency tables. Vertical line at RR = 1 separates RRs associated with a mild/asymptomatic status (left side) from those associated with a severe/extremely severe status (right side); p-value: probability level derived from Fisher's exact test; *$p \leq 0.05$ ***$p \leq 0.001$ q-value: probability level adjusted for multiple comparisons. (B) Flow cytometry analysis of HLA-DR expression on CD8+ T cells from two representative ICU patients (left); HLA-DR expression on CD8+ T cells in intensive care unit (ICU) patients expressing ($n = 7$) or not ($n = 12$) HLA-C*17. Data are shown as mean ± SD (right); *$p \leq 0.05$. (C) Analysis of KIR2DL1 and KIR2DS1 genes in HLA-C*17 ICU patients (C*17pos, $n = 7$). KIR genes presence (grey boxes) or absence (white boxes) is shown. (D) Serum levels of troponin-T (TnT), Procalcitonin, Interleukin-6 (IL-6), C-Reactive Protein (hs-CRP) and D-dimer in asymptomatic/mild (As/Mild) and ICU cohorts of COVID-19 patients. (E) Flow cytometry analysis of CD8+ and CD4+ T cell gating on total T cells in two representative ICU patients (left); CD4/CD8 ratio derived from HLA-C*17 ICU patients (C*17pos, $n = 7$) and ICU patients not expressing HLA-C*17 (C*17neg, $n = 12$). Data are shown as mean ± SD (right); *$p \leq 0.05$.

Table 1
Demographic and clinical characteristics of the patients from ICU.

Characteristics	HLA-C*17^{neg} ($n = 12$)	HLA-C*17^{pos} ($n = 7$)
Median age (range) - yr	65 (38–85)	65 (39–89)
Female- no. (%)	5 (42)	4 (57)
Male - no. (%)	7 (58)	3 (43)
Region -no. (%)		
Italy East-Sicily	11 (91)	6 (85)
Comorbidity- no. (%)		
Hypertension	10 (83)	6 (86)
Diabetes mellitus	2 (16)	2 (28)
Dysthyroidism	0 (0)	3 (43)
Obesity	2 (16)	2 (28)
Osteoporosis	2 (16)	2 (16)
Pulmonary fibrosis	1 (8)	1 (14)
Clinical outcome- no. (%)		
Remained in ICU	8 (67)	4 (57)
Died	4 (33)	3 (43)
Clinical laboratory results - GM * ($P^{\dagger}_{2.5}$-$P^{\ddagger}_{97.5}$)		
C-Reactive Protein (mg/dl)	11.20 (5.26–20.60)	11.90 (7.30–17.00)
D-dimer (μg/ml)	1.76 (0.80–4.00)	1.88 (1.10–3.41)
Troponin-T (pg/ml)	47.90 (15.70–130.80)	105.0 (18.60–345.4)
Interleukin-6 (pg/ml)	48.20 (3.70–247.70)	39.80 (2.02–101.00)
Procalcitonin (ng/ml)	0.62 (0.09–3.70)	1.22 (0.14–75.50)

*GM= geometric mean, $^{\dagger}P_{2.5}$ = 2.5th percentile, $^{\ddagger}P_{97.5}$ = 97.5th percentile, Categorical variables were reported as count (percentage), while continuous variable was reported as geometric mean.

accordance, we observed that HLA-C*17 patients showed an increased CD4/CD8 ratio compared to ICU patients not carrying the allele (Fig. 1E), suggesting a preferential recruitment of cytotoxic T cells in the inflammatory milieu.

Despite SARS-CoV-2 tropism for the lungs, several clinical observations clearly indicate that the cardiovascular system represents an additional relevant target of viral-mediated immune damage [9]. Altogether, our findings support the view that exaggerated cytotoxic response by CD8+ T cells might behave as a potential mechanism that contribute to myocardial injury, as well as, endothelial dysfunction. Further exploration of these current findings associated to HLA-C*17 allele are now required to confirm their usefulness for predicting patient's outcome as well as for the development of successful treatments.

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Disclosures

The authors have disclosed that they do not have any conflicts of interest.

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