A Greater increase in C5aR1-level at onset and a smaller decrease in IgG-level after recovery in severer COVID-19 patients: A new analysis of existing data with Ishida's t-test¹ and t-test²

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Original Research

Title:

A Greater increase in C5aR1-level at onset and a smaller decrease in IgG-level after recovery in severer COVID-19 patients: A new analysis of existing data with Ishida's t-test1 and t-test2

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Abstract

It is important to exactly identify the difference in the changes in a receptor C5aR1-levels at onset and Immunoglobulin G (IgG)-levels after recovery between severe (acute respiratory distress syndrome) and nonsevere (pneumonia) coronavirus disease 2019 patients to reduce the severity of the disease and prevent reinfection with the causative virus. 2012 literature studies were selected from the PubMed Central® databases with keywords of (((covid-19) AND individual) NOT review) AND C5a / IgG. Three studies showing individual expression levels of C5a/C5aR1 / antibody in the patients before and after the passage of time without significance were selected by assessing the literatures. We extracted dynamics where the slope values of the regression line between the initial (X) and changed level (C) with passage of time were not zero significantly ($p < 0.05$) using SAS-JMP-10. We examined the significance of C in the patients with Ishida's t-test¹ and that of the difference in C between the severe and nonsevere patients with Ishida's t-test², which used the values on the above regression line as the expected values of C . These tests suggested a greater increase in C5aR1-levels at onset and a smaller decrease in IgG-levels after recovery in severe patients than in nonsevere patients.

Introduction

COVID-19 (Coronavirus disease 2019) is a new pandemic disease caused by infection with SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). C5a anaphylatoxin and its receptor C5aR1 play a key role in the initiation and maintenance of several inflammatory responses by recruiting and activating neutrophils and monocytes in the lungs¹. It is important to know exactly the difference in changes in C5aR1 levels at onset and antibody, especially Immunoglobulin G (IgG), levels after recovery, between severe and nonsevere COVID-19 patients to reduce the severity of the disease and prevent reinfection with SARS-CoV-2. Carvelli et al.² reported that there was no statistically significant change in the concentration of C5a desArg (complement C5a removal of the C-terminal arginine) in plasma, or % C5aR1 (receptor for C5a)-expressing neutrophils and monocytes between < 72 h and days 5 - 10 after the beginning of hospital care from pneumonia (with two-tailed Wilcoxon signed-rank tests³) and ARDS (acute respiratory distress syndrome) patients with Kenward-Roger method^{4,5}. Chen et al.⁶ reported that there was no statistically significant change in the IgM, IgG, and IgA titers specific to the spike protein between hospital discharge and follow-up visit between 21 days and 28 days after discharge among nonsevere and severe patients except anti-RBD (receptor binding domain) IgA and neutralization

activities with paired t-test⁷ for normal distribution and Mann-Whitney U⁸ for non-normal distribution. Yang et al.⁹ reported that there were no significant changes in the levels of antibody against SARS-CoV-2 surface spike protein RBD in either recurrent-positive or nonrecurrent-positive patients between one and two weeks postdischarge. Additionally, there were no significant changes in the levels in recurrent-positive patients between one week before recurrent-positive detection and the time of the detection or between detection time and one week after detection with Mann-Whitney U tests⁸.

However, we found that some of these changes over time (*C*) were dependent on their initial levels (*X*) and not suitable for analysis by conventional statistical tests. We developed new statistical tests (Ishida's t-test1 and t-test2) that correctly examine the above changes and then, we analyzed the above changes with Ishida's t-test1 and t-test2 to obtain different results from their papers as follows (research funding was not provided by any organ. Approval Number of Clinical Ethics Committee in Suzuka University of Medical Science is No. 322 (Judgment that it does not correspond to clinical study). UMIN-CTR ID: UMIN000035831).

Results

70 Selection of literatures.

71

72 According to the procedure for selection of literatures described in Methods and in the

73 first half of Figure 1, we selected one study on C5a/C5aR1² and two studies on

74 antibodies^{6,9} in COVID-19 patients from 2012 literature studies selected from the PMC

75 (PubMed Central®) databases with keywords of (((covid-19) AND individual) NOT

76 review) AND C5a / IgG. We analyzed p-values of slope values (β) of 65 regression lines

between X and C made by the method of least squares (E -line) (Figure 2 and Extended Figure 1a and 1b) of 39 dynamics and 26 subdynamics (Table 1 and Extended Table 1) described in the three literatures.

Extraction of dynamics dependent on X .

According to the procedure for extraction of dynamics described in Methods and in the latter half of Figure 1, we extracted eighteen dynamics and twelve subdynamics dependent on X (Table 1). C -axis intercept (α), slope value (β), X -axis intercept ($\gamma (= -\alpha/\beta)$) of E -line, standard error (SE), standard deviation ($SD (=SE\sqrt{N})$), mean of C (Mc), p -value (p), and effect size (Cohen's d^{10} ($d (=|Mc|/SD)$) for β of extracted dynamics were shown on upper left of Table 2. All of β of E -line for 18 dynamics and 12 subdynamics were significantly ($p < 0.05$) negative. Thus, in these dynamics, E was significantly inversely proportional to X to the extent represented by p -value for β described on upper left of Table 2.

Analysis of the extracted dynamics with Ishida's test1

We analyzed positive (Mp) and negative (Mn) component of mean (Me) of expected value (E) of C , Me , standard deviation (SD_I), p -value (p_I), effect size (d_I) for Me of the extracted dynamics with Ishida's t-test1 (upper right of Table 2). When $p_I < 0.05$, C also significantly inversely nearly proportional to X to the extent represented by p -value for

Me described on upper right of Table 2. In one dynamic (109) and five sub-dynamics (101s, 109n, 109s, 10yn and 10ws), the *E* of all subjects decreased with passage of time from hospital discharge to days 21-28 after hospital discharge. However, in 13 dynamics (081, 091, 092, 093, 094, 101, 107, 108, 10y, 10w, 211, 212 and 221) and 5 sub-dynamics (107n, 108n, 10ys, 10wn and 10ws), the *E* of those subjects whose *X* were less than γ increased and those of other subjects decreased with passage of time significantly to the extent represented by p-value for *Me* described on upper right of Table 2.

Generally, measuring of values before and after treatment included *RTM* (regression to the mean)¹¹. Therefore, the above *Me* should subtract *Me* of placebo control for the passage of time. However, no placebo control for passage of time was described in all of the three studies^{2,6,9} and others (33 studies on C5a/C5aR1 and 1976 studies on antibodies) described in Figure 1. Therefore, we could not get *Me* free from *RTM*¹¹.

Analysis of the extracted dynamics with Ishida's test²

As we could not get *Me* free from *RTM*¹¹, we analyzed the difference between *Me* of particular group *k* and *Me* of particular group *l* (*dMe*) under the assumption that at least one group is significantly different from *RTM*¹¹, if there is a significant difference between the two groups.

We analyzed the difference between dMe , standard deviation (SD_2), p-value (p_2), effect size (d_2) for dMe of the related two dynamics with Ishida's t-test² (lower of Table 2). The dMe of pneumonia (081, 091 and 093) and ARDS (082, 092 and 094) patients; and nonsevere (107n - 109n and 10yn) and severe (107s - 109s and 10ys) patients was significant to the extent represented by p-value for dMe described in Table 2. There was risk of bias due to limitation of databases, RTM^{11} by double measurements, limitation of numbers of subjects whose data were able to be estimated from the spots or lines drawn in figures and its estimation errors, and limitation of validity of Ishida's t-test¹ and t-test².

Discussions

The E -line crossed the C axis at α . When $\beta \neq 0$ with $p < 0.05$, this line also crossed the X axis at γ . Thus, the equation for this line was described as $E = \beta(X - \gamma)$. A value obtained from this equation in which X_i (X of particular subject i) was put was set as E_i . As deviation of C_i (C of particular subject i) from Mc is $C_i - Mc$. Thus, that of C_i from E_i was set as $C_i - E_i$. The equation showed that E was dependent on X . When $\beta \neq 0$ with $p < 0.05$ and p-value of Me was less than 0.05, C_i exists on or near E_i . Thus, C was also significantly nearly dependent on X . The E -line is made by the method of least squares.

Thus, $\sum_{i=1}^N (Ci - Ei)^2$ was minimum and $\leq \sum_{i=1}^N (Ci - Mc)^2$. Thus, When C was nearly significantly dependent on X , the expected value of C was not Mc but Ei . For that reason, Ishida's t-test1 was developed by replacing Mc and $[Ci - Mc]$ of paired t-test⁷ with Me and $[Ci - Ei]$ and Ishida's t-test2 was developed by replacing dMc (Mc of particular group $k - Mc$ of particular group l) and $d[Ci - Mc]$ ($[Ci - Mc]$ of particular group $k - [Ci - Mc]$ of particular group l) of unpaired t-tests (Student's t-test⁷, Welch's t-test¹², and Tukey's multiple comparison test¹³) with dMe and $d[Ci - Ei]$ ($[Ci - Ei]$ of particular group $k - [Ci - Ei]$ of particular group l) under the condition that C is significantly nearly dependent on X . The following was demonstrated using 092 dynamics as an example.

Figure 2 showed the E line for 092 (dynamics of % C5aR1-expressing neutrophils in ARDS patients between < 72 h and days 5-10 after the beginning of hospital care shown in extended Fig. 2a 2nd from left by Carvelli et al.²).

Figure 2.

The β for 092 was significantly negative ($p = 0.0002$) and p_I for Me was 0.00004.

Figure 2 showed that C existed on or near by the E -line and far from the Mc (Compare the difference between “●” and “+” and the difference between “●” and “x” in Figure 2.). Thus, $\sum_{i=1}^N (Ci - Ei)^2 < \sum_{i=1}^N (Ci - Mc)^2$ in 092. Thus, the expected value of Ci was Ei but not Mc and the expected value of Mc was Me because C of 092 was significantly nearly dependent on X .

As previously mentioned, measuring values before and after the passage of time included RTM^{11} but placebo control for the passage of time was not described in 2012 literatures on C5a/C5aR1 and antibodies selected from the PMC databases. We should plan clinical trials including placebo control for the passage of time, which will be easily obtained by measuring marker values again immediately after measuring their initial values.

Unlike the conclusions of Carvelli et al.², analysis of their paper with Ishida’s tests suggested the following under the assumption that at least one group was significantly different from RTM^{11} , as there was a significant difference between the two groups. 1) C5a levels of pneumonia patients decreased significantly ($p = 0.004$) with a large effect size ($d = 0.77$) but C5a levels of ARDS patients increased without significance ($p = 0.068$) and with a moderate effect size ($d = 0.38$) for at least 10 days after the beginning

of hospital care. 2) There was significant ($p = 0.002$) difference in changes in C5a levels between pneumonia patients and ARDS patients for the passage of time mentioned above with large effect size ($d = 1.01$). 3) The % C5aR1-expressing neutrophils of pneumonia patients increased slightly but significantly ($p = 3e-05$) with large effect size ($d = 9.78$) but those of ARDS patients increased ten times more than those of pneumonia patients significantly ($p = 4e-05$) with a large effect size ($d = 8.90$) for the passage of time mentioned above. 4) There was a significant ($p = 10e-08$) difference in the % C5aR1-expressing neutrophils between pneumonia patients and ARDS patients at the passage of time mentioned above, with a large effect size ($d = 11.4$). 5) The % C5aR1-expressing monocytes of pneumonia patients increased significantly ($p = 0.0002$) with a large effect size ($d = 1.29$) but those of ARDS patients increased two and half times more than those of pneumonia patients significantly ($p = 7e-10$) with large effect size ($d = 3.13$) at the passage of time mentioned above. 6) There was a significant ($p = 1e-05$) difference in the % C5aR1-expressing monocytes between pneumonia patients and ARDS patients at the passage of time mentioned above, with a large effect size ($d = 1.87$).

Unlike the conclusions of Chen et al.⁶, analysis of their paper with Ishida's t-test¹ suggested that not only anti-RBD IgA (109) but also other antibodies (anti-RBD IgM

(101), anti-S1 IgG (107), anti-NP (nucleoprotein) IgG (108), and anti-S1 IgA (10y))
 reduced with the passage of time by each Me with each p-value described on upper right
 of Table 2 and these reduced antibodies might be responsible for the declining trend of
 neutralizing activities (10w) by 1973 titers ($p = 3e-09$ with $d = 3.10$). Analysis with
 Ishida's t-test² suggested that anti-S1 IgG (107) and anti-NP IgG (108) of nonsevere
 patients decreased more than those of severe patients ($p = 6e-07$ with $d = 4.65$ and $p =$
 $1e-09$ with $d = 8.23$), respectively, under the same assumption described above.
 There were no significant differences in antibody levels between 211 and 212, between
 221 and 222, and between 229 and 22x with Ishida's t-test². Our results could not be
 differentiated from the conclusion of Yang et al.⁹, because we could not assuming that
 at least one of the two groups compared was significantly different from RTM^{11} .

Conclusion

When clinical trials observing effects of passage of time will be planed, placebo control
 for the passage of time and analysis of β of the regression line between X and C should
 be included. When $\beta \neq 0$ with $p < 0.05$, the clinical trials should be analyzed with
 Ishida's t-test¹ and t-test².

Methods

197 Approval by the Clinical Research Ethics Review Committee:

198 Approval for the study was granted by the Clinical Research Ethics Review Committee
199 at Suzuka University of Medical Science (Approval No. No322, Approval date:
200 2020/07/30).

201 Registration of study:

202 The study was registered in the UMIN Clinical Trials Registry (CTR ID:
203 UMIN000035831) as follows: Analysis of the effects of treatment with alternative
204 medicine and administration of medicine on the physiological value before treatment or
205 administration. The date of disclosure of the study information was 2019/02/12

206 Ethical Guidelines:

207 All experiments were performed in accordance with Ethical Guidelines for Medical and
208 Health Research Involving Human Subjects by Ministry of education and Ministry of
209 Health, Labor and Welfare in Japan and the Declaration of Helsinki.

210 Informed consent:

211 Our studies were only proposals concerning new t-tests and analysis of anonymized
212 data described in literatures concerning clinical studies with the t-tests without any

213 contact with patients. In that case, "written informed consent" was not required by the
214 Ethical Guidelines or able to be obtained. Thus, the authors did not obtain written
215 informed consent from any participants.

216 Data Sources:

217 The data source of this meta-analysis is all field of the PMC databases (up to June 1st,
218 2021).

219 Selection of literatures:

220 We searched literatures with keyword of “(((covid-19) AND individual) NOT review)
221 AND C5a” and “(((covid-19) AND individual) NOT review) AND IgG”. Then, we
222 selected literatures obtained by keyword search with questioning “whether did the
223 literature show individual initial expression levels and the levels after passage of time of
224 C5a/C5aR1 during onset and antibody after recovery in patients without significance.

225 Data Extraction and Synthesis:

226 α , β , its SE , and its p -value of dynamics described in selected literatures were obtained
227 with SAS JMP 10 (Corporate Headquarters 100 SAS Campus Drive Cary, NC 27513,
228 USA). γ , SD , and d of the dynamics were obtained from $\gamma = -\alpha/\beta$, $SD = SE\sqrt{N}$, and d
229 $= |Mc|/SD$, respectively, where N indicated number of subjects. The dynamics where β

230 was not zero with significance ($p < 0.05$) were extracted from the above selected
 231 literatures.

232 Ishida's t-test1:

233 Ishida's t-test1 is a new two-tailed t-test fit for pair samples where $\beta \neq 0$ with $p < 0.05$.

234 Mp , Mn and Me , and SD_I , SE_I , t_I , and d_I were calculated using Excel where the
 235 following formulae were incorporated.

236 $Mp = \frac{1}{N} \sum_{i=1}^{Np} (Epi)$, where Np = the numbers of subjects having Ep , $Epi = Ep$ of
 237 particular subject i , and Ep = positive expected value of C .

238 $Mn = \frac{1}{N} \sum_{i=1}^{Nn} (Eni)$, where Nn = the numbers of subjects having En , $Eni = En$ of
 239 particular subject i , and En = negative expected value of C .

240 $Me = \frac{1}{N} \sum_{i=1}^N (Ei) = \frac{1}{N} \sum_{i=1}^{Np} (Epi) + \frac{1}{N} \sum_{i=1}^{Nn} (Eni) = Mp + Mn$.

241 $SD_I = \left[\frac{1}{N-1} \sum_{i=1}^N (Ci - Ei)^2 \right]^{\frac{1}{2}}$,

242 $SE_I = \left[\frac{1}{N-1} \sum_{i=1}^N (Ci - Ei)^2 \right]^{\frac{1}{2}} / \sqrt{N}$,

243 $t_I\text{-value} = |Me|/SE_I$ and $d_I = |Me|/SD_I$

244 The p_I were determined by inputting the number of degrees of freedom and t_I into Excel
 245 2019 T.DIST.2T function. A p_I of <0.05 was considered statistically significant.

246 In general, C include the measurement errors due to double measurement called RTM by

247 Galton¹¹. Thus, the marker values without the passage of time should be observed as
 248 placebo control for C . C should be cut off by the values of placebo control.

249 When there was no placebo for the changes with the passage of time, we examined the
 250 significance of the difference between C of the dynamics in patients in any two related
 251 groups with Ishida's t-test² described later to estimate significance of C under the
 252 assumption that at least one group is significantly different from RTM^{11} , if there is a
 253 significant difference between the two groups.

254 Ishida's t-test²:

255 Ishida's t-test² is a new two-tailed t-test fit for unpaired samples where $\beta \neq 0$ with $p <$
 256 0.05 . dMe , SD_2 , SE_2 , t_2 , and d_2 are calculated with using Excel where the following
 257 formulae are incorporated.

258 $dMe = Me \text{ of particular group } k - Me \text{ of particular group } l$

259 $SD_2 = \left\{ \sum_{j=1}^m \left[\sum_{i=1}^{Nj} (Ci - Ei)^2 \right]_j / \sum_{j=1}^m (Nj - 1) \right\}^{\frac{1}{2}}$, where m and Nj indicate the
 260 number of groups and the number of subjects of a particular group j respectively.

261 $SE_2 = \left\{ \sum_{j=1}^m \left[\sum_{i=1}^{Nj} (Ci - Ei)^2 \right]_j / \sum_{j=1}^m (Nj - 1) \right\}^{\frac{1}{2}} * \left[\frac{1}{Nk} + \frac{1}{Nl} \right]^{\frac{1}{2}}$

262 $t_2 = |dMe|/SE_2$ and $d_2 = |dMe|/SD_2$

263 When the number of groups was two, the p₂-values were determined by inputting the

number of degrees of freedom and t_2 into Excel 2019 T.DIST.2T function. When the number of groups is three or more than three, the p₂-values were determined by inputting the number of degrees of freedom, the number of groups, and t_2 into function “>ptukey (t-value*sqrt(2), the number of groups, the number of degrees of freedom,lower.tail=FALSE))” of open software R. A p_d-value of <0.05 was considered statistically significant.

Risk of bias in Methods:

There was risk of bias due to limitation of databases, RTM¹¹ by double measurements, limitation of numbers of subjects whose data were able to be estimated from the spots or lines drawn in figures and its estimation errors, and limitation of validity of Ishida’s t-test1 and t-test2.

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Author contributions statement

T.I. designed two new statistical tests, run the study and wrote the manuscript. K.T.,

G.W., N.T., J.K., H.T., Y.G., N.M. contributed to discussions for the study. All authors

reviewed the manuscript.

Additional information

Competing interests

The authors declare no competing interests associated with this manuscript. Funding

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Data availability

The authors declare that all data supporting the findings of this study are available

within the article and in a public (doi:10.1038/s41586-020-2600-6,

325 <https://doi.org/10.1371/journal.ppat.1008796>,
326 <https://doi.org/10.1080/22221751.2020.1837018>) or from the corresponding author
327 upon reasonable request.

328 **Figure legend**

329 **Figure1.** Procedures for literatures selection and extraction of C5a/C5aR1 or antibody
330 dynamics in COVID-19 patients.

331 **Figure 2.** *E*-line for 092. The solid diagonal line indicated *E*-line and that p-value of β
332 was < 0.05 . The dotted horizontal line indicated *Mc*-line and that p-value of *Mc* was $\geq$
333 0.05. ●, +, and x indicated points at (X_i, C_i) , (X_i, E_i) , and (X_i, Mc) , respectively. *E*-line,
334 092, β , X_i , C_i , E_i , and *Mc* were described in text.

335

Table 1. Contents of the included dynamics with their source.

081, 082	Dynamics of concentration (ng/mL) of C5a desArg in plasma from pneumonia (green: 081, n=19) and ARDS (red: 082, n=25) patients between T0 and T1 in extended Fig. 1b by Carvelli et al. ²
091 092	Dynamics of % C5aR1-expressing neutrophils in pneumonia (green: 091, n=5) and ARDS (red: 092, n=5) patients between T0 and T1 in extended Fig. 2a by Carvelli et al. ²
093 094	Dynamics of % C5aR1-expressing monocytes in pneumonia (green: 093, n=15) and ARDS (red: 094, n=17) patients between T0 and T1 in extended Fig. 2a by Carvelli et al. ²
101	Dynamics of the IgM titer specific to RBD from non-severe (101n, n=15) and severe (101s, n=5) patients between T3 and T4 in Fig. 6a anti-RBD IgM by Chen et al. ⁶
107	Dynamics of the IgG titer specific to S1 from non-severe (107n, n=11) and severe (107s, n=5) patients between T3 and T4 in Fig. 6a anti-S1 IgG by Chen et al. ⁶
108	Dynamics of the IgG titer specific to NP from non-severe (108n, n=11) and severe (108s, n=5) patients between T3 and T4 in Fig. 6a anti-NP IgG by Chen et al. ⁶
109	Dynamics of the IgA titer specific to RBD from non-severe (109n, n=10) and severe (109s, n=5) patients between T3 and T4 in Fig. 6a anti-RBD IgA by Chen et al. ⁶
10y	Dynamics of the IgA titer specific to S1 from non-severe (10yn, n=11) and severe (10ys, n=5) patients between T3 and T4 in Fig. 6a anti-S1 IgA by Chen et al. ⁶
10w	Dynamics of the NAb titer (IC50) from non-severe (10wn, n=11) and severe (10ws, n=5) patients between T3 and T4 described in Fig. 6a Nab by Chen et al. ⁶
211 212	Dynamics of IgM levels specific to RBD (titers) from non-recurrent-positive (211, n=35) and recurrent-positive (212, n=6) patients between T5 and T6 described in Fig. 4a IgM level by Yang et al. ⁹
221 222	Dynamics of IgM levels specific to RBD (titers) from recurrent-positive patients between T7 and T8 (221, n=27) and between T8 and T9 (222, n=23) described in Fig. 4c IgM level by Yang et al. ⁹
229 22x	Dynamics of NAb levels specific to RBD (titers) from recurrent-positive patients between T7 and T8 (229, n=21) and between T8 and T9 (22x, n=18) described in Fig. 4c Nab level by Yang et al. ⁹
n; number of subjects, T0: < 72 h after the beginning of hospital care, T1: days 5-10 after the beginning of hospital care, T3: the time point of hospital discharge, T4: days 21-28 after hospital discharge, T5: week 1 hospital discharge, T6: weeks 2 hospital discharge, T7: one week before recurrent-positive detection, T8: the time point of recurrent-positive detection, T9: one week after recurrent-positive detection, ARDS: acute respiratory distress syndrome, C5a des-Arg: Complement C5a removal of the C-terminal arginine, C5aR1: receptor for the C5a anaphylatoxin; S1, NP, and RBD: S1 protein, nucleoprotein, receptor binding domain of the spike proteins of SARS-CoV-2; Ig: Immunoglobulin, and NAb: neutralizing antibody.	

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Table 2. Analysis of the included dynamics in three studies^{2,6,9} with regression line and Ishida's t-test1 and t-test2.

Grp	β	γ	SD	p	d	Mp	Mn	Me	SD_1	p_1	d_1
081	-0.61	47.0	0.51	<.0001	1.19	2.60	-13.91	-11.30	14.77	0.004	0.77
082	-0.87	81.1	1.11	0.0007	0.79	12.64	-3.89	8.75	22.88	0.068	0.38
091	-0.36	101.5	0.05	0.0005	7.12	1.02	-0.02	1	0.10	3e-05	9.78
092	-0.94	99.9	0.09	0.0002	10.9	11.29	-0.87	10.42	1.17	4e-05	8.90
093	-0.65	94.6	0.40	<.0001	1.61	5.73	-0.41	5.32	4.11	2e-04	1.29
094	-0.86	94.1	0.24	<.0001	3.62	13.73	-0.67	13.06	4.17	7e-10	3.13
101	-0.81	3x10 ⁴	0.71	0.0007	1.15	8381	-8x10 ⁴	-7x10 ⁴	1x10 ⁵	0.017	0.70
101n	-0.87	4x10 ⁴	0.98	0.0162	0.89	1x10 ⁴	-7x10 ⁴	-5x10 ⁴	1x10 ⁵	0.174	0.44
101s	-0.72	-9583	0.11	0.0053	6.87	0	-1x10 ⁵	-1x10 ⁵	2x10 ⁴	6e-04	7.68
107	-1.01	4x10 ⁴	0.09	<.0001	11.3	2x10 ⁴	-4x10 ⁵	-3x10 ⁵	8x10 ⁴	3e-11	4.25
107n	-1.00	1x10 ⁴	0.02	<.0001	60.9	5310	-5x10 ⁵	-4x10 ⁵	2x10 ⁴	1e-15	25.77
107s	-1.18	1x10 ⁵	0.68	0.0306	1.73	6x10 ⁴	-2x10 ⁵	-1x10 ⁵	1x10 ⁵	0.128	0.86
108	-1.00	2x10 ⁵	0.05	<.0001	19.5	7x10 ⁴	-2x10 ⁶	-2x10 ⁶	4x10 ⁵	1e-12	6.13
108n	-1.00	2x10 ⁵	0.05	<.0001	18.9	1x10 ⁵	-3x10 ⁶	-3x10 ⁶	4x10 ⁵	2e-09	7.38
108s	-1.00	1x10 ⁵	0.60	0.0333	1.67	2x10 ⁴	-2x10 ⁵	-2x10 ⁵	1x10 ⁵	0.059	1.17
109	-0.42	-306	0.17	<.0001	2.47	0	-3260	-3260	2125	4e-05	1.53
109n	-0.23	-1346	0.27	0.0303	0.83	0	-1127	-1127	1167	0.014	0.97
109s	-0.41	-3275	0.18	0.0139	2.32	0	-7526	-7526	2960	0.005	2.54
10y	-0.82	90.2	0.28	<.0001	2.96	8.24	-2x10 ⁴	-2x10 ⁴	9263	2e-06	1.90
10yn	-0.70	-52.0	0.28	<.0001	2.55	0	-1x10 ⁴	-1x10 ⁴	8674	8e-04	1.43
10ys	-1.01	1303	0.03	<.0001	39.7	228	-3x10 ⁴	-3x10 ⁴	781	1e-07	37.35
10w	-0.94	546	0.13	<.0001	7.28	120	-2093	-1973	637	3e-09	3.10
10wn	-0.94	679	0.13	<.0001	7.27	254	-2380	-2126	748	3e-06	2.84
10ws	-0.93	255	0.04	<.0001	25.2	0	-1636	-1636	42	1e-07	38.92
211	-0.26	0.87	0.16	<.0001	1.66	0.04	-0.56	-0.53	0.57	4e-06	0.92
212	-0.34	0.72	0.17	0.0073	2.06	0.06	-0.72	-0.67	0.36	0.006	1.85
221	-0.23	0.33	0.11	<.0001	2.14	0.01	-0.12	-0.11	0.11	1e-05	1.04
222	-0.45	0.94	0.77	0.011	0.58	0.22	-0.13	0.10	0.82	0.579	0.12
229	-0.27	46.1	0.30	0.0047	0.70	5.33	-18.13	-12.80	57.92	0.323	0.22
22x	-0.80	69.57	0.29	<.0001	2.78	38.65	-53.97	-15.32	70.12	0.367	0.22
Gr k	Gr l	dMe	SD_2	p_2	d_2	Gr k	Gr l	dMe	SD_2	p_2	d_2
081	082	-20.1	19.8	0.002	1.01	109n	109s	6399	1908	4e-05	3.35
091	092	-9.42	0.83	1e-07	11.4	10yn	10ys	2x10 ⁴	7343	8e-04	2.72
093	094	-7.74	4.14	1e-05	1.87	10wn	10ws	-489	633	0.173	0.77
101n	101s	7x10 ⁴	1x10 ⁵	0.276	0.66	211	212	0.14	0.55	0.559	0.26
107n	107s	-3x10 ⁵	7x10 ⁴	6e-07	4.65	221	222	-0.21	0.56	0.198	0.37
108n	108s	-3x10 ⁶	4x10 ⁵	1e-09	8.23	229	22x	2.52	63.8	0.903	0.04

Abbr.: C-axis intercept (α), slope value (β), X-axis intercept (γ ($= -\alpha/\beta$)) of the regression line between the initial (X) and changed (C) value with treatment of a maker made by the method of least squares (E-line); positive (Mp) and negative (Mn) compartment of mean of expected value of C (Me); difference between Me of Group (Grp) k and Group (Grp) l (dMe); standard deviation and p-value of conventional (SD, p), Ishida's t-test1 (SD₁, p₁), t-test2 (SD₂, p₂); |Mc (mean of C)|/SD (d), |Me|/SD₁ (d₁), |dMe|/SD₂ (d₂); Contents and source of 081 - 22x were described in Table 1. Unit of Mp, Mn, Me, and dMe: ng/mL (081, 082), % (091, 092, 093, 094), titers for IC50 (10w, 10wn, 10ws, 229, 22x), titers (Others).

340 **Extended Figures and an Extended Table**

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342 **Extended Figure 1a. *E*-line for 091 - 109s. Contents of the dynamics with their source**

343 were described in Table 1 or Extended Table 1. Solid and dotted lines indicated that p-
 344 value of slope of *E*-line was < 0.05 and ≥ 0.05 , respectively.

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Extended Figure 1b. *E*-line for 10x - 22x. Contents of the dynamics with their source were described in Table 1 or Extended Table 1. Solid and dotted lines indicated that *p*-value of slope of *E*-line was < 0.05 and ≥ 0.05 , respectively.

Extended Table 1. Contents of the excluded dynamics with their source.

102	Dynamics of the IgM titer specific to ECD from non-severe (102n, n=11) and severe (102s, n=5) patients between T3 and T4 in Fig. 6a anti-ECD IgM by Chen et al. ⁶
103	Dynamics of the IgM titer specific to S1 from non-severe (103n, n=11) and severe (103s, n=5) patients between T3 and T4 in Fig. 6a anti-S1 IgM by Chen et al. ⁶
104	Dynamics of the IgM titer specific to NP from non-severe (104n, n=11) and severe (104s, n=5) patients between T3 and T4 in Fig. 6a anti-NP IgM by Chen et al. ⁶
105	Dynamics of the IgG titer specific to RBD from non-severe (105n, n=11) and severe (105s, n=5) patients between T3 and T4 in Fig. 6a anti-RBD IgG by Chen et al. ⁶
106	Dynamics of the IgG titer specific to ECD from non-severe (106n, n=11) and severe (106s, n=5) patients between T3 and T4 in Fig. 6a anti-ECD IgG by Chen et al. ⁶
10x	Dynamics of the IgA titer specific to ECD from non-severe (10xn, n=9) and severe (10xs, n=5) patients between T3 and T4 in Fig. 6a anti-ECD IgA by Chen et al. ⁶
10z	Dynamics of the IgA titer specific to NP from non-severe (10zn, n=11) and severe (10zs, n=5) patients between T3 and T4 in Fig. 6a anti-NP IgA by Chen et al. ⁶
213 214	Dynamics of IgG levels specific to RBD (titers) from non-recurrent-positive (213, n = 32) and recurrent-positive (214, n = 6) patients between T5 and T6 described in Fig. 4a IgG level by Yang et al. ⁹
215 216	Dynamics of IgA levels specific to RBD (titers) from non-recurrent-positive (215, n = 32) and recurrent-positive (216, n= 5) patients between T5 and T6 described in Fig. 4a IgA level by Yang et al. ⁹
217 218	Dynamics of all Ig levels specific to RBD (titers) from non-recurrent-positive (217, n=33) and recurrent-positive (218, n=6) patients between T5 and T6 described in Fig. 4a all Ig level by Yang et al. ⁹
219 21x	Dynamics of Nab levels specific to RBD (titers) from non-recurrent-positive (217, n=33) and recurrent-positive (218, n=6) patients between T5 and T6 described in Fig. 4a Nab level by Yang et al. ⁹
223 224	Dynamics of IgG levels specific to RBD (titers) from recurrent-positive patients between T7 and T8 (223, n=27) and between T8 and T9 (224, n=23) described in Fig. 4c IgG level by Yang et al. ⁹
225 226	Dynamics of IgA levels specific to RBD (titers) from recurrent-positive patients between T7 and T8 (225, n=21) and between T8 and T9 (226, n=20) described in Fig. 4c IgA level by Yang et al. ⁹
227 228	Dynamics of all Ig levels specific to RBD (titers) from recurrent-positive patients between T7 and T8 (227, n=27) and between T8 and T9 (228, n=25) described in Fig. 4c all Ig level by Yang et al. ⁹
Each abbreviation was described in Table 1.	