Antinuclear Autoimmunity Potentially Makes the Middle-aged and Female with COVID-19 Prone to Severe Progression: A Case-control Study

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Research Article

Keywords: Antinuclear autoimmunity, COVID-19, SARS-CoV-2, Severe progression, The middle-aged and female

Posted Date: August 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-806396/v1

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Abstract

Background

Elderliness is known risk factor for severe progression of COVID-19 due to compromised immunity, however aberrant hyperactive immune response including autoimmunity might be responsible for younger patients.

Methods

162 patients tested with autoimmunological detections were enrolled, and study of “Severe” cases and “Non-severe” controls was retrospectively analyzed.

Results

Multivariable analysis involving antinuclear autoimmunity manifests correlation of disease severity with middle age and attenuates the risk of age older than 65. Middle age (45≤age≤65) and female turn out to be the risk factors after hierarchical cluster analysis, before which however sex was not correlated. We find antinuclear autoimmunity to be strongly correlated with severity for the middle-aged (OR=21,000, 95% CI 4.893-90.126, p<0.001) and female (OR=16,044, 95% CI 4.717-54.568, p<0.001), especially for the middle-aged female (Pearson R=0.770, p<0.001). Incidence of symptoms fever and chest distress, and complication myocardial injury are statistically more frequent in patients with positive antinuclear antibody, compared with those negative. Severe patients with positive antinuclear antibody possess significantly shorter onset of symptoms to severity time (p=0.021), indicating quicker progression, and interestingly, present more incidence (21%) of post-remission aggravation, compared with those negative (6%).

Conclusions

The presence of antinuclear autoimmunity potentially makes COVID-19 prone to severe progression, especially for the middle-aged and female, probably even quicker.

Introduction

Since late December, 2019, the Coronavirus Disease 2019 (COVID-19) pandemic has been affecting 212 countries, areas or territories worldwide1,2. Most cases are mild, and less than 20% cases are severe or critical in China3,4. Although elderliness and chronic comorbidities such as hypertension, diabetes, cardiovascular diseases and respiratory diseases, are known risk factors for severe progression3,5,6, it still remains puzzling on why younger patients without any comorbidity advance to severity and even more rapidly, the underlying mechanisms for severe progression of COVID-19 still needs to be elucidated.

Based on current picturing of the COVID-193,5,7,8, similar to SARS9, besides direct viral toxicity, immune-mediated attack derived from either the release of pro-inflammatory cytokine perpetual cascade8,10, or secondary pathogen-induced autoimmunity response11 may also play important roles on disease progression and partly account for the multi-system injuries related with COVID-1912. Virus infection has been implicated in the initiation of autoimmunity13,14, which can attack multiple systems14. With the knowledge of characteristics of SARS, high level of autoimmune activity was shown to make severe injuries to lungs or other organs, leading to poor outcome including multi-system failure9. COVID-19 may also get autoimmunity involved which is of obviously younger and female population predominance15 during the pathogenesis, no matter pre-existing or secondary to viral infection. Zhou et al.16 reported preliminary observation that autoimmunity exists in severe or critical COVID-19 cases, and a prevalence of between 20% and 50% of anti-Ro-52, anti-Ro-60 and antinuclear antibodies. A 22 cases cohort study from Germany indicates COVID-19 may involve a form of organ specific autoimmunity in predisposed patients who tend to require invasive ventilation and present significantly more severe complications17. Particularly strong immune response to SARS-CoV-2 infection might not be protective, but perhaps, be harmful to the host, contributing to disease severe progression9.

Here in this study, we retrospectively collected and analyzed detailed clinical data from inpatients with laboratory-confirmed COVID-19 hospitalized at two centers in Wuhan, China, with the aim to ascertain whether autoimmunity contributes to the severe progression of COVID-19, and whether the autoimmunological detection could serve as indicator to it, especially for younger patients.

Methods
Study design and patients

The retrospective study includes two groups of adult inpatients from two centers, Zhongnan Hospital of Wuhan University and Thunder God Mountain Hospital, Wuhan, China. All patients who were diagnosed as COVID-19 with real-time PCR (RT-PCR) assay of pharyngeal swab specimens\(^\text{18}\) confirmed results were screened, and those tested with autoimmunological detections including either ANA+ENA (Antinuclear antibody + Anti-extractable nuclear antigen antibody), Anti-cardiolipin antibody (ACA), Rheumatoid factor (RF), or Anti-streptolysin O antibody (ASO) detection, were enrolled in our study from Jan 22, 2020 to Jun 24, 2020. The study protocol was registered at ClinicalTrials.gov with ID NCT04967781, and all methods involved were performed in accordance with the relevant guidelines and regulations.

Each enrolled patient was allocated into control group “Non-severe” or case group “Severe” as per the disease severity which was defined according to the Chinese novel coronavirus pneumonia prevention and control guideline (version 6.0)\(^\text{19}\), “mild” and “moderate” types were grouped into control group as “Non-severe”, “severe” and “critical” types were grouped into case group as “Severe”. Representative critical case with positive antinuclear autoimmunity and full spectrum information reflecting disease dynamics and features would be presented to support the study findings.

Data collection

Demographics, epidemiological, clinical characteristics, laboratory findings, imaging, treatment, and outcome data of all enrolled patients were collected and extracted from electronic medical records using a customized data collection form. Three investigators (ZC, JZ and CC) independently reviewed all the data collection forms and checked by a fourth researcher (FZ).

Laboratory procedures

All pharyngeal swab specimens were subjected to test for SARS-CoV-2 with officially recommended kit (BioGerm, Shanghai, China), following the standard protocol for RT-PCR\(^\text{18}\). Results from all qualified clinical laboratory centers were accepted. Laboratory confirmed positive cases were diagnosed as SARS-CoV-2 infection, no matter if symptoms or thoracic CT imaging results presented or not.

For all patients, complete blood count, serum biochemical tests including hepatic and renal function, electrolytes, coagulation function, myocardial enzymes, lactate dehydrogenase, C-reactive protein, procalcitonin were ordered routinely. Autoimmunological detections and immunoglobulin tests were not routinely ordered depending on the supervising physicians’ knowledge and judgement. ANA+ENA detection was performed with immunofluorescence and immunoblotting methods using EUROLINE ANA Profile examination kit and EUROBlotMaster system (EUROIMMUN Medizinische Labordiagnostika AG, Germany), and the titer of ANA IgG above 1:100 was reported as positive. RF and ASO detections were performed with turbidimetric inhibition immunoassay using the BN II nephelometric analyzer system (SIEMENS, Germany). Anti-cardiolipin antibody (ACA) was measured with Enzyme-linked immunosorbent assay (ELISA) using AESKULISA Cardiolipin A kit (AESKU DIAGNOSTICS GmbH&Co. KG, Germany).

Hepatic injury was defined as various liver function abnormalities including alanine aminotransferases (ALT), aspartate aminotransferase (AST), serum bilirubin, alkaline phosphatase (ALP), or gamma-glutamyl transferase (GGT)\(^\text{20}\). Renal injury was identified based on the highest serum creatinine level or urine output criteria according to the kidney disease improving global outcomes classification\(^\text{21}\). Myocardial injury was defined as abnormal blood levels of cardiac biomarkers (TNI or CK-MB) above the 99th percentile upper reference limit\(^\text{22}\).

Statistical analysis

Continuous and categorical variables were presented as median (IQR) and n (%), respectively. Mann-Whitney U test, Cochran-Mantel-Haenszel test (CMH test), Fisher’s exact test, univariable or multivariable logistics regression analysis was used to compare differences between control group “Non-severe” and case group “Severe” where appropriate. Univariable and multivariable logistic regression analysis, hierarchical cluster analysis, Pearson correlation analysis, Odds Ratio (OR) and Mantel-Haenszel Common OR evaluations were performed to verify whether specific variables affect severity outcome and to indicate association strength. All statistical analyses were performed with software SPSS (version 25.0). p< 0.05 was taken as statistically significant.
Results

Demographic and clinical characteristics

2869 laboratory confirmed COVID-19 inpatients were screened, and 162 patients tested with autoimmunological detections were enrolled. Of the enrolled patients, 120 patients were detected with ANA+ENA (36% positive rate), 75 patients were detected with ACA (32% positive rate), 114 and 110 patients were detected with RF and ASO with 8% positive rate for each, respectively (Figure 1). All positive results of autoimmunological detections were newly emerging after SARS-CoV-2 infection compared with patient's most recent result if any. There were 105 patients in the control group "Non-severe", and 57 patients in the case group "Severe" (Supplementary Table 1). Elderliness (older than 65) do exhibit significant risk for severity (p< 0.001), both univariable and multivariable logistic regression analyses indicate patients aged over 65 are more vulnerable to severe progression than those aged less than 45 (p= 0.002 for multivariable analysis, hereinafter the same), however, those aged between 45 and 65 suffer the most (p< 0.001). Sex (p= 0.393) and the onset of symptoms to treatment time (OTT) (p=0.106) seem to contribute no significant risk. Comorbidity do serve as risk factor (p= 0.004), hypertension (p= 0.003), diabetes (p = 0.003) or coronary heart disease (p= 0.001) contributes significant risk. As a treatment option, severe patients are more often administered corticosteroid or immunoglobulin (p< 0.001). Before the stratification analysis with antinuclear autoimmunity, the overall profiles of the enrolled patients are in accord with those of other large-scale study\(^3\), indicating the representativeness of the study objects.

Antinuclear autoimmunity and severity of COVID-19

Of the detected patients, ANA+ENA is shown significant correlation with severity overall (p< 0.001). For specific single item, ANA IgG and anti-SSA account for 22% and 13% positive rate, respectively, and contribute significantly (p< 0.001 for each, respectively), ACA detection is shown significant but inverse correlation with severity for only IgG antibody (p= 0.038). RF and ASO are demonstrated no correlations with severity (p= 0.752 and p= 0.999, respectively). (Supplementary Table 2)

Univariable and multivariable logistic regression analyses were performed to reveal the correlations between severity and multiple potential risk factors including ANA+ENA detection, age, sex, OTT and comorbidity (only risky chronic diseases included as hypertension or diabetes or CHD or COPD) (Table 1). Compared with model without ANA+ENA detection shown in Supplementary Table 1, ANA+ENA detection reflecting antinuclear autoimmunity was taken into account here and demonstrated significant correlation with severity in both univariable and multivariable analyses, and even higher risk in multivariable analysis (OR= 10.514, 95%CI 3.633- 30.424, p< 0.001). With the multivariable analysis involving antinuclear autoimmunity compared with univariable analysis, age over 65 loses the correlation with severity (p= 0.064), however, the middle age (45 \( \leq \) age \( \leq \) 65) (p= 0.011) and comorbidity (p= 0.002) are still strongly correlated, and interestingly, OTT becomes correlated instead, indicating antinuclear autoimmunity involvement attenuates the risk of elderliness and manifests the urgency of timely treatment. Sex is not correlated for either univariable or multivariable analysis.

Table 1

| Potential risk factors including antinuclear autoimmunity for severe progression of COVID-19 |
|---------------------------------------------|
| Since strongly correlated with severity, ANA+ENA detection was further subjected to hierarchical cluster analysis, and ANA+ENA is overall shown to possess 60.9% sensitivity and 79.7% specificity with moderate correlation when indicating severity (Pearson R= 0.412, OR= 6.119, 95% CI 2.696- 13.887, p< 0.001) (Table 2). It becomes further correlated for cluster middle-aged patients (45 \( \leq \) age \( \leq \) 65), the sensitivity and specificity are elevated to 78.9% and 84.8%, respectively, with strong correlation (Pearson R= 0.631, OR= 21.000, 95% CI 4.893- 90.126, p< 0.001), however not significantly correlated for patients aged over 65 (p= 0.452) and less than 45 (p= 0.057) (Table 2). For cluster female patients, stronger correlation with 79.2% sensitivity and 80.9% specificity between ANA+ENA and disease severity is observed (Pearson R= 0.581, OR= 16.044, 95% CI 4.717- 54.568, p= 0.001), however not for the males (p= 0.158). When cluster narrowed to middle-aged female, strongest correlation with 100.0% sensitivity and 81.0% specificity is observed (Pearson R= 0.770, p< 0.001). The time from onset of symptoms to treatment (OTT, days) is thought to reflect illness urgency, and underlying chronic comorbidities make patients vulnerable to severe progression, in this study, no matter OTT longer or shorter than 7 days, with or without pre-existing comorbidity, the ANA+ENA detection can both indicate severity significantly. |
| Table 2 |
| Hierarchical cluster analyses of correlations between ANA+ENA detection and severity of COVID-19 |

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|                      | Total (n=120) | Non-severe (n=74) | Severe (n=46) | Univariable OR (95% CI) | p value | Multivariable OR (95% CI) | p value |
|----------------------|---------------|-------------------|---------------|-------------------------|---------|---------------------------|---------|
| **ANA+ENA**          |               |                   |               |                         |         |                           |         |
| Positive             | 43 (36%)      | 15 (20%)          | 28 (61%)      | 6.119 (2.696-13.887)    | <0.001* | 10.514 (3.633-30.424)     | <0.001* |
| Negative             | 77 (64%)      | 59 (80%)          | 18 (39%)      |                         |         |                           |         |
| **Age**              |               |                   |               |                         |         |                           |         |
| >65                  | 36 (30%)      | 13 (18%)          | 23 (50%)      | 3.073 (1.270-7.437)     | 0.013*  | ..                         | 0.064   |
| 45≤Age≤65            | 52 (43%)      | 33 (44%)          | 19 (41%)      | 12.385 (3.552-43.185)   | <0.001* | 7.235 (1.579-33.140)      | 0.011*  |
| <45                  | 32 (27%)      | 28 (38%)          | 4 (9%)        | 1.000                   | <0.001* | 1.000                      | 0.030*  |
| **Sex**              |               |                   |               |                         |         |                           |         |
| Male                 | 49 (41%)      | 27 (36%)          | 22 (48%)      | ..                      | 0.219   | ..                         | 0.051   |
| Female               | 71 (59%)      | 47 (64%)          | 24 (52%)      |                         |         |                           |         |
| **OTT, days**        |               |                   |               |                         |         |                           |         |
| >7                   | 67 (56%)      | 37 (50%)          | 30 (65%)      | ..                      | 0.103   | 2.924                      | 0.042*  |
| ≤7                   | 53 (44%)      | 37 (50%)          | 16 (35%)      |                         |         |                           |         |
| **Comorbidity (Hypertension or Diabetes or CHD or COPD)** |                 |                   |               |                         |         |                           |         |
| With                 | 38 (32%)      | 12 (16%)          | 26 (57%)      | 6.717                   | <0.001* | 5.256                      | 0.002*  |
| Without              | 82 (68%)      | 62 (84%)          | 20 (43%)      |                         |         |                           |         |

Data are n (%), or n/N (%), unless otherwise specified; OTT = onset of symptoms to treatment time (days); CHD = coronary heart disease; COPD = chronic obstructive pulmonary disease. Univariable or multivariable logistics regression analysis was used to compare differences between control group "Non-severe" and case group "Severe". p< 0.05 was taken as statistically significant.
| Variables | ANA+ENA Severe (n) | Non-severe (n) | Sensitivity/Specificity (%/ %) | Pearson $R^a$ | p value | OR (95% CI) | Mantel-Haenszel Common OR$^b$ (95% CI), p value |
|-----------|---------------------|---------------|-------------------------------|---------------|---------|------------|---------------------------------|
| Overall   | + 28                | 15            | 60.9%/ 79.7%                 | 0.412         | <0.001* | 6.119      | (2.696-13.887)                  |
|           | - 18                | 59            |                               |               |         |            |                                 |
| Age, years |                    |               |                               |               |         |            |                                 |
| 65+       | + 10                | 4             | 43.5%/ 69.2%                 | ..            | 0.452   | ..         | ..                              |
|           | - 13                | 9             |                               |               |         |            |                                 |
| 45≤Age≤65 | + 15                | 5             | 78.9%/ 84.8%                 | 0.631         | <0.001* | 21.000     | (4.893-90.126)                  |
|           | - 4                 | 28            |                               |               |         |            |                                 |
| < 45      | + 3                 | 6             | 75.0%/ 78.6%                 | ..            | 0.057   | ..         | ..                              |
|           | - 1                 | 22            |                               |               |         |            |                                 |
| Sex       |                    |               |                               |               |         |            |                                 |
| Male      | + 9                 | 6             | 40.9%/ 77.8%                 | ..            | 0.158   | ..         | ..                              |
|           | - 13                | 21            |                               |               |         |            |                                 |
| Female    | + 19                | 9             | 79.2%/ 80.9%                 | 0.581         | <0.001* | 16.044     | (4.717-54.568)                  |
|           | - 5                 | 38            |                               |               |         |            |                                 |
| Female and 45≤Age≤65 | | | | | | | |
| Yes       | + 11                | 4             | 100.0%/ 81.0%                | 0.770         | <0.001* | ..         | ..                              |
|           | - 0                 | 17            |                               |               |         |            |                                 |
| No        | + 17                | 11            | 48.6%/ 79.2%                 | 0.292         | 0.006*  | 3.606      | (1.411-9.214)                  |
|           | - 18                | 42            |                               |               |         |            |                                 |
| OTT, days |                    |               |                               |               |         |            |                                 |
| 7+        | + 16                | 6             | 53.3%/ 83.8%                 | 0.393         | 0.001*  | 5.905      | (1.906-18.293)                 |
|           | - 14                | 31            |                               |               |         |            | (2.986-16.926), p< 0.001*      |
| ≤7        | + 12                | 9             | 75.0%/ 75.7%                 | 0.476         | 0.001*  | 9.333      | (2.400-36.296)                 |
|           | - 4                 | 28            |                               |               |         |            |                                 |
| Comorbidity (Hypertension or Diabetes or CHD or COPD) | | | | | | | |
| With      | + 15                | 1             | 57.7%/ 91.7%                 | 0.465         | 0.004*  | 15.000     | (1.679-134.025)                |
|           | - 11                | 11            |                               |               |         |            | (3.054-21.222), p< 0.001*      |
| Without   | + 13                | 14            | 65.0%/ 77.4%                 | 0.388         | <0.001* | 6.367      | (2.130-19.030)                 |

ANA= antinuclear antibody, ENA= extractable nuclear antigen, OTT= onset of symptoms to treatment time (days), OR= odds ratio.

*Cochran-Mantel-Haenszel test (CMH test), $X^2$
test, or Fisher’s exact test was used to compare differences between control group “Non-severe” and case group “Severe” where appropriate. p<0.05 was taken as statistically significant.

\[^a\text{Pearson correlation coefficient R: “very weak” 0.00-0.19, “weak” 0.20-0.39, “moderate” 0.40-0.59, “strong” 0.60-0.79, “very strong” 0.80-1.00.}\]

\[^b\text{If Homogeneity of OR was tested p>0.05, Mantel-Haenszel Common OR estimate was applied.}\]

For specific single item in the detection list of ANA+ENA, ANA IgG or anti-SSA is significantly correlated with severity of COVID-19 separately (p<0.001 for each, respectively) (Supplementary Table 2) with high specificity (93.2% and 95.9%, respectively), but low sensitivity (45.7% and 28.3%, respectively) (Supplementary Table 3 and supplementary Table 4). Among anti-SSA antibody positive cases, 13 present with anti-Ro-52 (81.3%), and 7 present with anti-Ro-60 (43.8%) (data not shown).

Demographic and clinical characteristics between cluster ANA+ENA positive and negative are compared and shown in Figure 2A. Compared with cluster negative, the complication myocardial injury (28%, p=0.007) and severe cases (65%, p<0.001) present significantly more frequently in the cluster ANA+ENA positive. Sex, age and some clinical characteristics such as OTT, comorbidity, and hepatic or renal injury complication, all exhibit no difference between the two clusters. However, when severe cases are picked out for hierarchical cluster analyses, female (68%, p=0.008) and middle-aged patients (45≤age≤65) (54%, p=0.035) present more frequently in the cluster ANA+ENA positive, and so does the severe middle-aged female patients (39%, p=0.041), none of this kind of patient is found in the cluster negative in this study. It seems more patients of positive ANA+ENA spent shorter OTT but statistically insignificant (p=0.152). For these two types of severe clusters, ANA+ENA positive and negative, the incidence of hepatic, renal or myocardial injury seems to be of no difference (Figure 2B). Incidence rates of symptoms between cluster ANA+ENA positive and negative are shown in Figure 2C. Incidence of fever and chest distress are statistically more frequent in cluster positive (84%, p=0.007, and 70%, p=0.015, respectively) compared with cluster negative (60% and 47%, respectively), and there is no statistical difference for other symptoms. However, when severe cases picked out, the incidences of all symptoms become statistically indistinguishable between the two clusters (Figure 2D).

In order to investigate if there is difference of the time-dependent progression of disease for severe cases between cluster ANA+ENA positive and negative, the timelines of different events are compared and demonstrated in Figure 3A and Figure 3B. The onset of symptoms to severity time (OTS, days) of the cluster positive, of which 89% is shorter than 12 days (p<0.001) (Figure 3E), appears to be significantly shorter than that of cluster negative (p=0.021) (Figure 3C). However, the overall disease courses, namely, the onset of symptoms to outcome time (OTO, days) are statistically the same for these two clusters (p=0.735) (Figure 3D). Interestingly, during treatment, 7 severe patients experienced post-remission aggravation, 6 of them (21%) present in the cluster positive, and only 1 (6%) presents in the cluster negative (Figure 3E), although the difference is not supported by statistics (p=0.220). The incidence of hepatic, renal or myocardial injury appears no statistical difference between the two clusters (Figure 2B, Figure 3A and Figure 3B). 3 patients died, all are senile (all aged 75), 2 in cluster negative and 1 in cluster positive who advanced quickly to death with only 12 days overall disease course (Figure 3A).

**Disease dynamics picturing**

Disease dynamics picturing of antinuclear autoimmunity involved COVID-19 was profiled by a representative case study. A 49-year-old woman was admitted on Feb 6, with 2-day history of fever, fatigue and intermittent dry cough, SARS-CoV-2 infection was confirmed by RT-PCR test of pharyngeal swab specimen. She stated 10-year hypertension history but denied any autoimmune diseases or other comorbidities. The thoracic CT imaging exhibited no sign of COVID-19 on Feb 5, but started to show multiple ground-glass opacities on Feb 9, especially in subpleural area. She progressed rapidly to acute respiratory distress syndrome (ARDS) and was transferred to ICU immediately on Feb 12. The ARDS progressed further and reached peak on Feb 14, ventilator was applied promptly. With intensive care and massive use of corticosteroid (total 2500 mg), immunoglobulin, antibiotics and other essential treatments, she gradually recovered with RNA turning negative since Feb 21 with a solo positive result in the middle on Feb 27, and eventually was discharged on Mar 11 (Figure 4, schemes are to scale).

The patient was detected presence of multiple serous antinuclear antibodies with positive ANA IgG, anti-SSA/Ro-52 and anti-AMA-M2 on Feb 10, which was only 6 days after onset of symptoms, 2 days before the initiation and 4 days before the peak of ARDS. The timing was as early as other sensitive inflammation indicators such as Interlukin-6 (IL-6) and Serum Amyloid A (SAA) which reached peaks as early as Feb 10 with the amount of 12.37 folds and 12.63 folds of ULN (upper limit of normal), respectively. C-reactive protein (CRP) and D-dimer followed, both reaching peaks on Feb 15 with 17.03 folds and 9.63 folds of ULN, respectively. Lactic dehydrogenase (LDH) acted...
much slower, peaking on Feb 20 with 1.85 folds of ULN. As myocardial damage indicators, creatine kinase-MB (CK-MB) elevated preceding to CK, peaking on Feb 20 with 2.00 folds of ULN, 2 days before CK's. The antinuclear antibodies still stayed positive with ANA IgG, anti-SSA/Ro-52, anti-SSA/Ro-60 and anti-PR3-ANCA on Feb 24 when a post-remission aggravation of disease occurred, and all turned negative on Mar 9 when disease recovered. Since the onset of symptoms, lymphocytes had kept dropping until Feb 15 reaching nadir (0.23 folds of LLN (lower limit of normal)), coincident with the ARDS peak. Eventually, most indicators went down to around baseline except SAA when disease recovered (Figure 4, Supplementary table 5).

Discussion And Conclusion

The COVID-19 pandemic has been developed into a global major crisis, and severe illness occurs in about 15.7% of the inpatients\textsuperscript{3}, mostly elderliness or those with comorbid chronic diseases. In our study, elderliness (older than 65) and pre-existing comorbidity with hypertension, diabetes or coronary heart disease indeed make patients prone to severity, which is in coincidence with most reports\textsuperscript{3,5,23,24}. Nevertheless, there were still many younger patients absent of any comorbidity advancing to severity and fatality, even more rapidly, which obviously could not be explained by previous knowledge. Meanwhile, it is a remarkable fact that, for not only SARS but COVID-19, there were few children under 12 years old\textsuperscript{25,28} and HIV patients\textsuperscript{26} developing into ARDS and fatalities. The immaturity of children's immune system and impaired immunocompetence of HIV patients appear to be the most plausible explanation\textsuperscript{26}. Both pathogen and host factors are important for the progression of an virus infection\textsuperscript{9}. Recent findings point to a major role of the host immune response, particularly of cytokine storm, as a determining factor for the life-threatening severe progression of COVID-19\textsuperscript{29}. Based on the knowledges of SARS and COVID-19\textsuperscript{7,9}, diffuse alveolar damage (DAD) and ARDS appear to be a common pathway of lung parenchyma damage initiated by virus itself and aggravated by host's immune system, once an inflammatory process reaches a certain intensity, it will self-perpetuate to form cascade making graver damage to the lung parenchyma locally or attacking other organs more diffusely\textsuperscript{9}. Similar to SARS-CoV, it is proven that SARS-CoV-2 also triggers an exaggerated hyperactive cytokines response in patients at severe end\textsuperscript{10,30}. In this study, the presence of antinuclear antibody is thought as manifestation of aberrant hyperactive immune response, severe patients with positive antinuclear antibody appear to possess significantly shorter OTS indicating quicker progression, and patients with positive antinuclear antibody present with more incidence of symptom fever and chest distress reflecting graver inflammation. Current understanding indicates that patients with more intense immune response stand more chance to be at risk of a poor outcome\textsuperscript{31}, which can explain why some younger patients advanced more quickly and gravely.

Viruses have been long associated with autoimmunity with four possible mechanisms involving as molecular mimicry, epitope spreading, bystander activation and cryptic antigens\textsuperscript{13}. Both viral infection and host's genetic susceptibility facilitate autoimmunity to varied extent. SARS-CoV-2 may get a couple of models above involved to induce autoimmunity to either exaggerate the local pulmonary injury, or spread the immune damage to multiple other systems, which can be evidenced by the pathological finding of a critical COVID-19 patient reported by Xu\textsuperscript{7}. They found the counts of peripheral CD4 and CD8 T cells substantially reduced but hyperactivated, and there was an increased concentration of highly proinflammatory CCR4+ CCR6+ TTh17 in CD4 T cells, which implied overactivation of T cells with highly inflammatory and cytotoxic activity. In addition, virus-specific histological changes were not seen in the heart and liver tissue, indicating SARS-CoV-2 infection might not directly impair those organs\textsuperscript{7}. These findings were also in coincidence with those found in SARS\textsuperscript{9}. Post-remission aggravation is an interesting phenomenon observed in this study for severe cases, of whom 21% presents in cluster ANA+ENA positive and 6% in cluster negative. It is characterized by a re-aggravated disease after initial remission during effective treatment, which could be possibly attributed to the cytokine storm, the timing would be the moment at which the shift from a controlled immune response to a host-damaging reaction began to manifest clinically\textsuperscript{29}. This is currently thought to most likely related to the so called "long COVID-19"\textsuperscript{32}, and would be another profile reflecting antinuclear antibody might be one of indicators for hyperinflammatory immune activation or cytokine release syndrome (CRS).

ANA+ENA detection represents the measuring of a series of autoantibodies majorly antinuclear antibodies, which are closely related with many autoimmune diseases, and involved in the pathogenesis of many kinds of viruses\textsuperscript{33,34}. Lately, Zhong et al. revealed patients with autoimmune rheumatic disease might be more susceptible to COVID-19 infection than the general population\textsuperscript{15}. In our study, the ANA+ENA detection can indicate severity of COVID-19 for the middle-aged and female patients sensitively and specifically. Female, both adults\textsuperscript{35} and children\textsuperscript{36}, are known more vulnerable to autoimmunity. Our results do show strong bond between middle-aged female and antinuclear autoimmunity related severe progression, which is in coincidence with facts that autoimmunity is more prevalence in middle-age and female population. In ANA+ENA detection list, ANA IgG and anti-SSA presented more frequently, which was also reported by
Zhou et al.\textsuperscript{16}, and were significantly correlated with severity for the middle-aged and female. When these two were used separately to indicate severity, the sensitivity dropped dramatically indicating the whole ANA+ENA list should be used to keep satisfactory accuracy.

Disease dynamics picturing can help profile the pathogenesis evolution and alarm events of COVID-19. Although a single case described above, we can still see from the representative case most potential major features of antinuclear autoimmunity involved COVID-19, such as middle-aged female susceptibility, quicker progression (only 8 days of OTS), and presence of post-remission aggravation. Antinuclear antibody acts most sensitively as other sensitive inflammatory indicators like IL-6 and SAA, and seems to dynamically go with the disease activity indicating prognosis. It enlightens that antinuclear antibody may be potentially used as a novel sensitive disease indicator probably for the middle-aged and female, and for the best, shall be detected in the acute phase in case of false-negativity by delayed detection and dynamically for follow-up. As a matter of fact, 4 other female stated pre-existing rheumatic disease, and 2 developed into severity, consistent use of immunosuppressor may help to prevent deterioration of COVID-19, on the contrary, poor control of rheumatic disease would be an even greater risk factor\textsuperscript{11,37}. It is worth noting that Chloroquine or Hydroxychloroquine, as widely used for malaria and autoimmune diseases, was useful but controversial for treating COVID-19\textsuperscript{38-40}, and might be more favorable for autoimmunity involved cases\textsuperscript{15,37}. Virus-induced secondary autoimmunity instead of comorbid autoimmune disease has been gradually reported in COVID-19 like Guillain–Barré syndrome\textsuperscript{41}, chilblain-like acral lesions\textsuperscript{42}, autoimmune myocarditis and immune thrombocytopenic purpura with positive antinuclear antibodies\textsuperscript{43} and etc., and might also play roles and contribute to the severe progression during disease pathogenesis, illustrated by this study. It is interesting that one female patient aged 57 encountered immune thrombocytopenia with positive ANA IgG, anti-SSA/Ro-52, anti-SSA/Ro-60 and anti-CEN P-B during the pathogenesis and treatment of COVID-19, sustaining on discharge, which rings a bell that severe SARS-CoV-2 infection may stand a chance to induce secondary autoimmune disease. The surveillance follow-up is still ongoing for revealing of the possible secondary autoimmune disease derived from SARS-CoV-2 infection for all patients with positive ANA+ENA detection.

Anti-cardiolipin antibody (ACA) targets against anionic phospholipids and inhibits the effect of the prothrombin-activator complex to cause arterial and/or venous thrombosis\textsuperscript{44}, transiently appears positive around times of acute infections and acute thrombosis which is insidious and indiscoverable. In this study, surprisingly, ACA presents majorly in the control group “Non-severe” with majorly IgA and IgG. Zhang\textsuperscript{45} reported 3 critical ill patients presenting antiphospholipid, coagulopathy and multiple infarcts simultaneously. Hence, it doesn’t necessarily mean ACA is a benign factor but a potential risk factor concealing in the non-severe population, especially when combined with thrombocytopenia\textsuperscript{45,46}. It is nearly absent in the case group “Severe” most likely can be attributed to the detection timing.

The limitations of this study include as followed. First, the sample size is limited by insufficient autoimmunological detection ordering and bias might be introduced thereby. Since these detections are not included in the guideline for COVID-19, few physicians realize the necessity and only 4.2% of all patients were ordered the ANA+ENA detection. Second, the retrospective nature of this study limits the accurate profiling of patients’ immunological status. Third, the ANA+ENA detection is qualitative, the relationship between the titer of autoantibodies and severity is still not established. Fourth, for most patients, there is lack of dynamic follow-up of the autoimmunological detections.

So far, this is the largest case-control study to ascertain that presence of antinuclear autoimmunity potentially makes patients with COVID-19 prone to severe progression, and the first to define the middle-aged and female as the relevant high-risk population and to describe the post-remission aggravation phenomenon with a potential explain. Based on this study, we can deduce that antinuclear autoimmunity involved COVID-19 might possess some potential features as severe progression susceptibility, the middle-age and female prevalence, graver inflammation, quicker progression and more incidence of post-remission aggravation. For patients at risk, timely and appropriate use of corticosteroid, immunoglobulin or other immune modulators shall be considered to prevent poor outcomes or multi-system failure.

**Abbreviations**

ANA= Antinuclear antibody,  
ENA= Extractable nuclear antigen antibody,  
ACA= Anti-cardiolipin antibody,  
RF= Rheumatoid factor,
ASO= Anti-streptolysin O antibody,
OTT= Onset of symptoms to treatment time (days),
OTS= Onset of symptoms to severity time (days),
OTO= Onset of symptoms to outcome time (days).

Declarations

Ethics approval and consent to participate

This study was reviewed and approved by the Medical Ethics Commission of Zhongnan Hospital of Wuhan University (approval number 2020074), the requirement for informed consent of the study was waived by the Ethics Commission, and the written consent of the patient involved in the case study was acquired and ready for review.

Consent for publication

Not applicable.

Availability of data and materials

With the permission of the corresponding author, we can provide participant data without names and identifiers, but not the study protocol, statistical analysis plan, or informed consent form. Data can be provided after the article is published. Once the data can be made public, the research team will provide an email address for communication. The corresponding authors have the right to decide whether to share the data or not based on the research objectives and plan provided.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funded by the Key Project for Anti-2019 novel Coronavirus Pneumonia from the Ministry of Science and Technology, China (grant number 2020YFC0845500). The funding organization did not participate in study design, data collection, analysis and interpretation, or writing of the report. The corresponding authors (XW and YW) were responsible for all aspects of the study to ensure that issues related to the accuracy or integrity of any part of the work were properly investigated and resolved. The final version of the report was approved by all authors.

Authors’ contributions

ZC made substantial contributions to the study concept and design, data analysis and interpretation, and was in charge of the manuscript draft. YW participated in study design and data analysis, drafting and revising the manuscript, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. ZC, JZ and CC made substantial contributions to the data collection, reviewing and analysis. FZ made substantial contributions to checking the data collection form, obtaining ethical approval, and participated in data analysis and manuscript drafting. ZZ, TC, KL, KW, ZSC, ZP and YZ were the supervising physicians who provided valuable cases. CX and YZ made substantial revisions to the manuscript.

Acknowledgements

We acknowledge all health-care workers participated in the diagnosis and treatment of COVID-19 patients in Zhongnan Hospital of Wuhan University and Thunder God Mountain Hospital, Wuhan, China, thank the patient’s consent to permit our report of this valuable
case, and appreciate Chinese Government for salvaging every COVID-19 patient without any charge, and Prof. Guiling Li for valuable suggestions.

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**Figures**
2963 inpatients diagnosed with COVID-19 in electronic medical record system from two centers screened

94 excluded without laboratory confirmed PCR result

2707 excluded without autoimmunological detections

162 enrolled with autoimmunological detections (ANA+ENA, ASO, RF, ACA)

120 with ANA+ENA detection

- 43 (36%) positive
- 77 (64%) negative

110 with ASO detection

- 9 (8%) positive
- 101 (92%) negative

114 with RF detection

- 8 (8%) positive
- 93 (92%) negative

75 with ACA detection

- 24 (32%) positive
- 51 (68%) negative

Figure 1

Workflow of the study. ANA= Antinuclear antibody, ENA= Anti-Extractable nuclear antigen antibody, ACA= Anti-cardiolipin antibody, RF= Rheumatoid factor, ASO= Anti-streptolysin O antibody.
Figure 2

Demographic and clinical characteristics comparison between cluster ANA+ENA positive and cluster ANA+ENA negative. Compared with cluster negative, there are significantly more severe cases (65%, p < 0.001) and more cases with myocardial injury (28%, p = 0.007) presented in the cluster ANA+ENA positive (A), and symptoms fever and chest distress are statistically more frequent in the cluster ANA+ENA positive (84%, p = 0.007, and 70%, p = 0.015, respectively) (C). Among severe cases, female (68%, p = 0.008) and middle-aged patients (45 ≤ age ≤ 65) (54%, p = 0.035) present significantly more in the cluster ANA+ENA positive (B), however, the incidences of all symptoms are statistically indistinguishable between the two clusters (D). Note: OTT = onset of symptoms to treatment time (days). * was taken as p < 0.05, ** was taken as p < 0.01.
Figure 3

Timeline of different events indicating time-dependent progression of COVID-19 for severe cases between cluster ANA+ENA positive and cluster ANA+ENA negative. The timelines of different events for severe cases of cluster ANA+ENA positive and negative are compared and demonstrated in (A) and (B), respectively. The frequency distribution graphs of OTS and OTO are demonstrated in (C) and (D) for cluster ANA+ENA positive and negative. The OTS appears to be significantly shorter in cluster ANA+ENA positive of which 89% is shorter than 12 days (E), compared with that of cluster negative (C), but the OTOs are statistically the same between the two clusters (D). Notes: OTS = onset of symptoms to severity time (days), OTO = onset of symptoms to outcome time (days). *Was taken as p < 0.05, **was taken as p < 0.01.
Figure 4

Disease dynamics of a critical female patient with representative antinuclear autoimmunity involved COVID-19. Thoracic X-ray imaging combined with clinical manifestation indicated a post-remission aggravation for this patient on Feb 24. The amounts of indicators were demonstrated as folds of ULN (/ULN) or LLN (/LLN) (only for lymphocytes) to facilitate comparisons of varied indicators. ANA=Antinuclear antibodies, ENA=Extractable nuclear antigen antibody, Schemes are to scale. a: Positive for ANA IgG (Speckled pattern), Anti-SSA/Ro-52 and Anti-AMA-M2 on Feb 10; b: Positive for ANA IgG (Speckled pattern), Anti-SSA/Ro-52, Anti-SSA/Ro-60, Anti-AMA-M2 on Feb 24; c: All autoantibodies turned negative on Mar 9.

Supplementary Files

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- Supplementary0321.docx