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Low Percentage of Perforin-Expressing NK Cells during Severe SARS-CoV-2 Infection: Consumption Rather than Primary Deficiency

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1 **Low percentage of perforin-expressing NK cells during severe**
2 **SARS-CoV-2 infection : consumption rather than primary**
3 **deficiency**

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45 Abstract

46 Genetic defects in the ability to deliver effective perforin have been reported in patients with
47 hemophagocytic lymphohistiocytosis. We tested the hypothesis that a primary perforin
48 deficiency might also be causal in severe SARS-CoV-2 infection. We recruited 56 volunteers
49 confirmed as being SARS-CoV-2-infected by RT-PCR and admitted to Intensive Care Units
50 (ICU) or non-ICU, and age- and sex-matched healthy controls (HCs). Compared with HCs,
51 the percentage of perforin-expressing CD3⁻CD56⁺ NK cells quantified by flow cytometry was
52 low in COVID-19 patients (69.9 ± 17.7 vs 78.6 ± 14.6 %, $p = 0.026$). There was no
53 correlation between the proportions of perforin-positive NK cells and T8 lymphocytes.
54 Moreover, the frequency of NK cells producing perforin was neither linked to disease severity
55 nor predictive of death. Although IL-6 is known to downregulate perforin production in NK
56 cells, we did not find any link between perforin expression and IL-6 plasma level. Yet, we
57 unveiled a negative correlation between the degranulation marker CD107A and perforin
58 expression in NK cells ($r = -0.488$, $p = 10^{-4}$). *PRF1* gene expression and the frequency of NK
59 cells harboring perforin were normal in patients one year after acute SARS-CoV-2 infection.
60 A primary perforin defect does not seem to be a driver of COVID-19 since NK perforin
61 expression is (i) linked neither to T8 perforin expression nor to disease severity, (ii) inversely
62 correlated with NK degranulation, and (iii) normalized at distance from acute infection. Thus,
63 the cause of low frequency of perforin-positive NK cells appears, rather, to be consumption.

64

65 Key points

66 COVID-19 present low percentages of perforin-expressing NK cells.

67 This was not linked to disease severity but anticorrelated with NK degranulation.

68 A primary perforin deficiency does not seem to be a driver of COVID-19.

69

70 **Introduction**

71 NK cells are type 1 innate lymphoid cells playing a key role in defense against tumors and
72 intracellular pathogens, particularly via perforin- and granzyme B-mediated cytotoxicity (1).

73 A panel of activating and inhibitory receptors displayed at their surface and sensing molecules
74 at the surface of infected and tumoral cells determine their propensity to kill. The signals
75 delivered by these receptors provoke the release of cytotoxic factors by NK cells, including
76 perforin, resulting in the apoptosis of the target cells. Moreover, NK cells regulate T cell-
77 mediated antiviral immune response (2). NK cells are particularly well-known for being
78 important actors in the immune defense against respiratory viral infections, including
79 coronaviruses (3).

80 Severe forms of COVID-19 have many similar features to hemophagocytic
81 lymphohistiocytosis (HLH) (4-6). The frequency of HLH symptoms in COVID-19 is
82 discussed, some authors reporting it as low (7-9), but others as higher than in non-COVID-19
83 sepsis (10). Various articles (8, 11), but not all (9), established the prognostic value on
84 mortality of these symptoms. Differences in the level of different markers have also been
85 observed between severe COVID-19 and HLH. For instance, interleukin (IL)-12, IL-15, IL-
86 18, IL-21, interferon (IFN)- γ , soluble Fas Ligand, were shown lower, and IL-8, IL-1 Receptor
87 Antagonist, InterCellular Adhesion Molecule-1 higher in the former than in the latter (12,
88 13). HLH is defined by the presence of at least 5 of the following criteria: fever,
89 splenomegaly, cytopenia of at least 2 lineages, hyperferritinemia, an increase in soluble
90 CD25, high triglyceridemia and low fibrinogenemia, decrease in NK activity, and
91 hemophagocytosis (14). HLH may be primary, caused by a genetic deficiency in perforin or
92 in factors involved in exocytosis, or secondary to malignant hemopathies, auto-immune
93 disorders or infections, particularly viral infections. Even in secondary HLH, a deficiency in
94 perforin or mutations in proteins mediating exocytosis have been found in up to 40% of

95 patients (15). This raises the possibility that cytotoxic deficiency is a key driver of HLH and,
96 potentially, of the severity of COVID-19. This may be explained by the fact that perforin-
97 deficient cytotoxic cells produce more cytokines than wild-type cytotoxic cells (16). In fact, it
98 is the target cell which triggers the detachment of the cytotoxic cell via a caspase-dependent
99 signal. When perforin is deficient, the caspase pathway is not fully activated in the target cell
100 so the contact time is drastically increased. This increase results in an overproduction of IL-2,
101 tumor necrosis factor (TNF)- α , and IFN- γ by the cytotoxic cell (17). IFN- γ induces the release
102 of large amounts of IL-6 by myeloid cells, a marker of HLH and COVID-19 (4). In line with
103 this observation, NK cells secrete greater quantities of inflammatory cytokines in perforin-
104 deficient mice infected with mouse cytomegalovirus (18). In addition, NK cells may kill T
105 cells (19). Consequently, in mice infected with the lymphocytic choriomeningitis virus,
106 perforin deficiency has been shown to result in the accumulation of exhausted CD8⁺ T cells,
107 responsible for immune-mediated damage and death, as in severe forms of COVID-19 (20,
108 21). NK cells may also kill NK cells and thereby downregulate immune activation (22).
109 Finally, perforin has also been involved in NK and CD8⁺ T cell killing by regulatory T cells
110 (23). Altogether, these findings argue for perforin's role in reducing the intensity of immune
111 responses. Moreover, the hypothesis of defective cytotoxicity as a causal factor of COVID-19
112 ties in with the fact that aging (24) and comorbidities like diabetes (6), conditions known to
113 reduce perforin expression (25), are predictive of a poor prognosis in SARS-CoV-2 infection.
114 To better understand the pathogenic mechanisms of this disease, we analyzed perforin
115 expression in the NK cells of COVID-19 patients, seeking arguments in favor of perforin
116 deficiency having an etiologic role in the severity of this disease.

117 In the present study, we show a low frequency of perforin-expressing NK cells in COVID-19
118 patients, but we report arguments against a causal role of this impairment in COVID-19.

119

120 **Materials and methods**

121 **Study design**

122 All patients were diagnosed as being SARS-CoV-2-infected by RT-PCR. Their blood was
123 drawn on the first day of hospitalization at an intensive care unit (ICU) or a non-ICU in the
124 University Hospital of Nîmes, France. ICU participants presented with oxygen saturation
125 <90% in ambient air or <95% with 5L/mn of oxygen therapy and/or arterial oxygen tension of
126 less than 60 mm Hg. Non-ICU participants presented with oxygen saturation <96% in
127 ambient air. The French Île-de-France 1 Ethics Committee approved this study and all
128 volunteers had provided written informed consent. The trial was registered on
129 ClinicalTrials.gov under the reference NCT04351711.

130 **Flow cytometry**

131 Perforin expression was quantified by intracellular PBMC labeling. Frozen cells were first
132 thawed, and washed twice. 200,000 cells were surface-labeled with the following antibody
133 panel: CD3-allophycocyanine/Alexa750 (Beckman Coulter) + CD16-allophycocyanine
134 (Beckman Coulter) + CD56-phycoerythrin/cyanine 5.5 (Beckman Coulter) + CD107A-
135 phycoerythrin (Biolegend). IgG1- fluorescein isothiocyanate (clone REA293, Myltenyi
136 Biotec) and IgG1- phycoerythrin (clone MOPC-21, Biolegend) were used as isotypic controls.
137 The cells were then fixed using IMMUNOPREP Reagent System Kit and TQ Prep automate
138 (Beckman Coulter), and permeabilized for Perforin labeling (perforin-fluorescein
139 isothiocyanate, Myltenyi Biotec) using a cytofix-cytoperm kit (Becton-Dickinson). A
140 minimum of 20,000 lymphocytes were run on a Navios flow cytometer and results were
141 analyzed by using Kaluza software (Beckman Coulter).

142 **Biomarkers**

143 The plasma levels of IL-6, IL-12p70, and IFN- α were determined by Luminex/xMAP
144 immunoassay (ProcartaPlex, ThermoFisher scientific, Saint Aubin, France), and those of C-

145 reactive protein (CRP) and lactate dehydrogenase (LDH) by turbidimetry. Peripheral blood
146 lymphocytes and monocytes were counted by a hemocytometer (Sysmex XN-10).

147 **Perforin mRNA expression**

148 RNA was extracted with Trizol on a frozen dry pellet of 100,000 PBMCs from volunteers and
149 resuspended in 20 μ l of DEPC-treated water. 8 μ l were then reverse-transcribed with random
150 hexamers using the Superscript III kit (Invitrogen). The cDNA was then amplified on a
151 LightCycler 480 (Roche) with the Roche Sybr green master kit with the appropriate primers
152 as technical triplicate. Perforin expression is estimated relative to GAPDH. Primer sequences:
153 perforin: AACTTTGCAGCCCAGAAGACC and GTGCCGTAGTTGGAGATAAGCC.
154 GAPDH: AGTTAAAAGCAGCCCTGGTG and AGTTAAAAGCAGCCCTGGTG.

155 **Statistical analysis**

156 Normality was assessed by the d'Agostino and Pearson test. A two-sided unpaired Student *t*
157 test or Mann-Whitney test was used to compare groups, as appropriate. A two-sided Pearson
158 or Spearman test evaluated correlations, as appropriate. A p-value of <0.05 was considered
159 statistically significant.

160

161 **Results**

162 **Patient characteristics**

163 Twenty-eight non-ICU volunteers (15 females and 13 males, 67.5 ± 20.8 years old,
164 symptomatic for 6.3 ± 9.5 days) and 26 ICU volunteers (13 females and 13 males, 70.6 ± 13.4
165 years old, symptomatic for 12.3 ± 7.3 days) were recruited. Their bioclinical characteristics
166 had been previously reported (26). Twenty-nine age- and sex-matched healthy controls (HCs)
167 were recruited in parallel (12 females and 17 males, 63.7 ± 19.0 years old).

168 **NK cell perforin expression in the course of severe SARS-CoV-2 infection**

169 Perforin expression was quantified in permeabilized $CD3^+CD56^+$ NK cells by flow cytometry
170 (Fig. 1). As compared with HCs, we observed a decrease in the percentage of perforin-
171 positive NK cells in patients (69.9 ± 17.7 vs 78.6 ± 14.6 %, $p = 0.026$), particularly in ICU
172 patients (67.5 ± 19.9 vs 78.6 ± 14.6 %, $p = 0.037$), as shown in Figure 2A. The frequency of
173 perforin-expressing NK cells was independent of corticotherapy, either dexamethasone,
174 prednisolone, methylprednisolone or hydrocortisone (supplementary Fig. 1A), and
175 oxygenotherapy, either supplemental oxygen therapy or mechanical ventilation
176 (supplementary Fig. 1B).

177 If this perforin deficiency were genetically determined, one would expect patients with low
178 NK perforin expression to also have low T cell perforin expression. We previously measured
179 perforin expression in the T8 cells of patients we recruited (26). Yet, we observed no
180 correlation between the proportion of perforin-positive NK cells and the proportion of
181 perforin-positive T8 cells in ICU and non-ICU patients ($r = 0.195$, $p = 0.167$, Fig.2B). This
182 observation is a first argument against the hypothesis of a primary perforin deficiency as a
183 driver of COVID-19.

184 In addition to the frequency of perforin-positive NK cells, we evaluated the amount of
185 perforin per NK cell using the medianmean fluorescence intensity (MFI) of NK cells labeled

186 with the anti-perforin monoclonal antibody. As shown in Figure 2C, perforin expression in
187 NK cell was elevated in ICU patients ($59,599 \pm 63,168$ vs $54,380 \pm 86,750$ arbitrary units, $p =$
188 0.059), and even more in non-ICU patients ($120,130 \pm 75,926$ vs $54,380 \pm 86,750$ arbitrary
189 units, $p < 10^{-4}$), as compared with healthy controls. These findings, in line with previous data
190 (27), are a second argument against the hypothesis of a primary perforin deficiency as a driver
191 of COVID-19.

192 **Perforin expression and COVID-19 severity**

193 We tested the hypothesis of a causal link between the ability to produce perforin and the
194 severity of SARS-CoV-2 infection. As shown in Fig. 2A, there was no difference in the
195 percentage of perforin-positive NK cells between ICU and non-ICU patients (67.5 ± 19.9 %
196 vs 72.0 ± 15.5 %, $p = 0.497$), whereas COVID-19 was more severe in the former than in the
197 later. Secondly, we looked for correlations between perforin expression and established
198 markers of severity (28). The frequency of perforin-expressing NK cells was linked neither to
199 C-reactive protein ($r = 0.231$, $p = 0.136$, Fig. 3A), nor to lactate dehydrogenase ($r = 0.131$, $p =$
200 0.507 , Fig. 3B), nor to lymphocyte count ($r = 0.025$, $p = 0.867$, Fig. 3C), nor to monocyte
201 count ($r = 0.093$, $p = 0.531$, Fig. 3D). There was not any negative correlation between the
202 median level of perforin expression in NK cells and CRP ($r = 0.086$, $p = 0.588$, supplementary
203 Fig. 2A), LDH ($r = -0.240$, $p = 0.228$, supplementary Fig. 2B), lymphocyte count ($r = 0.270$, p
204 $= 0.069$, supplementary Fig. 2C), or monocyte count ($r = 0.088$, $p = 0.560$, supplementary
205 Fig. 2D).

206 Finally, we rationalized that, if a primary defect in perforin production was involved in the
207 severity of COVID-19, low perforin expression should be predictive of an adverse prognosis.
208 Yet, as shown in Figure 3E and 3F, the percentage of perforin-positive NK cells (63.8 ± 21.9
209 vs. 78.3 ± 11.4 %, $p = 0.243$) and the median perforin expression per NK cell ($34,139 \pm$

210 31,258 vs. $124,770 \pm 137,681$, $p = 0.110$) were not higher in ICU patients who survived than
211 in those who did not.

212 **Perforin expression and cytokines**

213 As we did not find any argument for a primary deficiency in perforin production in patients,
214 we looked for other potential causes of the low frequency of NK cells harboring perforin we
215 had observed. IFN- α and IL-12 are known to induce perforin expression in NK cells (25, 29).
216 We therefore looked for, but did not find any positive correlation between the plasma level of
217 each cytokine and the frequency of perforin-positive NK cells ($r = -0.283$, $p = 0.049$, Fig. 4A
218 and $r = -0.190$, $p = 0.196$, Fig. 4B, respectively) or the NK cell content in perforin ($r = 0.021$,
219 $p = 0.890$, Fig. 4D and $r = -0.255$, $p = 0.084$, Fig. 4E, respectively). By contrast, IL-6 is
220 known to inhibit perforin expression in NK cells (30). Therefore, we sought a link between
221 NK cell perforin expression and IL-6 production. To this aim, we measured IL-6 in plasma.
222 However, we did not find a significant negative correlation between the percentage of
223 perforin-positive NK cells ($r = -0.198$, $p = 0.181$, Fig. 4C) or the density in perforin in NK
224 cells ($r = 0.129$, $p = 0.397$, Fig. 4F) and IL-6 plasma levels.

225 **Perforin expression and perforin consumption**

226 Another explanation for the low frequency NK cells expressing perforin we unveiled might be
227 consumption. To test this possibility, we determined cell surface expression of the
228 degranulation marker CD107a. Indeed, we observed a clear negative correlation between the
229 percentage of CD107A-positive NK cells and the percentage of perforin-positive NK cells (r
230 $= -0.488$, $p = 10^{-4}$, Fig. 5A). There was also a negative correlation between CD107A cell
231 surface expression and the intracellular perforin level ($r = -0.269$, $p = 0.051$, Fig. 5B). This is
232 a strong argument in favor of perforin release as a cause of the low frequency of perforin-
233 positive NK cells. Of note, as compared with controls, the frequency of perforin
234 degranulation, as evaluated by CD107a expression, was elevated in ICU patients (31.7 ± 25.3

235 % vs 17.3 ± 20.2 %, $p = 0.014$), but not in non-ICU patients (6.2 ± 9.7 % vs 17.3 ± 20.2 %, p
236 $< 10^{-4}$) where it was even low (Fig. 5C). The same tendency, but less significant, was
237 observed for T8 cells (Fig. 5D). In our cohort, the mean duration of symptomatology was 7
238 days for non-ICU patients and 12 days for ICU patients. Therefore, the low percentage of
239 CD107A-positive NK cells we observed in non-ICU participants is in line with the previous
240 report of a defect in NK degranulation during the first week of severe disease (31).

241 **Perforin expression in NK cells at distance from infection**

242 To definitively rule out the hypothesis of a constitutive quantitative deficiency in perforin as a
243 cause of severe COVID-19 in cases of SARS-CoV-2 infection, we measured *PRF1*
244 expression in 6 volunteer PBMCs one year after the acute episode of infection. At that time,
245 their PRF1 mRNA was no different from that of 5 HCs (102.3 ± 60.6 vs. 59.4 ± 54.7 arbitrary
246 units, $p = 0.248$, Fig. 6A). Likewise, there was no difference in the frequency of perforin-
247 positive NK cells (78.0 ± 10.0 % vs. 78.6 ± 14.6 %, $p = 0.893$, Fig. 6B) between 12 patients a
248 year after the acute infection and HCs.

249

250

251 **Discussion**

252 SARS-CoV-2 appears to be able to block interferon production and signaling at the early
253 phase of infection (32). Consequently, COVID-19 patients present with an impaired
254 interferon type 1 (IFN1) activity (33). This allows its replication during an asymptomatic
255 phase. In some patients, this results in an acute immune activation including a cytokine storm
256 that might be co-responsible for the lung lesions that determine the prognosis (6). A key
257 question is to unravel the reasons why individuals who develop a severe form of COVID-19
258 fail to control this virus-induced immune response. Paradoxically, among the possibilities is a
259 faint cytotoxic activity. Perforin impairment may result in inflammatory cytokine
260 overproduction by the cytotoxic cells, insufficient clearance of infected cells and activated
261 immune cells, and a regulatory T cell deficiency contributing to an acute immune activation
262 (25). Accordingly, a deficiency in perforin production or release may be responsible for the
263 HLH syndrome. As COVID-19 has fever, cytopenia, hyperferritinemia, increase in soluble
264 CD25, high triglyceridemia and low fibrinogenemia, and hemophagocytosis in common with
265 HLH (5), it is logical to propose cytotoxic deficiency as a driver of severe forms of COVID-
266 19.

267 In the present study, we did observe a decrease in the frequency of perforin-expressing NK
268 cells in patients hospitalized for COVID-19. Now, contradictory data on perforin expression
269 in NK cells and NK cytotoxicity have previously been reported in COVID-19 patients. Most
270 authors observed a decrease in NK perforin protein (34) (35) or mRNA (36) expression as
271 well as a decrease in NK cytotoxicity (37, 38) (39, 40), whereas Y. Jiang et al. found an
272 increase (41). Yet, in this latter study, mild COVID-19 was included, and the percentage of
273 perforin-positive NK cells was very low in healthy controls.

274 The low percentage of perforin-expressing NK cells we observed does not seem to be a
275 primary etiologic factor in severe forms, since it was (i) not concurrently present in T8 cells

276 and even contrasted with the high frequency of perforin-positive T4 cells we previously
277 reported (26), (ii) negatively correlated with a marker of degranulation, an argument in favor
278 of perforin consumption, (iii) neither linked to bioclinical markers of severity, nor predictive
279 of death, and (iv) not observed one year after the episode of acute infection. Interestingly,
280 earlier on we described a decrease in NK perforin expression in HIV-1-infected individuals of
281 approximately the same magnitude as what we are now reporting in COVID-19 patients (42).
282 Yet, HIV patients do not suffer from cytokine storms. Altogether these data argue against a
283 genetic background impairing perforin expression as a cause of severe COVID-19. In line
284 with this, various authors have underlined differences in COVID-19 and HLH immune
285 activation profiles. In particular, IL-6 and HScore (43) are usually lower in the former than in
286 the latter. Nonetheless, the perforin gene variant A91V, which encodes for a perforin protein
287 with impaired processing, was observed three times more frequently in 22 young patients with
288 severe forms of COVID-19 (44). Moreover, the two A91V-positive patients who presented
289 with a high HScore, a score of severity in HLH, progressed rapidly and died. It is therefore
290 possible that perforin deficiency plays a role in young patients, but not in older ones.

291 Lymphocyte DNA damage and apoptosis we have previously described in severe COVID-19
292 might contribute to the low frequency of perforin-positive NK cells we observed here (45-47).
293 This low frequency might also be due to the TGF β overproduction (40), to anti-IFNI
294 autoantibodies (48), or to the impaired IFNI production and signaling observed in COVID-19
295 patients (32, 33, 49), as IFNI is a major inducer of perforin expression (25). Yet, in opposition
296 to this scenario, we observed a negative - rather than positive - correlation between IFN- α
297 plasma levels and perforin expression in NK cells. As IFN- α plasma levels have been linked
298 to SARS-CoV-2 viral load (50) and, as a high viral load should provoke NK activation, this
299 anticorrelation might be explained by the fact that low perforin expression is the consequence
300 of NK degranulation.

301 Consequently, the most probable hypothesis is indeed that the reduced number of NK cells
302 harboring perforin in COVID-19 is the consequence of the release of this cytolytic factor in
303 the course of NK cytotoxicity. A major way in which NK cells kill is by delivering perforin
304 and granzyme B to inside the target cells, thereby triggering apoptosis (25). Prager et al.
305 reported that target cell contact reduced perforin in NK cells over time (51). Moreover,
306 exposure to target cells has been reported to downregulate perforin mRNA in NK cells (52).
307 Consequently, within 8 hours of their contact with the target cell, NK cells lose their cytotoxic
308 efficiency (53). Accordingly, we have shown here an inverse correlation between perforin
309 expression and degranulation in COVID-19 NK cells. It is therefore logical to expect that *in*
310 *vivo* SARS-CoV-2-infected cell elimination also results in a decrease in intra-NK perforin
311 level. Strikingly, the amount of perforin in COVID patients NK cells is increased. This might
312 be a consequence of NK cell activation reported by various authors (54-56). Yet, here again,
313 the inverse correlation between perforin load in NK cells and their degranulation argues for
314 the consumption of this cytotoxic mediator.

315 Whatever the causes of low perforin expression might be, our data argue for the
316 administration of IFNI at the early stage of SARS-CoV-2 infection, in order not to increase
317 the acute immune activation that may occur at later stages. In fact, we have already
318 demonstrated that pegylated-IFN- α 2 administration restores NK perforin expression in people
319 living with HIV-1 (42). It may also be noted that the positive effects of IFN treatment on the
320 discharge rate and mortality have been reported (57, 58).

321

322

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327 **Author Contributions**

328 LK, EB, SA, CL, and TV contributed to the conception and design of the flow cytometry
329 study, and acquired, analyzed and interpreted the cell surface marker data.

330 RC acquired, analyzed and interpreted the soluble marker data.

331 CM designed, performed, analyzed, and interpreted the RT-PCR analyses.

332 MM, LM, JYL, CR, PGC, SD, PL, AS, and TAT contributed to the conception and design of
333 the study, to patient enrollment and acquired, analyzed and interpreted the clinical data.

334 JE and PC contributed to the conception and design of the study, analyzed and interpreted
335 data, and wrote the first draft of the manuscript. All authors reviewed and approved the final
336 version.

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589 **Figure legends**

590 **Figure 1.** Representative gating strategy for the identification of CD56⁺ NK cells expressing
591 perforin and/or CD107A (lower right hand). The isotypic control is shown (upper right hand).

592 **Figure 2.** (A) Perforin expression in COVID-19 patient cytotoxic cells. Frequency of
593 perforin-expressing NK cells in patients and controls. Two to two comparisons were carried
594 out using the Student t-test or the Mann-Whitney test, as appropriate. (B) Correlation between
595 perforin expressions in NK cells and CD8⁺ T lymphocytes in all COVID-19 patients using
596 the Pearson test. (C) Perforin density in patient and control NK cells. Perforin content was
597 expressed in arbitrary units of median fluorescence intensity (MFI). Two to two comparisons
598 were carried out using the Student t-test or the Mann-Whitney test, as appropriate. Each dot
599 represents a participant.

600 **Figure 3.** (A, B, C, D) Absence of link between the frequency of perforin expression in NK
601 cells and markers of disease severity. Lack of correlation between the percentage of perforin-
602 positive NK cells and CRP (A), LDH (B), lymphocyte count (C), and monocyte count (D).
603 The Pearson test or the Spearman test were used, as appropriate. (E, F) Absence of difference
604 in the frequency of perforin-positive NK cells (E) and in the perforin density in NK cells (F)
605 between ICU patients who survived or not. MFI, median fluorescence intensity. Differences
606 were evaluated using the the Mann-Whitney test. Each dot represents a participant.

607 **Figure 4.** Correlations between the frequency (A, B, C) and intensity (D, E, F) of perforin
608 expression in NK cells on one hand and IFN- α (A, D), IL-12 (B, E), and IL-6 (C, F) plasma
609 levels on the other hand, as calculated with the Spearman test. MFI, median fluorescence
610 intensity. Each dot represents a participant.

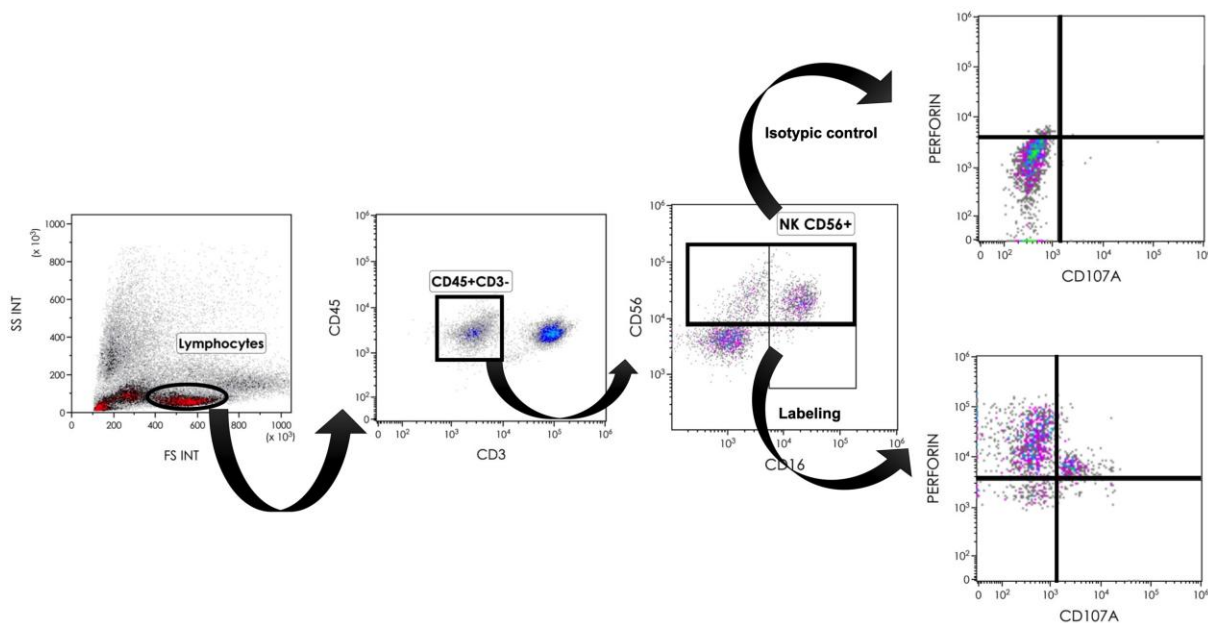
611 **Figure 5.** (A, B) Correlation between perforin expression in NK cells and NK cell
612 degranulation. The frequencies of perforin-harboring NK cells and of NK cells displaying the
613 degranulation marker CD107A at their surface are shown (A). The median perforin content in

614 NK cells and the frequency of NK cells displaying the degranulation marker CD107A at their
615 surface are shown (B). MFI, median fluorescence intensity. The Spearman test was used to
616 estimate the correlations. (C, D) Frequency of NK cells (C) and T8 cells (D) expressing the
617 cell surface marker of degranulation CD107A. Two to two comparisons were carried out
618 using the Student t-test or the Mann-Whitney test, as appropriate. Each dot represents a
619 participant.

620 **Figure 6.** (A, B) Normalization of perforin expression in patient NK cells one year after the
621 acute phase of SARS-CoV-2 infection. PRF1 mRNA (A) and the frequency of perforin (B) in
622 the NK cells of HCs and patients who had recovered. The Student t-test or the Mann-Whitney
623 test were used, as appropriate. Each dot represents a participant.

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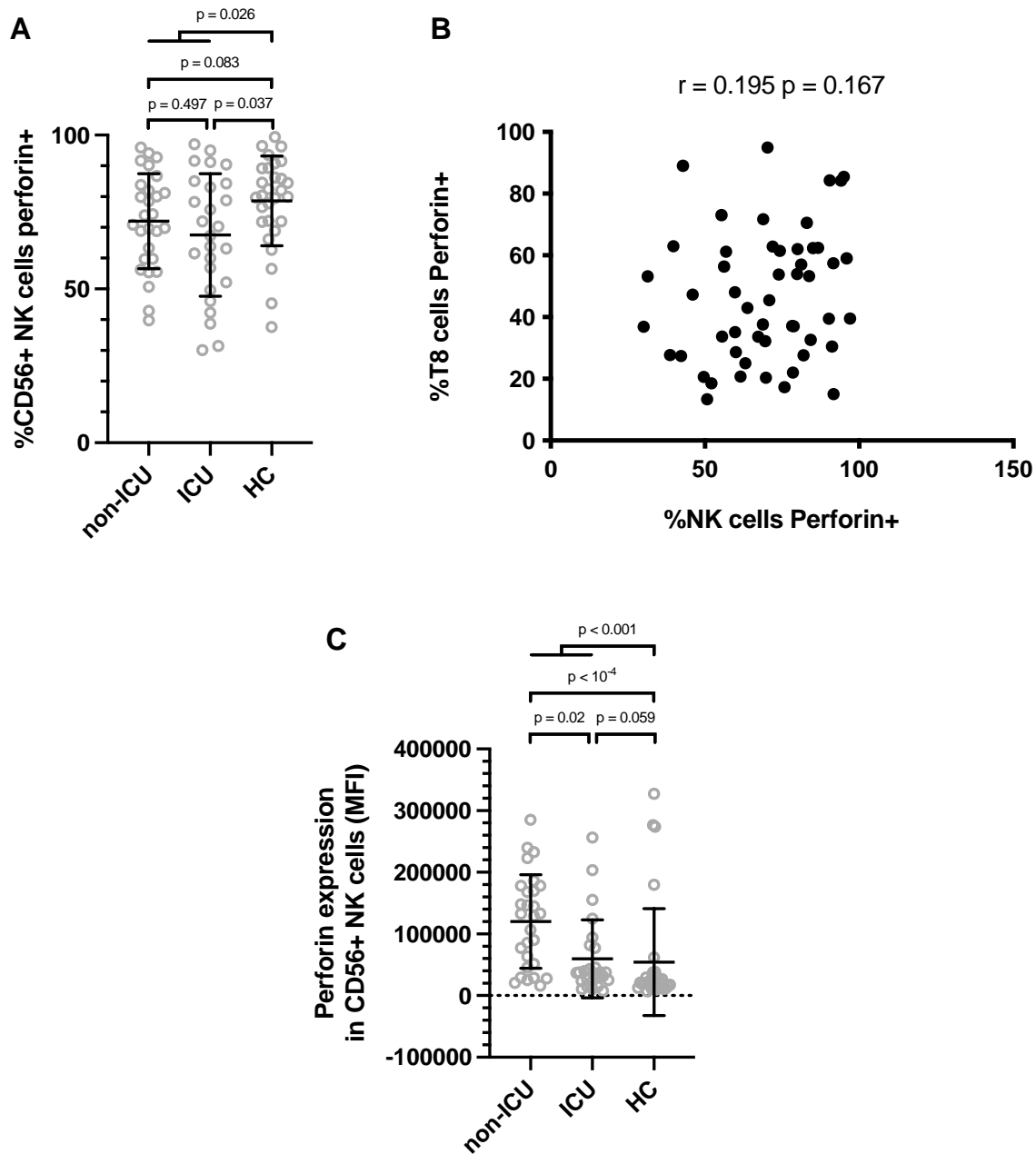
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Figure 1

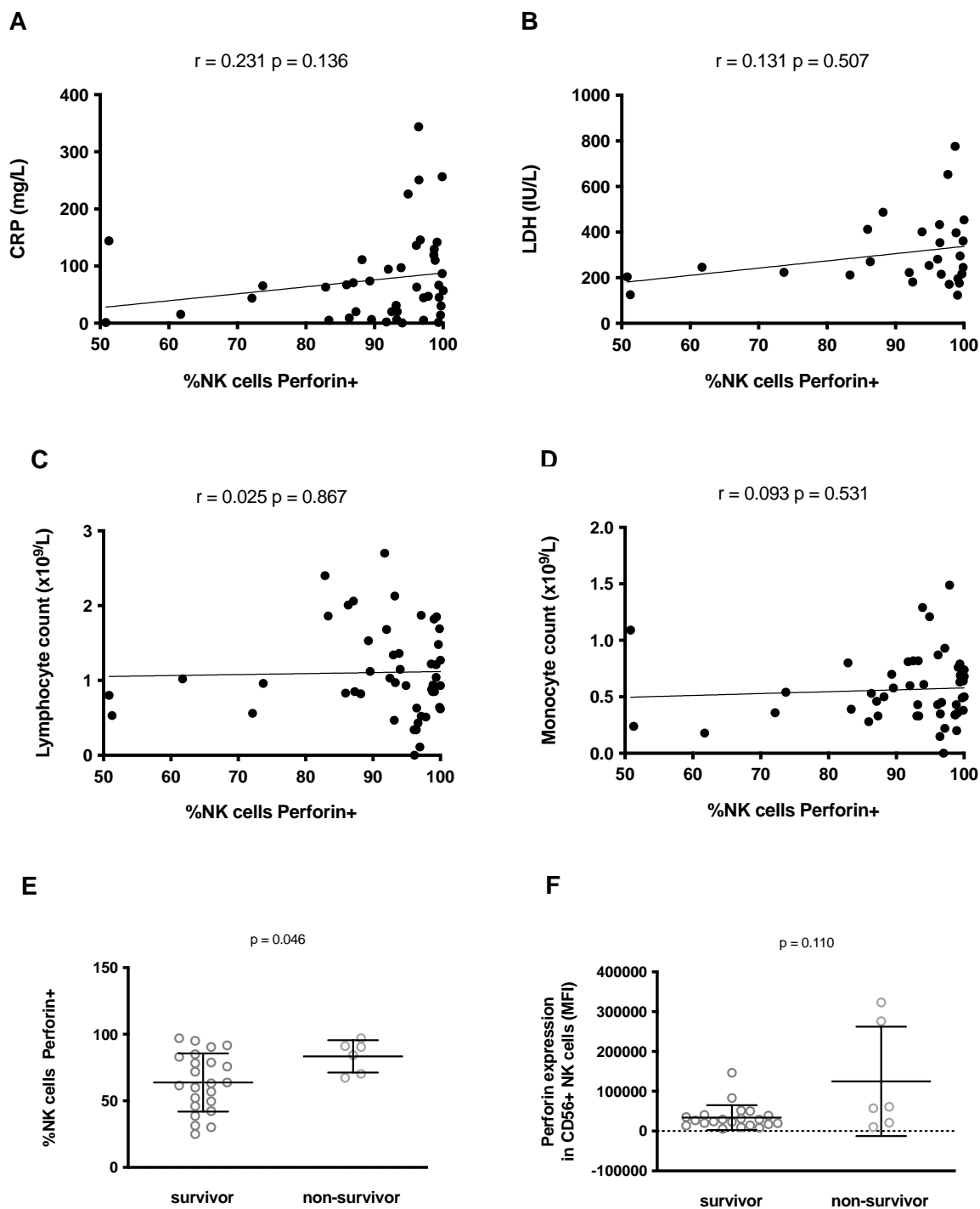
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Figure 2

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Figure 3

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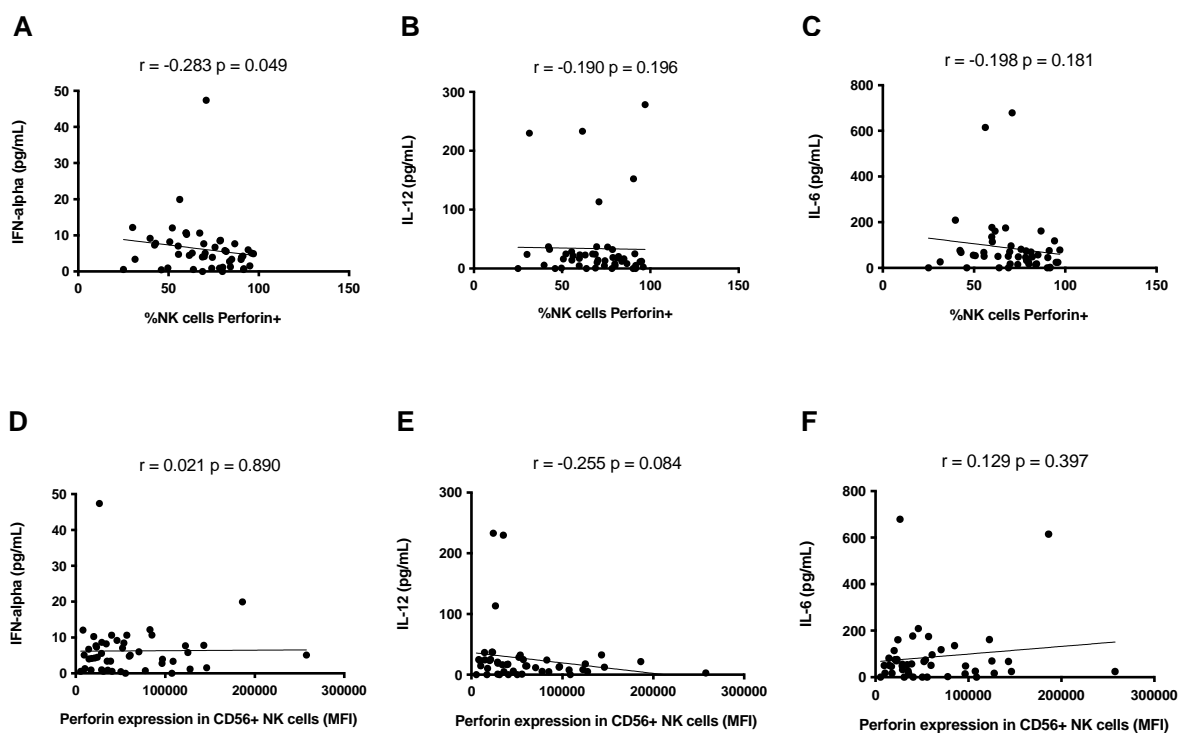
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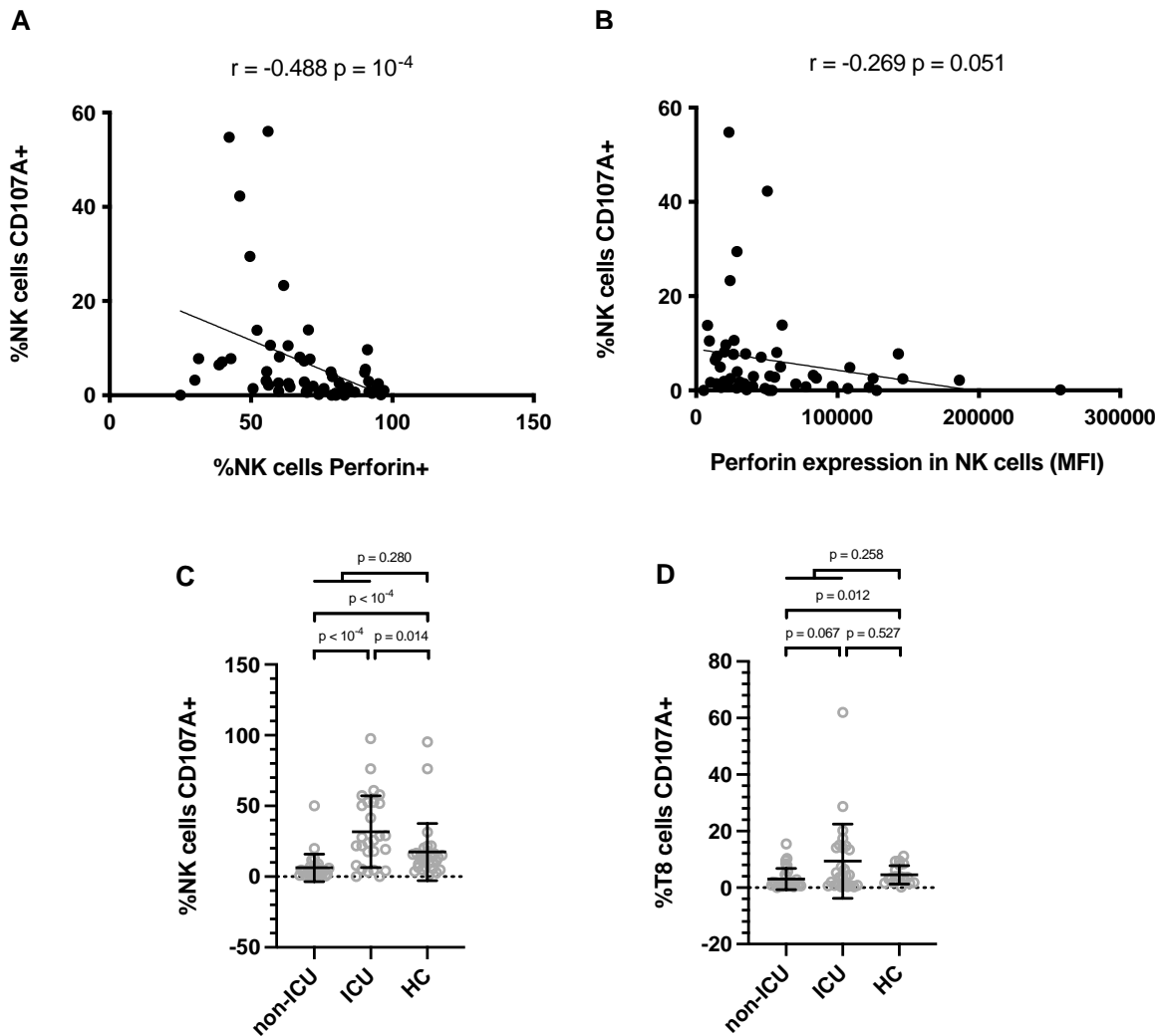
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Figure 4

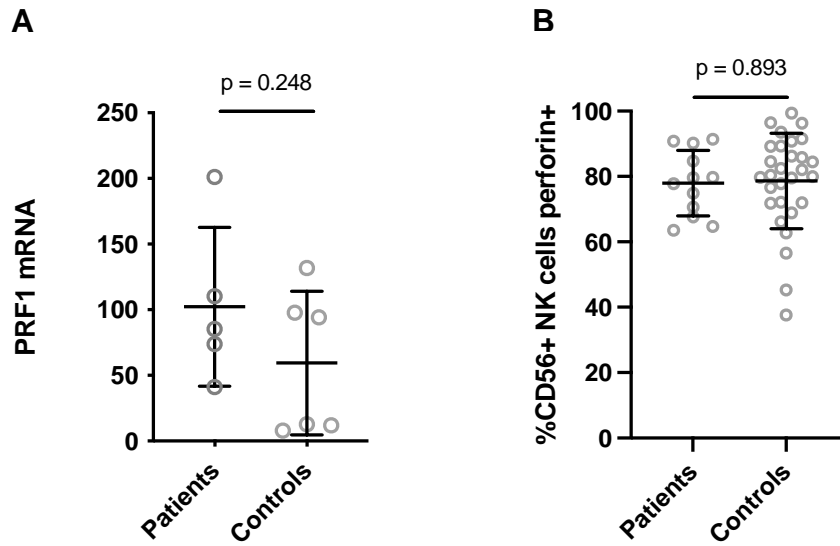
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Figure 5

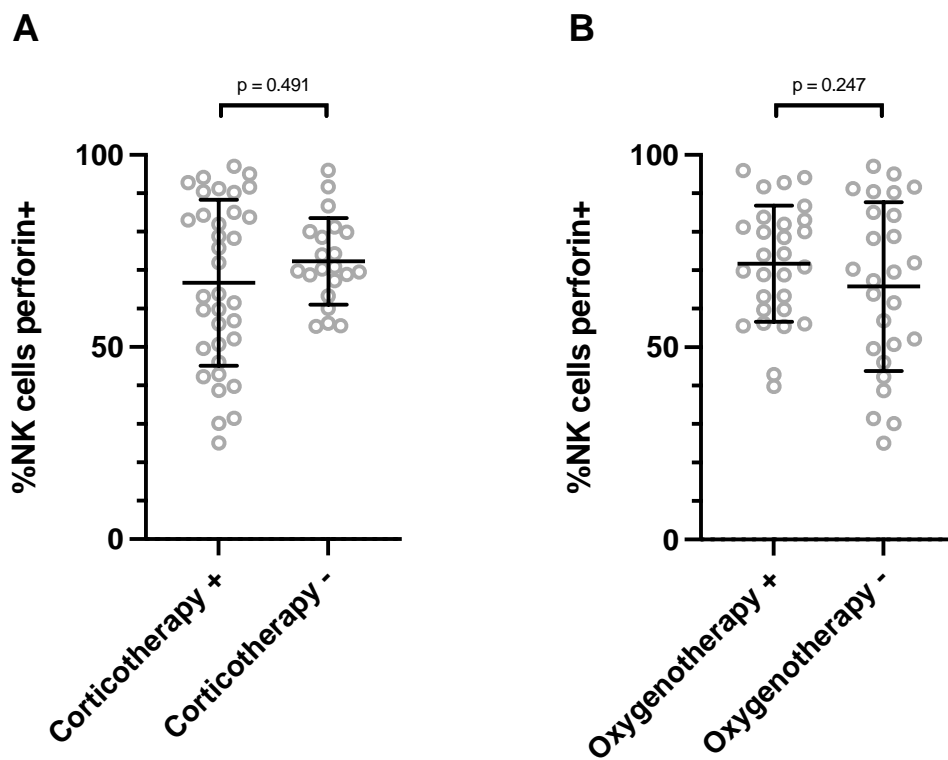
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Figure 6

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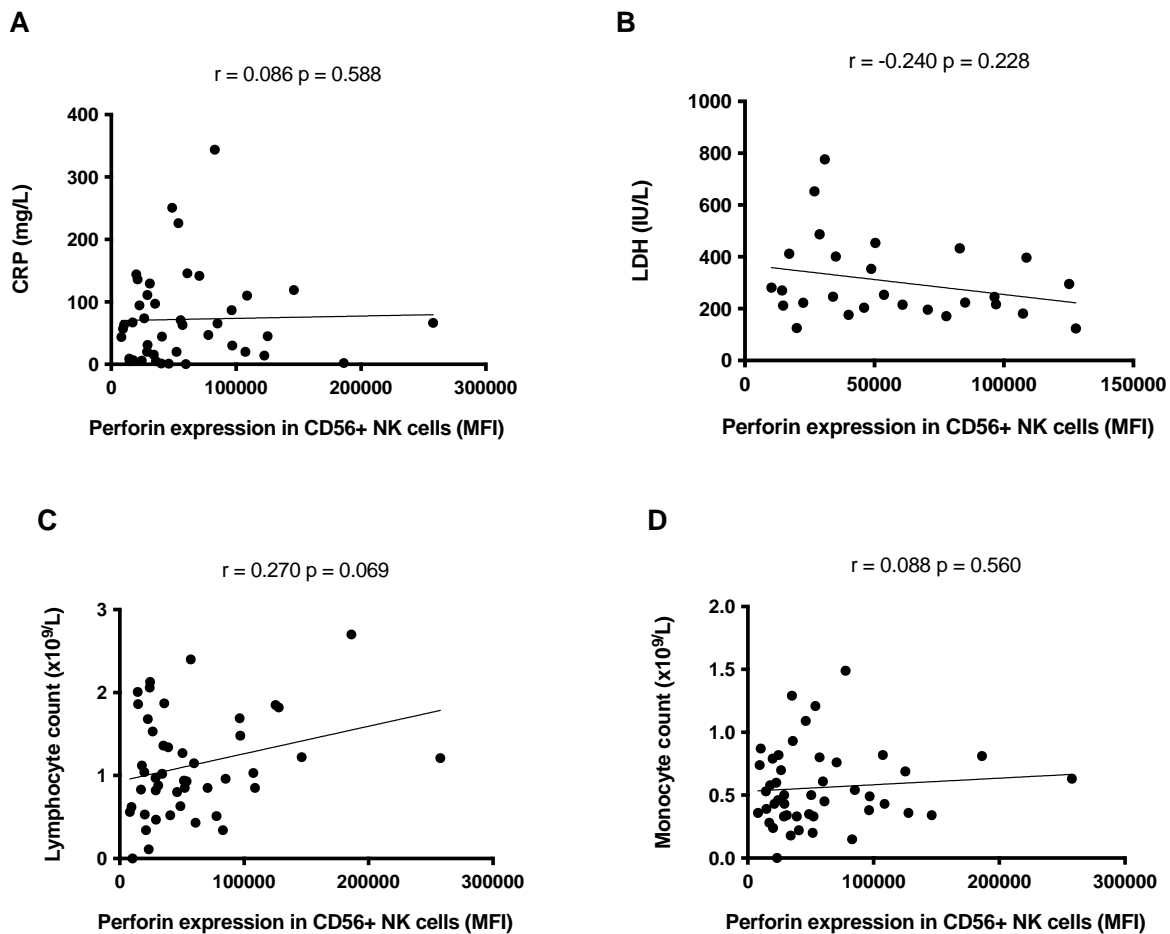


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Supplementary Figure 1

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Supplementary Figure 2

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