1
2

Long-Term Persistence of IgG Antibodies in SARS-CoV Infected Healthcare Workers

3

4   Xiaoqin Guo[1,#], Zhongmin Guo[2,#], Chaohui Duan[3,#], Zeliang Chen[1#], Guoling Wang[1], Yi Lu[4], Mengfeng

5   Li[5], Jiahai Lu[1,*]

6

7   [1] One Health Center, School of Public Health, Sun Yat-sen University, Guangzhou, 510080, P. R.

8   China

9   [2] Animal Experiment Center, Sun Yat-sen University, 510080, P. R. China

10   [3] Clinical Laboratory, The Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou,

11   510080, P. R. China

12   [4] Department of Health Law, Policy and Management, School of Public Health, Boston University,

13   USA.

14   [5] Department of Microbiology, Zhongshan School of Medicine, Sun Yat-sen University, 510080, P. R.

15   China

16

17   # the authors contributed equally to this work

18   * Corresponding author. lujiahai@mail.sysu.edu.cn for Jiahai Lu.

19

20

21

22

23   **ABSTRACT**

24   **BACKGROUND**

25   The ongoing worldwide outbreak of the 2019-nCoV is markedly similar to the severe acute

26   respiratory syndrome (SARS) outbreak 17 years ago. During the 2002-2003 SARS outbreak,

27   healthcare workers formed a special population of patients. Although virus-specific IgG play

28   important roles in virus neutralization and prevention against future infection, limited information is

29   available regarding the long term persistence of IgG after infection with SARS-like coronavirus.

30   **METHODS**

31   A long-term prospective cohort study followed 34 SARS-CoV-infected healthcare workers from a

32   hospital with clustered infected cases during the 2002-2003 SARS outbreak in Guangzhou, China,

33   with a 13-year follow-up. Serum samples were collected annually from 2003-2015. Twenty

34   SARS-CoV-infected and 40 non-infected healthcare workers were enrolled in 2015, and their serum

35   samples were collected. All sera were tested for IgG antibodies with ELISA using whole virus and a

36   recombinant nucleocapsid protein of SARS-CoV, as a diagnostic antigen.

37   **RESULTS**

38   Anti SARS-CoV IgG was found to persist for up to 12 years. IgG titers typically peaked in 2004,

39   declining rapidly from 2004-2006, and then continued to decline at a slower rate. IgG titers in

40   SARS-CoV-infected healthcare workers remained at a significantly high level until 2015. Patients

41   treated with corticosteroids at the time of infection were found to have lower IgG titers than those

42   without.

43   **CONCLUSIONS**

44   IgG antibodies against SARS-CoV can persist for at least 12 years. The presence of SARS-CoV IgG

45   might provide protection against SARS-CoV and other betacoronavirus. This study provides valuable

46   information regarding humoral immune responses against SARS-CoV and the 2019-nCoV.

47

48   Keywords: SARS-CoV; IgG antibodies; Cohort Study

49

1. INTRODUCTION

On December 31, 2019, an outbreak of coronavirus causing infections in Wuhan, China, was officially reported. The outbreak rapidly spread to several provinces of China and other countries. Currently, the ongoing outbreak in China caused by a new coronavirus, named 2019-nCoV by WHO, is becoming a global crisis. Notably, the 2019-nCoV is highly similar to the severe acute respiratory syndrome coronavirus (SARS-CoV), which is characterized by high mortality and infectivity. Therefore, information on SARS-CoV will be valuable for the prevention and control of the current outbreak.

SARS was first confirmed in Guangdong, China in 2002-2003, and subsequently became a pandemic, spreading rapidly to 31 countries and regions, causing panic and a global public health crisis.[1-3] In total, 8,422 SARS cases were reported to the World Health Organization (WHO) with a case fatality rate of 10.88% (916/8,422); of these total cases, 5,327 cases and 349 deaths were reported from 29 provinces of China. Guangdong was among the most affected provinces, with 1,511 reported cases, 22.90% (346/1,511) of which occurred in healthcare workers.[3-5] During emergency disease control, frontline healthcare workers, including physicians, nurses, and other healthcare workers, who were exposed to the virus while treating and caring for SARS patients in the early stages of the outbreaks, were mainly affected. During the later stages of the outbreak, although precautions to prevent the infection were enhanced, infection of healthcare workers still occurred.[6,7] No natural SARS-CoV infections have been identified since the last confirmed case in May 2004.[8] The reasons why SARS caused a transient epidemic and was experienced as an isolated event, remain unelucidated.[9] There are

69   concerns that SARS-CoV will re-emerge in the future; consequently, studies are underway to

70   determine the mechanisms through which SARS-CoV interacts with the host immune system, crosses

71   host barriers, and other related aspects.

72   SARS-CoV was initially identified as having occurred through interspecies transmission from small

73   carnivores (e.g., palm civets and raccoon dogs) to humans in 2003.[10] Recently, studies indicate that

74   SARS-CoV may have originated from bats. Bat coronavirus isolates have been shown to be able to

75   recognize both human and bat ACE2 receptors.[11] Coronaviruses can exploit numerous cellular

76   receptors and potentially cause various illnesses such as respiratory and gastrointestinal diseases.[12]

77   Particularly, the spike protein is an important viral determinant of virus-receptor interaction,

78   interspecies transmission, and host tropism, and its mutation can facilitate cross-species infection and

79   human-to-human transmission.[13] Another novel coronavirus similar to SARS-CoV, the middle-east

80   respiratory syndrome coronavirus (MERS-CoV), emerged in the Middle East in 2012. MERS-CoV

81   has been shown to be transmissible from camels to humans, highlighting the ongoing threat posed by

82   SARS-CoV or other SARS-like viruses.[14,15]

83   SARS-CoV IgG antibodies have neutralization activities and provide protection against infections.

84   Presence of IgG antibodies against virus and nucleocapsid protein represents protective immune

85   responses. While some previous studies reported longitudinal profiles of SARS IgG antibodies for up

86   to 5 years,[16-20] there has been a lack of knowledge on humoral immune responses for longer durations.

87   In our current study, we conducted a 13-year follow-up study on a cohort of SARS-CoV-infected

88   healthcare workers for analyzing the presence and persistence of antibodies against SARS-CoV.

89 **2. METHODS**

90 **2.1 STUDY SITE AND PARTICIPANT ENROLLMENT**

91 The study site was a teaching hospital and the main center for treating clustered SARS cases in

92 2002-2003 in Guangzhou, China. The hospital employed 4,797 staff, possessed 2,140 annual open

93 beds, and treated over 3.2 million patients annually. During the outbreak (from February to March,

94 2003), a total of 95 healthcare workers, out of all the staff in this hospital, were diagnosed with SARS

95 according to the diagnostic criteria promulgated by the Health Bureau of Guangdong (published on

96 February 3, 2003), and 34 of these cases were enrolled in this study. The majority of the participants

97 were aged between 20 and 30, in 2003, and 94.11% (32/34) of them were females (Table S1). The

98 enrollment criteria included clinical diagnosis of SARS-CoV infection in a healthcare worker treated

99 in the above mentioned hospital, the date of onset being prior to April 16, 2003, and an estimated

100 long-term follow-up of more than 9 years.

101 The sera used in this study is derived sample of the annual physical examination of the SARS-CoV

102 infected healthcare workers. Antibody surveillance is a part of the examination. The annual physical

103 examination has been reviewed and supported by the hospital ethics review committee. Written

104 consent was signed at the first examination.

105 **2.2 SAMPLE COLLECTION**

106 A total of 362 serum samples was collected. This included samples collected at the time of hospital

107 admission in 2003, from all study participants, and yearly sample collection, when the participants

108 attended their annual phyical examinations (April to June from 2004 through 2015). Eighty samples

109   were missing in the follow-up (Table S2): there was an interruption of sample collection in 2012 or

110   2013 due to an unexpected absence of the investigators in charge of sample collections; samples of 6

111   subjects were not collected in 2014 (Patient No. 29 - 34) and 2015 (Patient No. 26 - 28, 31-33)

112   respectively, for failing to arrange a physical examination or due to resignation. In addition, serum

113   samples from another 20 subjects, from the 95 SARS cases, were collected in 2015 (Table S3).

114   Another 40 non-SARS infected healthcare workers from the same hospital were enrolled and serum

115   samples were collected, as the control group, to verify the end result data of the 34

116   SARS-CoV-infected healthcare cohort in 2015 (Table S4).

117   **2.3 DETECTION AND QUANTIFICATION OF IgG ANTIBODIES**

118   Serum IgG antibodies were detected with a SARS-CoV IgG antibody enzyme-linked immunosorbent

119   assay (ELISA) Kit (BGI-GBI Biotech Co. Ltd., Beijing, China), which used the whole SARS-CoV as

120   the detection antigen. Briefly, the serum samples were added at a 1:10 diluion and incubated in

121   pre-coated plates. After the processes of washing, reacting with HRP-labeled goat anti-human IgG,

122   color developing, and stopping the reaction, the absorbance was measured at 450 nm using a plate

123   reader. The IgG antibodies against nucleocapsid protein were detected with a recombinant SARS-CoV

124   N199 antigen assay developed in our laboratory as described previously[21]. Indirect ELISA was

125   performed to detect and quantify antibodies (at 1:10 dilution) against SARS-CoV N199 in the serum

126   samples, as previously described by Guo et al[21].

127 **2.4 STATISTICAL ANALYSIS**

128 Boxplots and heatmaps were used to show the temporal trends and variations in the antibody levels.

129 Non-linear exponential decay models were used to estimate the average decay rates of the antibody

130 level. A non-parametric Mann Whitney U test was used to compare the IgG values of samples from

131 SARS-CoV-infected healthcare workers with those from the control in 2015. The test was also used to

132 compare the anti-SARS-CoV IgG titers from SARS-CoV-infected healthcare workers who were

133 treated with or without corticosteroids in 2003. We used the Kappa test to estimate the consistency of

134 the results of a positive rate for both methods in the samples of the SARS-CoV-infected healthcare

135 worker group from 2003 to 2005 and non-infected healthcare worker group in 2015. Missing values

136 were replaced with estimated values from exponential decay models. Additionally, we conducted a

137 comparison for the decay trend of the data with and without the missing value replacements.

138 **3. RESULTS**

139 **3.1 PROFILES OF IgG AGAINST SARS-CoV FROM 2003 TO 2015**

140 ELISA detection results showed that the IgG levels peaked in 2004, declined quickly from 2004 to

141 2006, and further decreased at a slower rate to a mean OD_{450nm} value of 0.286 (against whole virus)

142 and 0.330 (against N199) in 2015 (Figure 1, A and B). IgG levels against SARS-CoV varied between

143 individuals, with the OD_{450nm} range from 1.162 to 0.48 for IgG against whole virus, and 2.374 to

144 0.581 for IgG against N199. Notably, two healthcare workers, who were diagnosed as

145 SARS-CoV-infected patients, were negative for IgG against both the whole virus and N199, over the

146   entire study period (2003 – 2015), suggesting a possibility of misdiagnosis during the initial

147   admission in 2003; therefore, they were excluded from further statistical analyses (Figure 1, C and D).

148   The IgG levels against whole virus in 4 SARS infected healthcare workers increased notably after

149   2004, two of which also showed an increase in IgG antibodies against N199, while their levels in

150   other SARS infected healthcare workers decreased continuously during the following years (Figure 1,

151   C and D).

152   In the 13-year follow-up cohort, 80 out of 442 (18.10%) serum samples were lost. We used the

153   exponential regression of curve estimation method to estimate the missing values (Figure 1). Next, we

154   adopted the exponential decay models to define antibody decay, and the results indicated that the

155   antibody titers exhibited an overall declining trend in IgG against both whole virus and N199 (Figure

156   2, A and B). A similar decay trend inf IgG titers was found, although the missing values were not

157   replaced in the model (Figures S1 and S2). Furthermore, the proportion of study participants with IgG

158   titer against whole virus (> cutoff value) was 81.25% (26/32) in 2003, peaked at 100.00% (32/32) in

159   2007, and decreased to 69.23% (18/26) in 2015 (Figure 2C). For IgG antibody against N199, the

160   initial positive (higher than the cutoff value) was 59.38% (19/32) in 2003, peaked at 87.50% (28/32)

161   in 2005, and finally decreased to 19.23% (5/26) in 2015 (Figure2D). The proportion of both the IgGs

162   exhibited a reduced trend, and the higher level (>2 cutoff value) declined annually until it reached

163   26.92 % (IgG against whole virus) and 0.00% (IgG against N199) in 2015 (Figure 2, C and D).

164   Two healthcare workers, patient No. 11 and No. 26, who had previously been diagnosed with

165   SARS-CoV infection, were identified not to have detectable levels of IgG against N199 over the

166   entire study period (2003-2015). Anti-N199 IgG titers for patient No. 33 were also negative for all the

167   samples collected except the ones collected in 2005. The IgG titer against the whole virus in these

168   patients were weakly positive.

169 **3.2 DIFFERENCES IN ANTI-SARS-CoV IgG TITERS OF DIFFERENT**

170 **SARS-CoV-INFECTED GROUPS**

171 To analyze the general IgG distributions, subjects were divided into 2 groups: group 1 (n=46),

172 including SARS-CoV-infected healthcare workers enrolled in the original cohort (n=26) and

173 an additional group of SARS-CoV-infected healthcare workers who were enrolled in 2015

174 (n=20), and group 2 (n=40) of non-infected healthcare worker, selected as the control. The

175 IgG against whole virus was above the cutoff value, and the IgG titers were significantly

176 higher in group 1 than in group 2 ($p < 0.001$; Figure 3A). A similar trend was observed for

177 IgG against N199 titers, as group 1 had significantly higher IgG titers than group 2 (p=0.034;

178 Figure 3B). Moreover, in group 1 the positive rate of IgG binding to whole virus and N199

179 was 58.70% and 28.26% with statiscical significance ($p < 0.005$), respectively.

180 Kappa test was used to estimate the consistency between the methods for testing IgG. The

181 consistency test was mainly focused on the period from 2003-2005, as the IgG titers remained

182 stable during this period. Over this period, only 10 of the 102 cases showed inconsistent

183 results between the two detection methods, and the Kappa coefficient was 0.712, suggesting a

184 good general consistence between the results obtained through the whole virus and N199

185 ELISA methods. Similarly, in the control group, only 5 of the 40 cases displayed inconsistent

186 results, and the Kappa coefficient was -0.42, also indicating a general data consistency

187 between the two methods (Table S5).

188 3.3 THE EFFECT OF CORTICOSTEROID TREATMENT ON IgG ANTIBODY

189 RESPONSES

190 During the outbreak, some SARS-CoV-infected healthcare workers received corticosteroid

191 treatment, while others did not. The cohort was divided into two groups to assess the effect of

192 corticosteroid use on IgG titers in 2003. Of the 32 SARS-CoV-infected patients (with 2 cases

193 excluded as suspected to have been misdiagnosed), medical records were available for 27

194 patients, and the records showed that 17 of these patients were, and the rest were not, treated

195 with corticosteroids during the initial care for SARS in the 2002-2003 outbreak (Table S6). In

196 SARS patients with corticosteroids treatment and those not treated with corticosteroids, the

197 IgG against the whole virus was 0.35 ± 0.24 and 0.58 ± 0.29, respectively (p=0.03; Figure

198 4A), and the IgG against N199 was 0.56 ± 0.40 and 0.72 ± 0.51, respectively. Although IgG

199 against N199 of patients not treated with corticosteroids were higher than those treated with

200 corticosteroids, the difference was not statistically significant (Figure 4B; p=0.17).

201 4. DISCUSSIONS

202 SARS was first confirmed in Guangdong, China in 2003, and subsequently became a

203 pandemic as it spread rapidly to other countries and regions. Cases from Guangdong

204 accounted for nearly 20% of reported cases and approximately 17% of reported healthcare

205 worker cases worldwide. Healthcare workers, in particular, were exposed to an extremely

206 high risk in combating SARS due to limited knowledge of the etiology and other natures of

207 SARS outbreak. Presently, 17 years after the last SARS outbreak, a new SARS-CoV-like

208 virus, 2019-nCoV, a new member of the betacoronavirus, is causing large scale outbreak in

209   China. Till January 21, 2010, 440 confirmed cases have been reported in China, and several

210   cases have been reported outside China, including the USA, Japan, and Thailand.

211   Unfortunately, a large cluster of healthcare worker infections was officially reported on

212   January 20, 2020 at an early stage of this outbreak. Since information regarding this new

213   betacoronavirus outbreak remains largely unknown, evidence and knowledge from

214   SARS-CoV may provide important clues, aiding our responses to the outbreak.

215   IgG antibodies are proven to be important for virus neutralization and immunological

216   protection. As the first sampling of sera was performed during the early stage of SARS, it was

217   not possible to determine the maximum antibody titer, as this would be expected to occur

218   approximately 4 months after the onset of the outbreak.[19] Knowledge of the persistence and

219   level of antibodies against SARS-CoV, particularly neutralizing antibodies, is important for

220   evaluating the immune protection duration and vaccine efficacy. Neutralizing antibodies are

221   normally tested by viral neutralization tests; however, as manipulation involving live

222   SARS-CoV is prohibited in China in the interest of preventing laboratory-acquired SARS

223   infection and potential outbreaks, an alternative approach was employed to detect specific

224   IgG antibodies using SARS-CoV whole virus as capture antigens, which has been suggested

225   in previous studies to correlate with neutralizing activity[16]. Moreover, the SARS-CoV N199

226   antigen was used to detect its specific antibody, which has been demonstrated to be specific in

227   clinical diagnosis and SARS-CoV reservoir screening.[21]   We therefore adopted the testing of

228   IgG against N199, combined with the titer analysis of IgG against the whole virus, to

229   comprehensively evaluate the SARS antibody duration.

230   We found that the SARS-CoV-infected healthcare workers who were treated with

231   corticosteroids, such as prednisone and methylprednisolone, exhibited lower IgG titers during

232   the early stages of the disease than those who were not treated with corticosteroids (although

233   not significantly for IgG against N199). However, no differences were found between the two

234   groups after 2003, suggesting that corticosteroids may suppress the early production of

235   SARS-CoV IgG antibodies.

236   In our cohort, we found that two healthcare workers might have been misdiagnosed with

237   SARS, as the IgG antibodies against both the whole virus and N199 antigen was persistently

238   lower than the cutoff value, for these two patients. Notably, all the cases enrolled in this study

239   were admitted to the hospital between February and March, 2003, and that all the diagnoses

240   made before WHO's announcement that SARS-CoV was the causative agent of SARS on

241   April 16, 2003[1], were based on the epidemiological features, clinical symptoms, and regular

242   laboratory blood tests, because serological and etiological diagnostic methods were not

243   available. Specifically, atypical pneumonia, as it was commonly known prior to adoption of

244   the SARS nomenclature, could also be caused by other pathogens.[22] Based on the results of

245   our current 13-year serological follow-up study, it is possible that the two healthcare workers

246   might have been misdiagnosed. Thus, it warrants further investigation to clarify whether these

247   two subjects were non-responders who did not develop anti-SARS-CoV antibodies at all or

248   simply represented misdiagnosed cases. In the latter scenario, the number of SARS cases in

249   the early stage of 2002-2003 SARS outbreak may need to be reassessed. Presently, for the

250   2019-nCoV outbreak, nucleic acid-based assay and particular real time PCR, are the

251   mainstream methods for etiological diagnosis. However, the sensitivity and specificity of

252   these assays need careful evaluation, because any false positive or negative results might be

253   harmful to the subjects.

254   During the SARS outbreak, the typical SARS patients mainly presented with antibodies

255   positive for SARS-CoV, fever, and symptoms of respiratory tract infections. Additionally,

256   there was a small group of asymptomatic SARS-CoV-nfected subjects, who only presented

257   with antibodies positive for SARS-CoV, but showed an absence of clinical symptoms.[23-25]. In

258   this study, we found that four subjects had an abnormal increase in the titers of SARS-CoV

259   antibodies during the follow-up. In contrast, the titers of SARS-CoV antibodies in most of the

260   other healthcare workers appeared to decrease slowly. It is unclear if these four healthcare

261   workers were reinfected with SARS-CoV or other coronaviruses with no signs or symptoms.

262   Thus, further investigation is needed to clarify whether these healthcare workers elicited IgG

263   response by SARS-CoV or other coronaviruses. In the former situation, the novel mechanism

264   of IgG generation without the clinical SARS symptoms needs to be elucidated.

265   Although IgG against SARS-CoV declined from 2003 to 2015 gradually, IgG levels in

266   SARS-CoV-infected healthcare workers were significantly higher than those in the

267   non-infected healthcare workers control group in 2015. The infected cases enrolled into the

268   study in 2015, exhibited detectable IgG against whole virus, and the positive rate was ~60%.

269   For IgG against N199, the positive rate was ~30% in 2015. Moreover, results of the

270   exponential decay curve revealed that IgG antibodies against the whole virus were positive

271   throughout the 13-year follow-up, while IgG antibodies against N199 were negative only in

272   2015.

273   Collectively, based on our results, we can infer that the IgG against SARS-CoV can persist at

274   least for 12 years. Presently, we are unaware whether the persistent IgG antibodies possess

275   virus neutralization activities and can provide complete protection against SARS-CoV

276   infection or cross protection against the new betacoronavirus, 2019-nCoV. From previous

277   studies, we predicted that the presence of IgG antibodies could at least provide a partial

278   protection against coronavirus infection. These data will be valuable for better understanding

279   the immune mechanisms and vaccine development strategies against SARS-CoV and

280   2019-nCoV, therefore being helpful for responses against the ongoing coronavirus outbreak.

281

282

283

287

288

289

AUTHORS' CONTRIBUTION

291

J. Lu designed the study, X. Guo, Z. Guo performed the ELISA tests, Z. Duan collected samples, Z.

Chen analyzed the data and revised the manuscript. G. Wang, Y. Lu and M. Li participated in data

analysis.

295

296

297
298

References

299

300 1. Peiris JS, Yuen KY, Osterhaus AD, Stohr K. The severe acute respiratory syndrome. N Engl J
301 Med 2003;349:2431-41.

302 2. Drosten C, Gunther S, Preiser W, et al. Identification of a novel coronavirus in patients with
303 severe acute respiratory syndrome. N Engl J Med 2003;348:1967-76.

304 3. Xu C, Wang J, Wang L, Cao C. Spatial pattern of severe acute respiratory syndrome in-out flow
305 in 2003 in Mainland China. BMC Infect Dis 2014;14:721.

306 4. Al-Ahdal MN, Al-Qahtani AA, Rubino S. Coronavirus respiratory illness in Saudi Arabia. J Infect
307 Dev Ctries 2012;6:692-4.

308 5. Centers for Disease C, Prevention. Prevalence of IgG antibody to SARS-associated coronavirus in
309 animal traders--Guangdong Province, China, 2003. MMWR Morbidity and mortality weekly report
310 2003;52:986-7.

311 6. Hsin DH, Macer DR. Heroes of SARS: professional roles and ethics of health care workers. J
312 Infect 2004;49:210-5.

313 7. Chen WQ, Lu CY, Wong TW, et al. Anti-SARS-CoV immunoglobulin G in healthcare workers,
314 Guangzhou, China. Emerg Infect Dis 2005;11:89-94.

315 8. Organization WH. China's latest SARS outbreak has been contained, but biosafety concerns
316 remain - Update 7, 18 May 2004. 2007.

317 9. Kondro W. Canadian officials watch SARS-like mystery bug. Lancet 2003;362:714.

318 10. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS
319 coronavirus from animals in southern China. Science 2003;302:276-8.

320 11. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that
321 uses the ACE2 receptor. Nature 2013;503:535-8.

322 12. Fields B, Knipe D, Howley P. Fields virology. 5th. Philadelphia: Wolters Kluwer
323 Health/Lippincott Williams & Wilkins; 2007.

324 13. Li F, Li W, Farzan M, Harrison SC. Structure of SARS coronavirus spike receptor-binding
325 domain complexed with receptor. Science 2005;309:1864-8.

326 14. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel
327 coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 2012;367:1814-20.

328 15. Memish ZA, Cotten M, Meyer B, et al. Human infection with MERS coronavirus after exposure
329 to infected camels, Saudi Arabia, 2013. Emerg Infect Dis 2014;20:1012-5.

330 16. Liu L, Xie J, Sun J, et al. Longitudinal profiles of immunoglobulin G antibodies against severe
331 acute respiratory syndrome coronavirus components and neutralizing activities in recovered patients.
332 Scandinavian journal of infectious diseases 2011;43:515-21.

333 17. Liu W, Fontanet A, Zhang PH, et al. Two-year prospective study of the humoral immune response
334 of patients with severe acute respiratory syndrome. J Infect Dis 2006;193:792-5.

335 18. Xie SY, Zeng G, Xia SC, et al. A three-year follow-up study on sera specific antibody in severe
336 acute respiratory syndrome cases after the onset of illness. Zhonghua Liu Xing Bing Xue Za Zhi
337 2007;28:343-5.

338 19. Cao WC, Liu W, Zhang PH, Zhang F, Richardus JH. Disappearance of antibodies to
339 SARS-associated coronavirus after recovery. N Engl J Med 2007;357:1162-3.

340   20.   Wang H, Jiang S, Wang E, Zhou X, Li Y, Zhao M. Follow-up study on serological detection of
341   anti-SARS-CoV IgG/IgM antibody and its significance. Infectious Disease Information 2009:235-7.
342   21.   Guo ZM, Lu JH, Han WY, et al. Comparison of effectiveness of whole viral, N and N199 proteins,
343   by ELISA for the rapid diagnosis of severe acute respiratory syndrome coronavirus. Chinese Medical
344   Journal 2007;120:2195-9.
345   22.   Liu YN, Tian Q, Hu H, et al. Misdiagnostic analysis of clinically diagnosed severe acute
346   respiratory syndrome after following up 197 convalescent patients. Chin Med J (Engl)
347   2005;118:1487-9.
348   23.   Wilder-Smith A, Teleman MD, Heng BH, Earnest A, Ling AE, Leo YS. Asymptomatic SARS
349   coronavirus infection among healthcare workers, Singapore. Emerg Infect Dis 2005;11:1142-5.
350   24.   Woo PC, Lau SK, Tsoi HW, et al. Relative rates of non-pneumonic SARS coronavirus infection
351   and SARS coronavirus pneumonia. Lancet 2004;363:841-5.
352   25.   Ip M, Chan PK, Lee N, et al. Seroprevalence of antibody to severe acute respiratory syndrome
353   (SARS)-associated coronavirus among health care workers in SARS and non-SARS medical wards.
354   Clin Infect Dis 2004;38:e116-8.
355   26.   Deng W, Wang Y, Liu Z, Cheng H, Xue Y. HemI: a toolkit for illustrating heatmaps. PLoS One
356   2014;9:e111988.

357

358

359
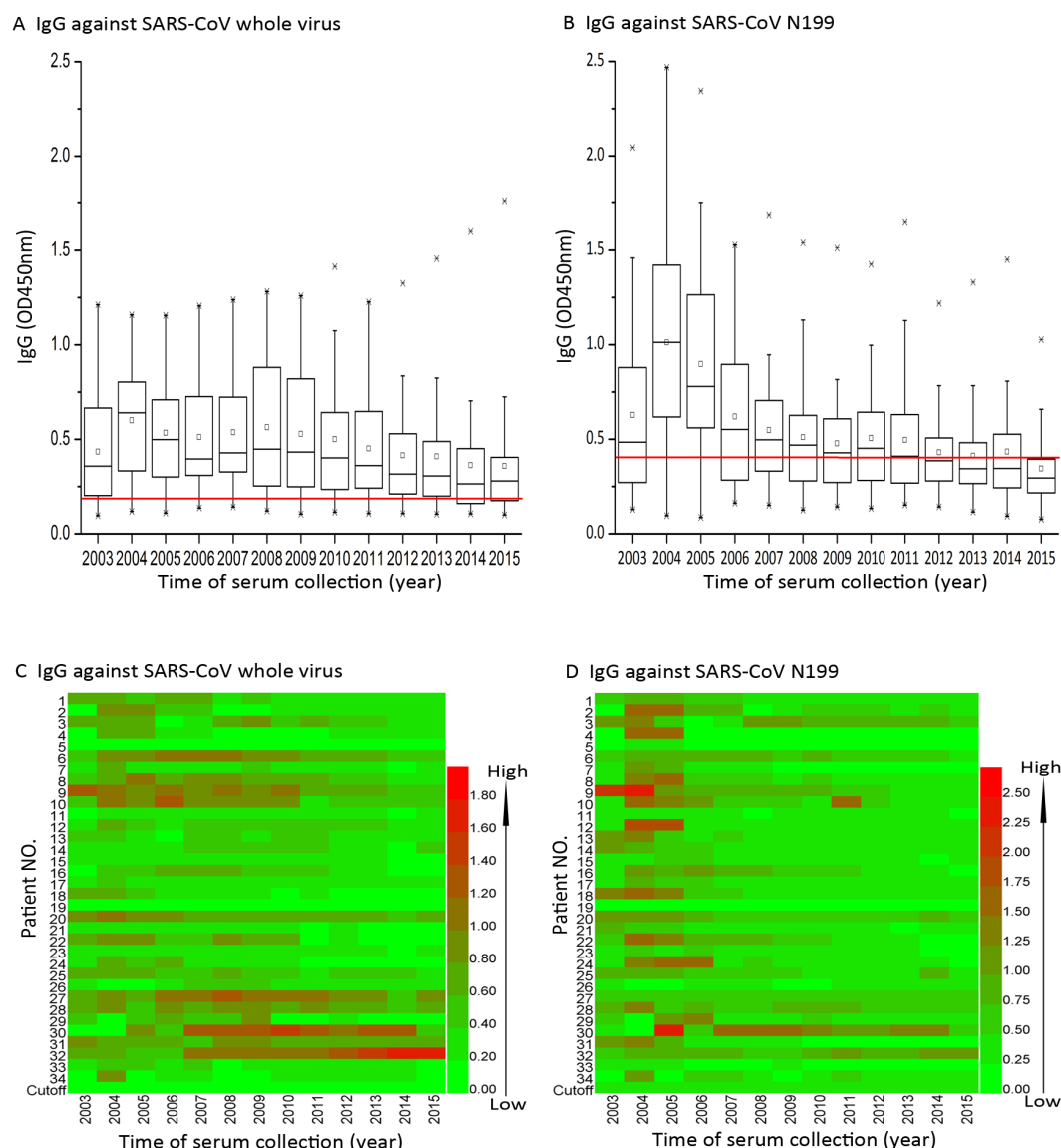
Figure 1. Temporal changes in IgG antibodies against SARS-CoV whole virus and N199 in SARS-Cov-infected healthcare workers

(A) The levels of IgG antibodies using SARS-CoV whole virus as antigen for ELISA detection. The boxplot displays the IgG titers of the 25th, 50th, and 75th percentiles. The whiskers extend to the most extreme data point <1.5 times the inter-quartile range. The cutoff value (red lines) was defined at 0.19 according to the manufacturer's instructions for the SARS-CoV whole virus ELISA kit. Eighty samples lost during follow up were estimated for their reading values using exponential regression of curve estimation method (The same as below). (B) Levels of IgG antibodies against SARS-CoV N199. The cutoff value (red lines) was calculated as follows: cutoff = mean of OD_{450nm} from 52 general population serum samples +3×standard deviations, which gave a value of 0.40. (C) The dynamics of IgG against SARS-CoV whole virus of each individual, from 2003 to 2015, illustrated by heatmap (HemI 1.0, Heatmap Illustrator).[26] (D) The dynamics of IgG against SARS-CoV N199 of each individual from

376 2003 to 2015.

377

378

379
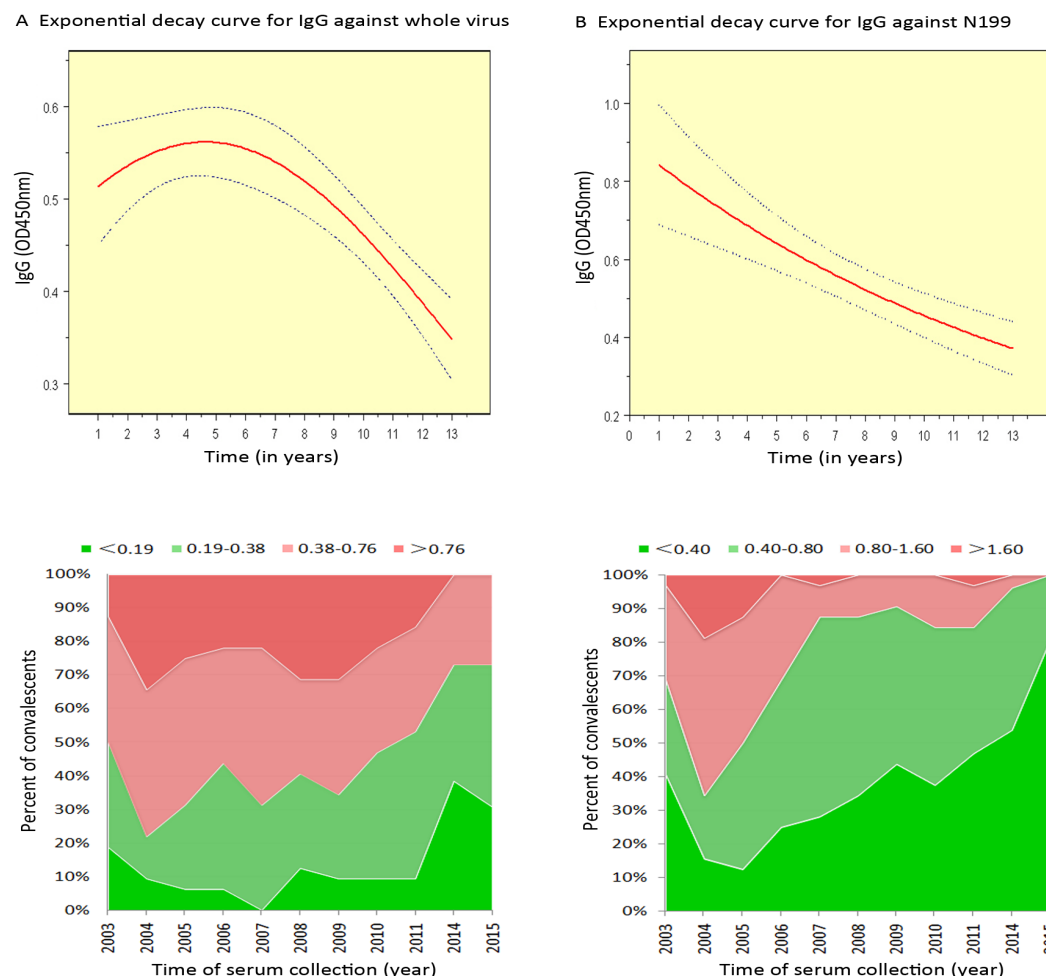
Figure 2. Population profiles of IgG against SARS-CoV whole virus and N199 from 2003 to 2015

(A) Predicted mean (red line) with 95% confidence limits (blue lines) of IgG against whole virus in the cohort from 2003 to 2015. Eighty specimens lost during follow up were estimated for the missing values using the exponential regression of curve estimation method (The same as Panel B). (B) Predicted mean with 95% confidence limits of IgG against N199 in the cohort from 2003 to 2015. (C) Proportions of different ranges of IgG against SARS-CoV whole virus in the SARS-CoV-infected healthcare workers. (D) Proportions of different ranges of IgG against SARS-CoV N199 in SARS-CoV-infected healthcare workers.
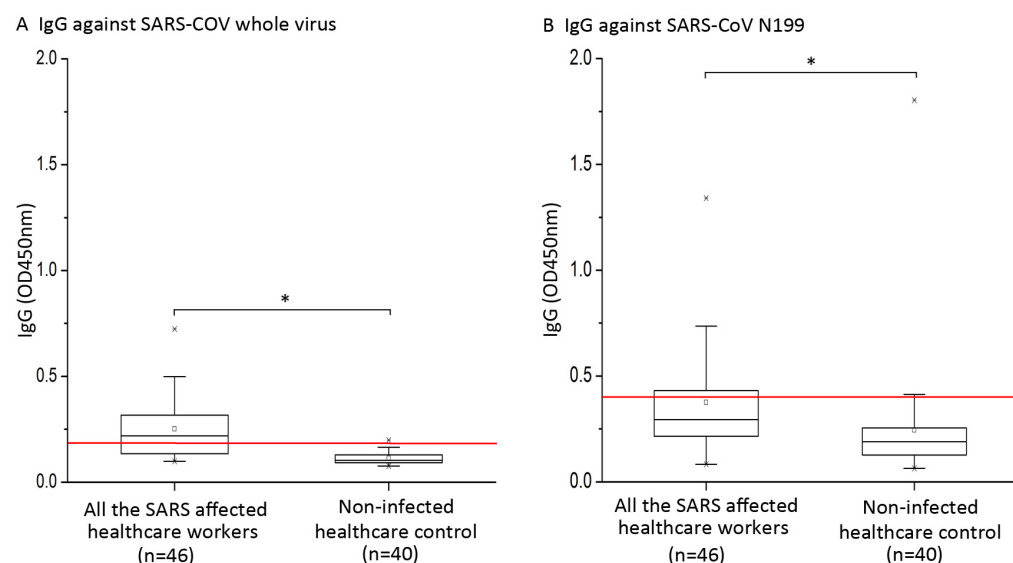
Figure 3. Distributions and differences of IgG in SARS-infected and non-infected healthcare workers (control)

(A) The distributions and differences of IgG antibodies against SARS-CoV whole virus between all SARS-CoV-infected (n=46) and non-infected healthcare workers (control) enrolled in 2015 (n=40). The boxplot displays the IgG titers of the 25th, 50th, and 75th percentiles. The whiskers extend to the most extreme data point <1.5 times the inter-quartile range. The red line represents the cutoff value (0.19). (B) The distributions and differences of IgG antibodies against SARS-CoV N199 between all the SARS-CoV-infected (n=46) and non-infected healthcare worker controls enrolled in 2015 (n=40). The red line is the cutoff value (0.40). P value of < 0.05 (two-tai) is considered to be statistically significant (*).

404

405
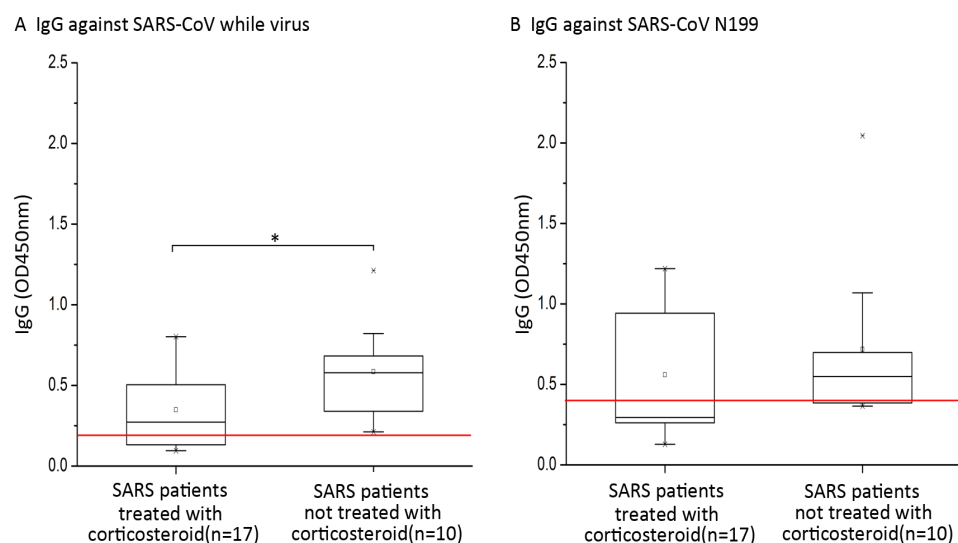
Figure 4. Differences of IgG titers between SARS patients treated with or without corticosteroids

Difference in IgG titers against SARS-CoV whole virus (A) or SARS-CoV N199 antigen (B) between SARS-infected healthcare workers treated with or without corticosteroids. The boxplot displays the IgG titers of the 25th, 50th, and 75th percentiles. The whiskers extend to the most extreme data point <1.5 times the inter-quartile range. The red line represents the cutoff value (0.19, Figure 4A; 0.40, Figure 4B), *P* value of < 0.05 (two-tai) is considered to be statistically significant (*).

406

407

408

409

410

411

412

413

414

415

416

417

Supplementary Appendix

419
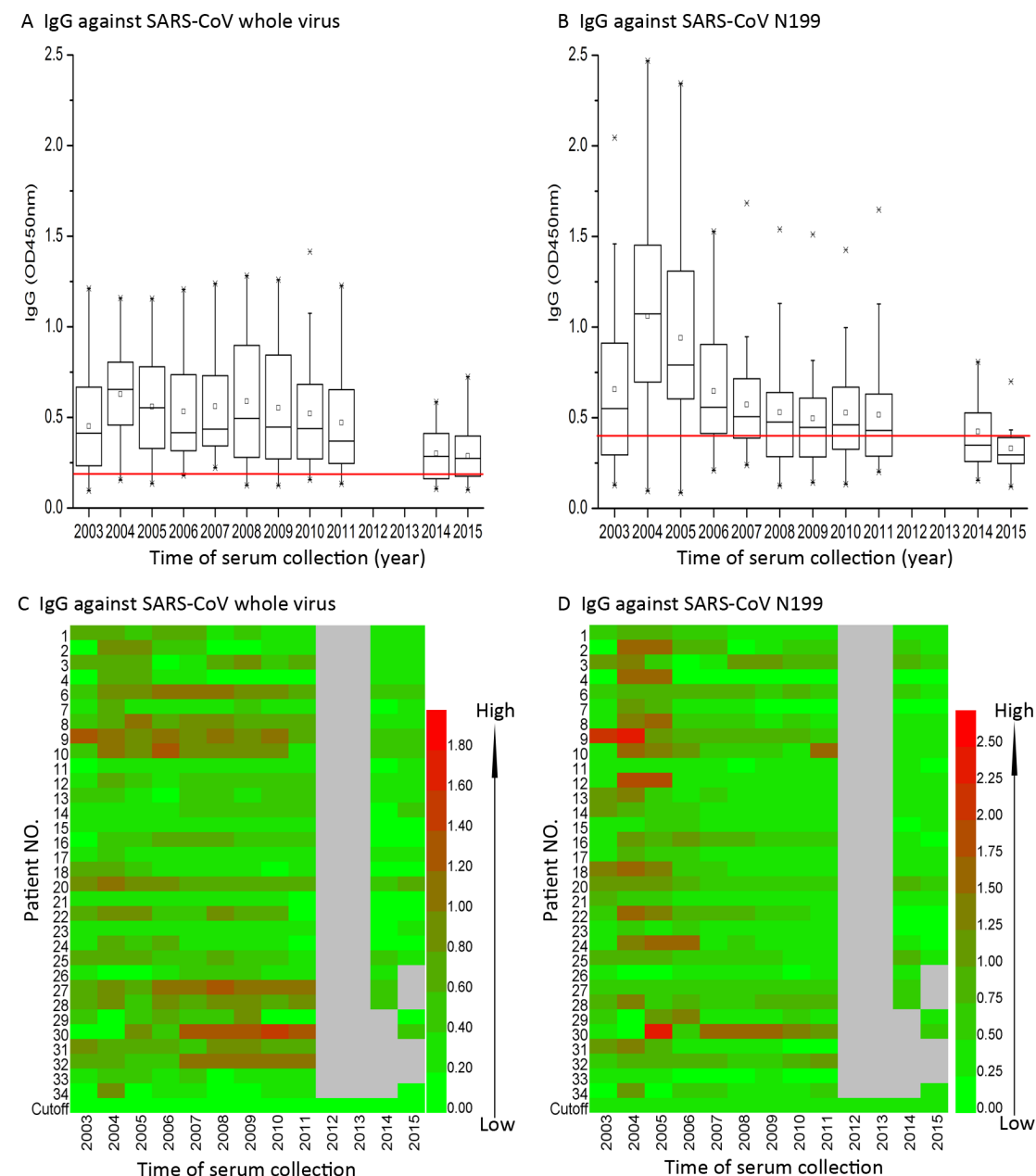
Figure S1. Temporal changes in IgG antibodies against SARS-CoV whole virus

and N199 in the SARS-infected healthcare workers (80 predicted missing values

excluded)

(A) Level of IgG antibodies against SARS-CoV whole virus. The boxplot displays the IgG tiers of

25

424   the 25th, 50th, and 75th percentiles. The whiskers extend to the most extreme data point <1.5

425   times the inter-quartile range. The cutoff value (red line) was defined as a value of 0.19 according

426   to the manufacturer's instruction of the SARS-CoV whole virus ELISA kit. (B) Levels of IgG

427   antibodies against SARS-CoV N199. The cutoff value (red line) was calculated as follows: cutoff

428   = mean of OD_{450nm} from 52 general population serum samples+3X standard deviations, which

429   gave a value of 0.40. (C and D) dynamics of IgG titers against SARS-CoV whole virus (C) or

430   SARS-CoV N199 (D) of each individual from 2003 to 2015 by the method described above.

431   Gray blanks represent the serum samples lost.

432

433

434

435
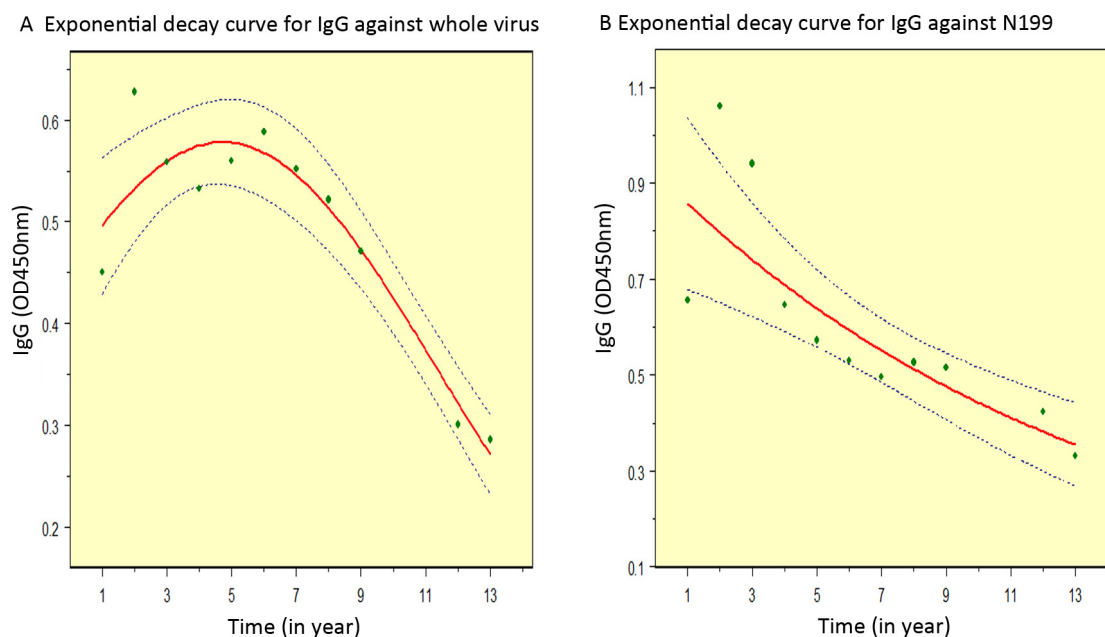
Figure S2. Predicted means of titers of IgG against SARS-CoV whole virus and

N199 during 2003 - 2015 (80 predicted missing values excluded)

Predicted means (red lines) and actual values (green solid circles) with 95% confidence limits

(blue lines) of IgG titers against the whole virus (A) and N199 antigen (B) in the cohort from 2003

to 2015.

441
442
443
444
445
446