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**Transient expression of antinuclear RNP-A antibodies in patients with acute COVID-19 infection**

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19 **ABSTRACT**

20 *Introduction:* Viral infections have been implicated in the initiation of the autoimmune

21 diseases. Recent reports suggest that a proportion of patients with COVID-19 develop

22 severe disease with multiple organ injuries. We evaluated the relationship between

23 COVID-19 severity, prevalence and persistence of antinuclear and other systemic and

24 organ specific autoantibodies as well as SARS-CoV2 infection specific anti-nucleocapsid

25 (N) IgG antibodies and protective neutralizing antibody (Nab) levels.

26 *Methods:* Samples from 119 COVID-19 patients categorized based on their level of care

27 and 284 healthy subjects were tested for the presence and persistence of antinuclear and

28 other systemic and organ specific autoantibodies as well as SARS-CoV2 and neutralizing

29 antibody levels.

30 *Results:* The data shows significantly increased levels of anti RNP-A, anti-nucleocapsid

31 and neutralizing antibody among patients receiving ICU care compared to non-ICU care.

32 Furthermore, subjects receiving ICU care demonstrated significantly higher nucleocapsid

33 IgG levels among the RNP-A positive cohort compared to RNP-A negative cohort.

34 Notably, the expression of anti RNP-A antibodies is transient that reverts to non-reactive

35 status between 20-60 days post symptom onset.

36 *Conclusions:* COVID-19 patients in ICU care exhibit significantly higher levels of transient

37 RNP-A autoantibodies, anti-nucleocapsid, and SARS-CoV2 neutralizing antibodies

38 compared to patients in non-ICU care.

39

40 **Keywords**

41 SARS-CoV-2**,** COVID-19, Antinuclear antibody, RNP-A, Neutralizing antibody,

42 Nucleocapsid protein

43 **Abbreviations**

44 SARS-CoV-2       severe acute respiratory syndrome coronavirus 2

45 COVID-19       coronavirus disease 2019

46 ACE2       angiotensin-converting enzyme 2

47 ANA       antinuclear antibody

48 sVNT       surrogate viral neutralization test

49 Nab       neutralizing antibody

50 RNP-A       ribonucleoprotein A

51 MCTD       mixed connective tissue disease

52 ICU       intensive care unit

53 PSO       post symptom onset

54 N       SARS-CoV-2 nucleocapsid protein

55 IFA       Immunofluorescence assay

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## 1. Introduction

There is a strong association between viral infections and autoimmune diseases although the underlying etiology is not fully understood. Shoenfeld et. al. have elegantly demonstrated that pathogenic viruses can trigger and initiate a host of autoimmune diseases [1, 2]. Autoimmunity may manifest itself through molecular mimicry, bystander activation or epitope spreading [3-5]. The emergence of novel severe acute respiratory syndrome-Coronavirus 2 (SARS-CoV-2) poses a serious global public health threat that has infected over 590 million people globally with over 6.4 million deaths. Coronavirus disease 2019 (COVID-19) exhibits similarities to systemic autoimmune conditions including an association with increased incidence of autoantibodies [6-8]. It has been suggested that SARS-CoV-2 infection triggers a form of organ specific autoimmunity in predisposed patients [9]. Recently, Lerma et al. reported autoantibodies to nuclear antigens in 30% of SARS-CoV-2 patients, however, strong reactive autoantibodies were only detected in patients with prior history of autoimmune disease [8]. It is not clear from these studies whether any relationship exists between COVID-19 severity and the prevalence and persistence of autoantibodies. We describe the prevalence and transient expression of antinuclear antibodies, particularly anti-RNP-A autoantibodies, along with other systemic and organ specific autoantibodies in patients with mild to severe COVID-19 based on their level of care.

## 2. Materials and methods

### 2.1 Serum specimens

Remnant serum samples from 119 COVID-19 patients with positive RT-PCR results were collected from a clinical hospital laboratory between March 2020 and September

82    2021. All samples were de-identified to ensure patient confidentiality. Use of remnant

83    samples from COVID-19 infected patients was approved by the University of California,

84    San Francisco Institutional Review board (IRB protocol number 20-30387). Patient-

85    reported symptom onset date and indicators of disease severity were extracted from

86    electronic health records. Patients were categorized based on their level of care; patients

87    admitted to an intensive care unit at any time during the disease course were classified

88    as ICU patients, whereas those admitted to a hospital or managed as outpatients were

89    considered non-ICU patients. Sera from 284 apparently healthy subjects were procured

90    from commercial vendors. All samples were maintained at -20$^0$C for the duration of the

91    study. After thawing at room temperature, samples were briefly vortexed before testing in

92    singlicate.

93    *2.2 Autoantibody detection*

94        All samples were tested by the BioPlex 2200 ANA screen assay that detects 13

95    IgG autoantibodies simultaneously against dsDNA, chromatin, ribosomal P, SSA-52,

96    SSA-60, SSB, Sm, the Sm/RNP complex, RNP-A, RNP-68, Scl-70, centromere B, and

97    Jo-1 within a single serum sample (10). Serum samples of COVID-19 patients were tested

98    for the presence of anti-cardiolipin,anti-β2GPI IgG, IgM and IgA isotype antibodies, anti-

99    MPO, anti-PR3 and anti-GBM-IgG antibodies, anti-tTG and anti-Gliadin IgA and IgG

100   antibodies, as well as anti-CCP IgG antibodies using the BioPlex 2200 anti-phospholipid

101   syndrome (APLS) IgG, IgM and IgA, vasculitis panel IgG, gastrointestinal IgG and IgA as

102   well as the BioPlex 2200 anti-CCP IgG kits. The BioPlex 2200 ANA reports an antibody

103   index (AI) value in the range of 0.2–8.0 AI for all antibodies except anti-dsDNA for which

104   IU/mL is used. The cutoff for the anti-dsDNA antibody is 10 IU/mL and for all other

105  autoantibodies is 1.0 AI. Results are considered positive when there is at least one

106  positive result for the antibodies detected by this panel. Patient samples with RNP-A

107  positive results were confirmed by Kallestad Hep-2 ANA IFA kit at 1:40 and 1:80 titers.

108  *2.3 Multiplex SARS-CoV-2 surrogate virus neutralization test (plex-sVNT)*

109      The BioPlex 2200 sVNT assay is a bead-based multiplex assay that detects the

110  presence of SARS-CoV2 neutralizing antibodies in serum and/or plasma [11]. Essentially,

111  neutralizing antibodies compete with biotinylated-human ACE2-Fc protein for binding to

112  trimeric spike proteins that are coupled to beads. An assay cutoff of 25% inhibition for

113  ACE2-trimeric spike protein binding was established based on 99th percentile cutoff using

114  commercially available healthy normal, pregnancy and potential cross reactant samples.

115  *2.4 BioPlex 2200 SARS-CoV-2 IgG Assay Panel*

116      The BioPlex 2200 SARS-CoV-2 IgG assay is a multiplex assay that detects IgG

117  antibodies against the receptor-binding domain (RBD), Spike 1 (S1), Spike 2 (S2), and

118  nucleocapsid protein (N) of the SARS-CoV-2 virus. The assay is commercially available

119  outside of the United States (OUS). Essentially, uniquely classified beads are coated with

120  one of the four antigens independently and the amount of antibody captured by each

121  antigen is determined by the fluorescence of the attached PE. Raw data was calculated

122  in relative fluorescent intensity (RFI). The assay is calibrated using six distinct calibrator

123  levels for each marker and semi-quantitative results expressed in U/mL using 4-PL curve

124  fit. The presence of RBD, S1 and S2 IgG antibodies appear in infected as well as

125  vaccinated uninfected subjects as opposed to the nucleocapsid antibodies that are

126  predominantly present in infected subjects only.

127    *2.5 Statistical analysis*

128    Difference in antinuclear and other systemic and organ specific autoantibody

129    prevalence levels were evaluated using Fisher's exact test where statistical significance

130    was defined as $p<0.05$. The differences in neutralizing SARS-CoV-2 antibodies and anti-

131    N antibodies for patients with positive and negative RNP-A levels were assessed by two-

132    tailed t-test, where statistical significance is defined as $p<0.05$. Statistical analysis was

133    performed using *GraphPad Prism* 9.0 (version 9.4.0).

134    **3.  Results**

135    403 samples obtained from 284 apparently healthy subjects and 119 SARS-CoV-2

136    RT-PCR positive patients were included in this study.  Of the 119 SARS-CoV-2 RT-PCR

137    confirmed patients, 41 (34.5%) were admitted to the ICU while 78 (65.5%) patients were

138    classified as non-ICU because they were either admitted to the hospital or managed as

139    outpatients. Samples from patients receiving ICU care were collected an average of 23.3

140    days (7 - 88 days) post symptom onset while non-ICU samples were obtained 44.2 days

141    (range 5 - 88 days) post symptom onset. Matched analysis between the two patient

142    cohorts was restricted to samples collected up to 90 days post symptom onset to reduce

143    the impact of confounding variables. While 10.2% (29/284) of the healthy population

144    demonstrated antinuclear autoantibodies (ANA), the non-ICU patient cohort displayed a

145    prevalence of 17.9% (14/78) compared to an exceptionally high prevalence of 43.9%

146    (18/41) in the ICU cohort (Table 1). The majority of patients displayed reactivity to one

147    target autoantigen: RNP-A. Only 3 of 78 non-ICU and 2 of 41 ICU samples demonstrated

148    reactivity to more than one target antinuclear autoantibodies (data not shown). Compared

149 to the healthy group with RNP-A prevalence of 3.9%, the non-ICU and ICU sample

150 cohorts displayed significantly higher prevalence of 10.3% and 31.7% (p value 0.0401

151 and <0.0001) respectively (Table 1). An overall increase in RNP-A autoantibodies among

152 ICU patients compared to the non-ICU cohort suggests a progressive increase in antibody

153 levels as a function of disease severity (Table 2). We also sought to determine whether

154 ICU and non-ICU cohorts correlate with SARS-CoV-2 anti-N IgG antibody levels, a

155 disease specific marker, as well as neutralizing antibody levels that prevent/protective

156 against the disease.  Our data shows that the means of both anti-N IgG and Nab levels

157 are significantly different between the ICU and non-ICU patient cohorts (Table 2).

158 The observation that both anti-N IgG and Nab levels reach statistical significance

159 among the combined disease group (ICU and non-ICU) between RNP-A positive and

160 RNP-A negative cohorts (Table 2) is suggestive of a relationship between disease

161 severity and expression of RNP-A autoantibodies. At the same time, all other systemic

162 and organ specific autoantibodies failed to exhibit any appreciable difference between the

163 two diseased cohorts (Table 3). It is important to note that 3/41 PCR positive ICU patients

164 and 5/78 non-ICU patients tested negative by the anti-N IgG assay. Lack of anti-N IgG

165 antibodies in approximately 7% of PCR positive patients is probably due to late sero-

166 conversion and/or higher sensitivity of RT-PCR assay.

167 Production and persistence of autoantibodies against RNP-A was examined by

168 analyzing multiple blood draws from 10 ICU patients with positive reactivity. Nine out of

169 ten patients sero-converted reaching peak levels between 13-31 days post-symptom

170 onset. Of these nine sero-conversion samples, six displayed RNP-A peak levels between

171 2-6 times the assay cutoff levels. Nonetheless, sero-conversion proved transient because

172  all nine samples reverted to non-reactive status between 20-60 days post-symptom onset

173  (Figure 1). Of the nine RNP-A positive samples, four patients also demonstrated transient

174  expression of other autoantibodies including anti-MPO IgG, anti-tTG IgG and anti-CCP

175  antibodies. Importantly, temporal profiles of RNP-A among serial draws parallel anti-N

176  IgG antibody expression levels, although the levels of anti-N antibody never became

177  negative (Figure 2). In contrast, other systemic and organ specific autoantibodies failed

178  to exhibit any appreciable change with disease progression. Next, we evaluated ANA in

179  RNP-A positive samples using an IFA assay as a confirmatory test. All RNP-A positive

180  samples were confirmed positive for ANA autoantibodies by IFA using Kallestad HEp-2

181  substrate at 1:40 and 1:80 titers. All samples demonstrated nuclear speckled pattern as

182  shown in the slide image (Figure 3). One patient with three blood draws taken between

183  days 27-60 post symptom onset maintained off-scale levels (>8.0 AI) and positive IFA

184  results. No additional blood draws were available for this patient.

185  **4. Discussion**

186  Recent work has demonstrated increased prevalence of anti-nuclear antibodies in

187  acute COVID-19 patients, however, these studies failed to demonstrate an association

188  between disease severity, autoantibody expression and long-term persistence (6-8). We

189  compared the prevalence of anti-nuclear and other systemic and organ specific

190  autoantibodies among acute (ICU care), mild (non-ICU care) COVID-19 patients, and

191  apparently healthy populations. We report progressive increases in anti-nuclear and more

192  specifically anti-RNP-A autoantibody, COVID-19 specific anti-N antibody and protective

193  Nab levels among patients receiving ICU care vs. non-ICU care. These observations lend

194  credence to the argument that disease severity plays a role in increased prevalence of

9

195  anti-RNP-A antibodies; however, the underlying mechanism is far from clear. None of the

196  patients in the ICU or non-ICU setting were previously diagnosed with autoimmune

197  disorder. It has been reported that ANA positive patients had a poor prognosis compared

198  to the negative patients with regards to COVID-19 disease [12, 13]. We could not confirm

199  these findings using hospital stay as a criterion. Indeed, the RNP-A autoantibody positive

200  ICU patients displayed longer hospitalization times; but this parameter didn't reach

201  statistical significance compared to the RNP-A negative ICU patients.

202  Garcia-Beltran [14] reported significantly diminished neutralizing potency in severely

203  ill patients. We evaluated neutralizing antibody levels among ICU and non-ICU patients.

204  Significantly higher levels of neutralizing and anti-N antibodies levels were observed in

205  the ICU group. The prevalence of ANA and RNP-A antibodies in healthy populations and

206  infectious disease patients has been reported extensively [15-18]. RNP-A is one of the

207  three RNP autoantigens (called A, C and 68 kD) located in the cell nucleus. High antibody

208  titers to nuclear ribonuclear protein is suggestive of mixed connective tissue disease

209  (MCTD), especially in the absence of other autoimmune antibodies such as anti-Smith,

210  anti-SSA/ro and SSB/la antibodies [19]. According to the Alarcon-Segovia criteria, MCTD

211  is diagnosed with an RNP antibody titer of >1:1600 and ≥3 clinical criteria, including

212  synovitis, myositis, edema in hands, Raynaud phenomenon, and arcosclerosis

213  [20].  Although the RNP titers of these patients met these criteria, unfortunately the clinical

214  criteria could not be assessed. The pathophysiology mechanism for the release of RNP

215  antibody during a SARS-CoV-2 infection is unknown.  The fact that we observed a higher

216  incidence of these antibodies for patients admitted to an ICU vs. hospital admission or

217  outpatients suggest that these antibodies instead may play a harmful role during the

218    infection. Excessive release of cytokines and various autoimmune antibodies have been

219    well described in patients with serious COVID-19 infections [21]. Ahmed et. al. [22] have

220    suggested that patients with pre-existing rheumatic diseases may flare during the SARS-

221    CoV-2 infection. However, none of the RNP-A positive COVID-19 patients in this study

222    revealed MCTD or other systemic autoimmune diseases. Coupled with these

223    observations is the fact that differences in other systemic and organ specific autoantibody

224    levels never reached statistical significance between ICU and non-ICU patient cohorts.

225    Whether this is due to the small cohort size remains to be explored.

226    RNP-A seroconversion panels serve as a valuable tool for investigating immune

227    responses. It has been argued that autoimmune responses may develop through virus

228    induced hyper-stimulation of the immune system or alternatively through molecular

229    mimicry due to resemblance between the virus and the host [23]. It is also possible that

230    amino acid sequences contained within SARS-CoV-2 resemble other sequences present

231    within human proteins to illicit a mimicry immune response. As viral proteins are cleared

232    from the circulation from a recovering infection patient, so too could the stimulus for

233    autoantibody production. Neutralization of HIV type I infectivity by serum antibodies from

234    a subset of autoimmune patients with mixed connective tissue disease was demonstrated

235    in earlier studies [24]. Whether a similar mechanism prevails among COVID-19 patients

236    due to retroviral nature of the virus, was outside the scope of this study, though early

237    recovery using hospital stay as a criterion would not support this conclusion. The fact that

238    nine out of ten ICU patients demonstrated transient RNP-A seroconversion on consecutive

239    sample draws suggests direct sequalae of COVID-19, however, long-term consequences

240    of SARS-CoV-2 infection in recovered patients need to be determined. Transient

241  expression of antinuclear antibodies has been reported for other medical conditions as

242  well [25]. It has been suggested that the risk of developing or increasing the autoimmune

243  response may enhance and adversely impact the outcome of COVID-19 patients [26].

244  Whether patients with transient RNP-A develop long COVID or new autoimmune

245  manifestations is unknown at this time.

246

**Author contributions**

247

248    SZ contributed to conducting the study, analyzing data, and writing the manuscript.

249 RK contributed to study design and writing the manuscript. KLL and AHBW contributed to

250 the patient sample and clinical information acquisition. RPW and AHBW reviewed and

251 edited the manuscript. All authors have approved the manuscript.

252 **Declaration of competing interest**

253    The authors declare that they have no known competing financial interests that

254 could have appeared to influence the work reported in this paper.

255 **Employment**

256    S. Zhou, R. Walker and R. Kaul are employees of Bio-Rad Laboratories.

257 **Stock Ownership**

258    S. Zhou, R. Walker and R. Kaul own Bio-Rad Laboratories stock.

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262

263 **References**

264 [1] Y. Shoenfeld, D.A. Isenberg, The mosaic of autoimmunity, Immunology Today 10

265 (1989) 123-126 . https://doi.org/10.1016/0167-5699(89)90245-4

266 [2] H. Amital, E.M. Gershwin, Y. Shoenfeld, Reshaping the mosaic of autoimmunity,

267 Semin Arthritis Rheum. 35 (2006) 341–343.

268 https://doi.org/10.1016/j.semarthrit.2005.09.002

269 [3] R. Fujinami, M.G. von Herrath, U. Christen, Molecular mimicry, bystander activation

270 or viral persistence: Infections and autoimmune disease, Clin Microbiol Reviews 19

271 (2006) 80-94.

272 [4] A.M. Ercolini, S.D. Miller, The role of infections in autoimmune disease, Clin Exp

273 Immunol 155 (2009)1-15.

274 [5] H.M. Hussein, Rahal EA, The role of viral infections in the development of

275 autoimmune diseases, Crit Rev Microbiol 45 (2019) 394-412.

276 [6] S.E.Chang, D. Minn, Y.K. Kim, Autoantibodies in moderate to critical cases of

277 COVID 19, Clin Transl Sci 14 (2021)1625-1626.

278 [7] P. Muratori, M. Lenzi, L. Muratori, A. Granito, Antinuclear antibodies in COVID19,

279 Clin Transl Sci 14 (2021) 1627-1628.

280 [8] L.A. Lerma, A. Chaudhary, A. Bryan, C. Morishima, M.H. Wener, S.L. Fink,

281 Prevalence of autoantibody response in acute coronavirus disease 2019 (COVID 19),

282 J Transl Autoimmun 3 100073 (2020) 1-6.

283  [9] D. Gagiannis, J. Steinestel, C. Hackenbroch, et al., COVID-19-induced acute

284     respiratory failure-an exacerbation of organ-specific autoimmunity? medRxiv. (2020)

285     https:// doi.org/10.1101/2020.04.27.20077180.

286  [10] O. Shovman, B. Gilburd, O. Barzilai, E. Shinar, B. Larida, G. Zandman-

287     Goddard, S.R. Binder, Y. Shoenfeld, Evaluation of the BioPlex 2200 ANA screen:

288     analysis of 510 healthy subjects: incidence of natural/predictive autoantibodies, Ann

289     N Y Acad Sci. 6 (2005) 1050 380-388. doi: 10.1196/annals.1313.120

290  [11] K.L. Lynch, S. Zhou, R. Kaul, R. Walker, A.H.B. Wu, Evaluation of neutralizing

291     antibodies against SARS-CoV-2 variants after infection and vaccination using a

292     multiplexed surrogate virus neutralization test, Clin Chem 68 (2022) 702-712.

293     https://doi.org/10.1093/clinchem/hvab283

294  [12] S. Pascolini, A. Vannini, G. Deleonardi, et. Al., COVID-19 and immunological

295     dysfuncion: can autoantibodies be useful? , Clin Trans Sci 14 (2021) 502-508. doi:

296     10.1111/cts.12908.

297  [13] Y. Liu, A.H. Sawalha, Q. Lu Q, COVID-19 and autoimmune disease, Curr Opin

298     Rheumatol 33 (2020) 155-162.

299  [14] W.F. Garcia-Beltran, E.C. Lam, M.G. Astudillo, D. Yang, T.E. Miller, J. Feldman,

300     B.M. Hauser, T.M. Caradonna, K.L. Clayton, A.D. Nitido, M.R. Murali, G. Alter, R.C.

301     Charles, A. Dighe, J.A. Branda, J.K. Lennerz, D. Lingwood, A.G. Schmidt, A.J.

302     Iafrate, A.B. Balazs, COVID-19-neutralizing antibodies predict disease severity and

303     survival, Cell 184 (2021) 476-488.

304 [15] O. Shovman, B. Gilburd, O. Barzilai, E. Shinar, B. Larida, G. Zandman-Goddard, et

305    al., Evaluation of the BioPlex™ 2200 ANA Screen: Analysis of 510 Healthy

306    Subjects: Incidence of Natural/Predictive Autoantibodies, Ann N Y Acad Sci 1050

307    (2005) 380-388.

308 [16] I. Katz, F. De Luca, B. Dzudor, B.K. Sarpong, B. Osei-Appiah, D. Azoulay, et al.,

309    Seroprevalences of autoantibodies and anti-infectious antibodies among Ghana's

310    healthy population, Scientific Reports 10 (2020) 2814,

311    https://doi.org/10.1038/s41598-020-59693-x

312 [17] C.M. Litwin, A.R. Rourk, Anti-ENA antibody profiles in patients with hepatitis C virus

313    infection, J Clin Lab Anal 32 (2018) e22279, https://doi.org/10.1002/jcla.22279

314 [18] W. Yuan, H. Cao, W. Li, X. Wu, J. Zheng, Comparison study of bead-based and

315    line-blot multiplex ANA immunoassays in the diagnosis of systemic autoimmune

316    rheumatic diseases, Clin Rheumatol 41 (2022) 899-909.

317    https://doi.org/10.1007/s10067-021-05946-7

318 [19] B. Combe, M. Rucheton, M. Graafland, V. Lussiez, C. Brunel, J. Sany, Clinical

319    significance of anti-RNP and anti-Sm autoantibodies as determined by

320    immunoblotting and immunoprecipitation in sera from patients with connective

321    tissue disease, Clin Exp Immunol 75 (1989) 18-24.

322 [20] D. Alacon-Segovia, M.H. Cardiel. Comparison between three diagnostic criteria for

323    mixed connective tissue disease. Study of 593 patients, J Rheumatol 16 (1989) 328-

324    334.

325 [21] Y. Zuo, S.K. Estes, R.A. Ali, A.A. Gandhi, S. Yalavarthi, H. Shi, et al., Prothrombotic

326    autoantibodies in serum from patients hospitalized with COVID-19, Sci Transl Med

327    (2020)12: https://doi.org/10.1126/scitranslmed.abd3876

328 [22] S. Ahmed, O. Zimba, A.Y. Gasparyan, COVID-19 and the clinical course of

329    rheumatic manifestation, Clin Rheumatol 40 (2021) 2611-2619.

330    https://doi.org/10.1007/s10067-021-05691-x

331 [23] A. Dotan, S. Muller, D. Kanduc, P. David, G. Halpert, Y. Shoenfeld, The SARS-

332    CoV2 as an instrumental trigger of autoimmunity.  Autoimmun Rev 20 (2021) 4 doi:

333    10.1016/j.autrev.2021.102792.

334 [24] A. Douvas, Y. Takehana, G. Ehresmann, T. Chernyovskiy, E.S. Daar,

335    Neutralization of HIV Type I infectivity by serum antibodies from a subset of

336    autoimmune patients with mixed connective tissue disease, AIDS Res. Human

337    Retroviruses 12 (16) (1996) 1509-1517. https://doi.org/10.1089/aid.1996.12.1509

338 [25] M. Koshy, J. Mathew, R. Alex, J.A. Jude, R. Ralph, T.D. Sudarsanam, S.

339    Sathyendra, J.  Visalakshi, J.V. Peter, Antinuclear antibodies in scrub typhus:

340    transient occurrence during acute illness, J. Vector Borne Dis 55 (2018) 52-57.

341 [26]. M.C. Sacchi, S. Tamiazzo, P. Stobbione, L. Agatea, P.D. Gaspari, A. Stecca , E.C.

342    Lauritano, A. Roveta, R. Tozzoli, R. Guaschino, R. Bonometti, SARS-CoV-2

343    infection as a trigger of autoimmune response, Clin Transl Sci 14 (2021) 898-907.

344

345

346 **Table 1**

347 Prevalence of antinuclear antibodies in COVID-19 patients and apparently healthy
348 subjects.

| Antinuclear antibody | COVID-19 Patients | | | Apparently Healthy (N=284) | p (healthy vs) | |
|---|---|---|---|---|---|---|
| | non-ICU (N=78) | ICU (N=41) | p | | non-ICU | ICU |
| ANA | 17.9% (14/78) | 43.9% (18/41) | 0.0042 | 10.2% (29/284) | 0.0748 | <0.0001 |
| dsDNA | 0.0% (0/78) | 0.0% (0/41) | >0.9999 | 1.4% (4/284) | 0.5813 | >0.9999 |
| Chromatin | 2.6% (2/78) | 2.4% (1/41) | >0.9999 | 0.7% (2/284) | 0.2042 | 0.3336 |
| RNP-A | 10.3% (8/78) | 31.7% (13/41) | 0.0053 | 3.9% (11/284) | 0.0401 | <0.0001 |
| SS-B | 1.3% (1/78) | 2.4% (1/41) | >0.9999 | 1.1% (3/284) | >0.9999 | 0.4185 |
| SS-A52 | 1.3% (1/78) | 4.9% (2/41) | 0.2725 | 1.1% (3/284) | >0.9999 | 0.1214 |
| Scl-70 | 1.3% (1/78) | 0.0% (0/41) | >0.9999 | 1.4% (4/284) | >0.9999 | >0.9999 |
| Sm | 0.0% (0/78) | 2.4% (1/41) | 0.3445 | 0.0% (0/284) | >0.9999 | >0.9999 |
| Cent B | 1.3% (1/78) | 2.4% (1/41) | >0.9999 | 0.0% (0/284) | >0.9999 | >0.9999 |
| SmRNP | 0.0% (0/78) | 4.9% (2/41) | 0.1168 | 0.0% (0/284) | >0.9999 | 0.0156 |
| Ribo P | 0.0% (0/78) | 0.0% (0/41) | >0.9999 | 0.4% (1/284) | >0.9999 | >0.9999 |
| RNP 68 | 1.3% (1/78) | 2.4% (1/41) | >0.9999 | 0.0% (0/284) | >0.9999 | >0.9999 |
| SS-A60 | 1.3% (1/78) | 0.0% (0/41) | >0.9999 | 1.4% (4/284) | >0.9999 | >0.9999 |
| Jo-1 | 0.0% (0/78) | 0.0% (0/41) | >0.9999 | 0.0% (0/284) | >0.9999 | >0.9999 |

349 Fisher's exact test, P < 0.05, significant difference

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357 **Table 2**

358 Comparison of anti-RNP-A, anti-N IgG and neutralizing antibody levels among SARS-
359 CoV-2 patients requiring ICU and non-ICU care and RNP-A positive and negative
360 patient cohorts

| Cohort | N | RNP-A (AI) | anti-N IgG (U/mL) | Nab inhibition (%) |
|--------|---|------------|-------------------|--------------------|
| ICU | 41 | 1.2 | 1044.0 | 87.1% |
| non-ICU | 78 | 0.4 | 192.8 | 58.6% |
| p value | | 0.0006 | 0.0002 | <0.0001 |
| RNP-A + | 21 | NA | 1609.9 | 83.2% |
| RNP-A - | 98 | NA | 234.0 | 63.2% |
| p value | | NA | <0.0001 | 0.0031 |

t-test, p value < 0.05, significant difference

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375 **Table 3**

376 Prevalence of systemic and organ specific autoantibodies in COVID-19 patients

377 receiving ICU and non-ICU care

| Autoantibodies | non-ICU | ICU | p* |
|---|---|---|---|
| ANA | 17.9% (14/78) | 43.9% (18/41) | 0.0042 |
| CCP | 1.3% (1/78) | 4.9% (2/41) | 0.2725 |
| β2-GP IgM | 3.8% (3/78) | 2.4% (1/41) | >0.9999 |
| Cardiolipin IgM | 3.8% (3/78) | 2.4% (1/41) | >0.9999 |
| β2-GP IgG | 2.6% (2/78) | 0.0% (0/41) | 0.5445 |
| Cardiolipin IgG | 0.0% (0/78) | 0.0% (0/41) | >0.9999 |
| β2-GP IgA | 3.8% (3/78) | 0.0% (0/41) | 0.5503 |
| Cardiolipin IgA | 5.2% (4/78) | 2.4% (1/41) | 0.6584 |
| GBM | 0.0% (0/78) | 0.0% (0/41) | >0.9999 |
| MPO | 1.3% (1/78) | 2.4% (1/41) | >0.9999 |
| PR3 | 0.0% (0/78) | 0.0% (0/41) | >0.9999 |
| DGP IgA | 3.8% (3/78) | 4.9% (2/41) | >0.9999 |
| tTG IgA | 0.0% (0/78) | 0.0% (0/41) | >0.9999 |
| DGP IgG | 2.6% (2/78) | 0.0% (0/41) | 0.5445 |
| tTG IgG | 0.0% (0/78) | 7.3% (3/41) | 0.0389 |
| Multiple autoantibodies | 10.3% (8/78) | 24.4% (10/41) | 0.0584 |
| Overall autoantibodies | 26.9% (21/78) | 48.8% (20/41) | 0.0251 |

Fisher's exact test, $p < 0.05$, significant difference
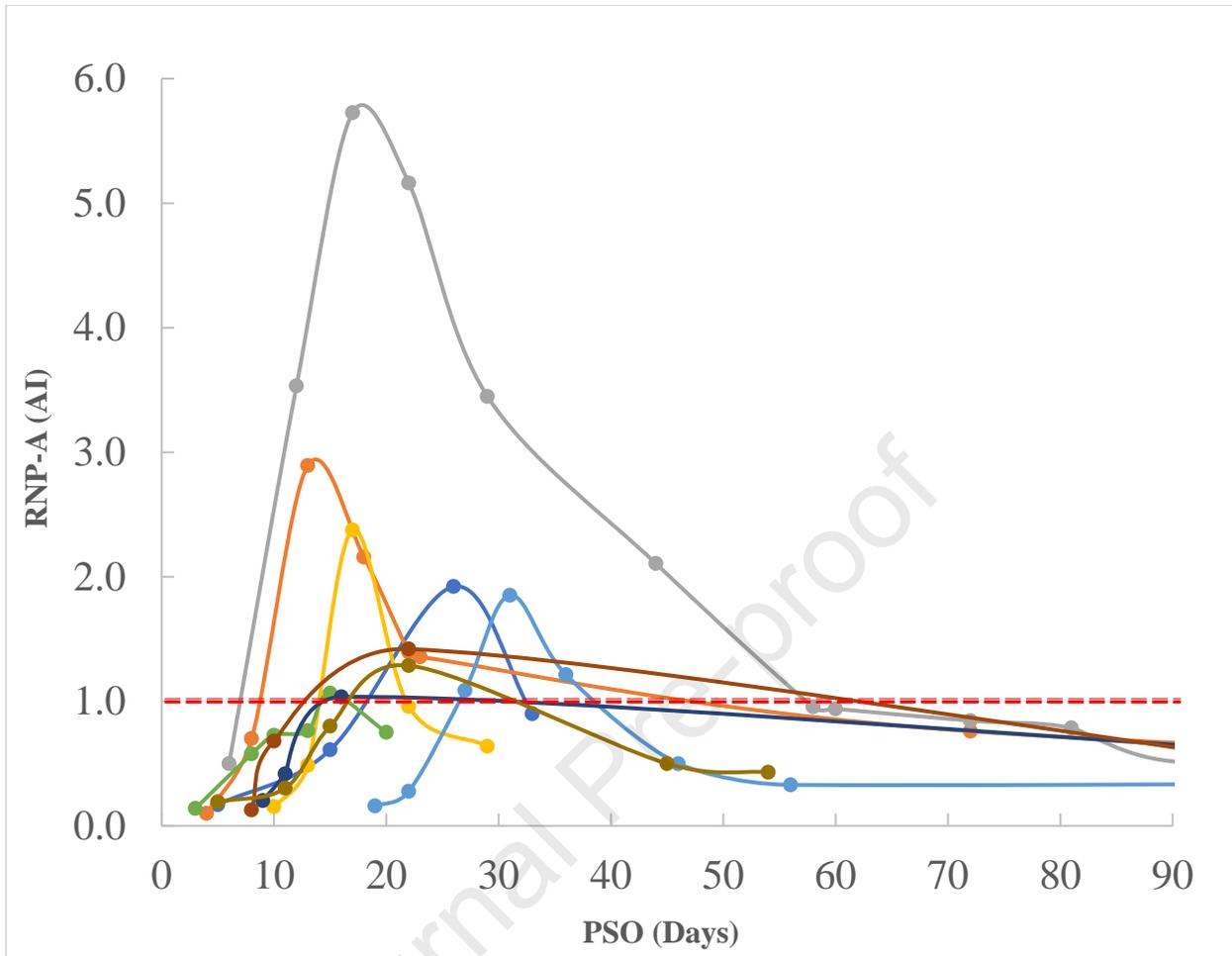
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**Fig. 1.** RNP-A seroconversion in ICU COVID-19 patients. The appearance and
persistence of autoantibodies was plotted vs. the days since symptom onset. Each
donor is shown in a different color. Exceptionally high RNP-A antibodies (> 8.0 AI) were
observed for one sample for which no additional sample draws were available. One
sample was excluded from this figure because all three blood draws exceeded the
assay range. Follow up draws from this patient were not available. Dotted line
represents assay cutoff level of 1.0 AI
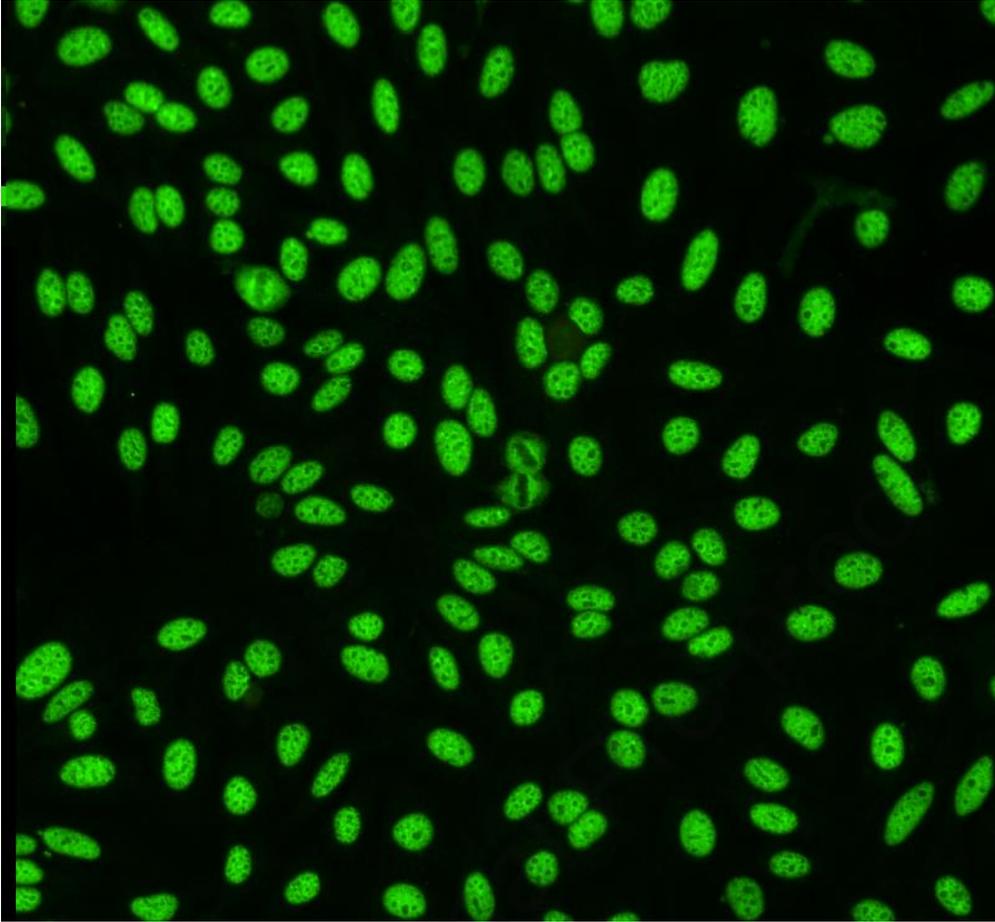
400
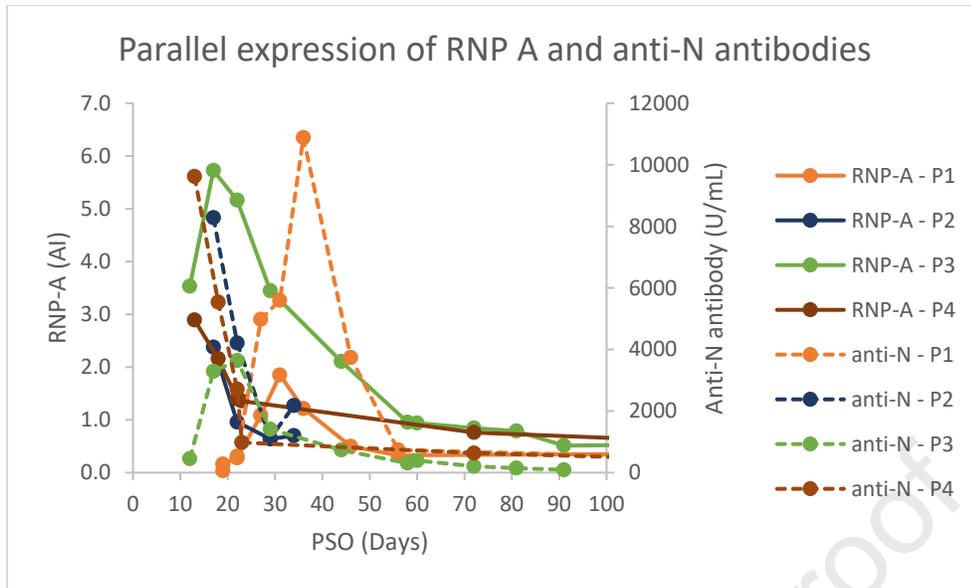
**Fig. 2.** Temporal profiles of four representative anti RNP-A and anti-N antibodies among

patient samples labeled P1 – P4 with serial draws.

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405 **Fig. 3.** Representative IFA ANA patterns on HEp-2 cells. Anti-RNP-A antibody positive

406 samples demonstrated a typical coarse speckled pattern at 1:80 titer.

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**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: