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Neutrophil Infiltration Induces Myocardial Injury in COVID-19 Post-Mortem Cases

Quanyu Zhang
General Hospital of Shenyang Military Region
Huarong Zhang
Army Medical University
Xiaowei Yan
Chinese Academy of Medical Sciences & Peking Union Medical College
Sicong Ma
General Hospital of Shenyang Military Region
Xiaohong Yao
Army Medical University
Yu Shi
Army Medical University
Yifang Ping
Army Medical University
Mianfu Cao
Army Medical University
Chengfei Peng
General Hospital of Shenyang Military Region
Shuai Wang
Army Medical University
Min Luo
Army Medical University
Chenghui Yan
General Hospital of Shenyang Military Region
Shuyang Zhang
Chinese Academy of Medical Sciences & Peking Union Medical College
Yaling Han (✉ hanyaling@163.net)
General Hospital of Shenyang Military Region
Xiuwu Bian
Army Medical University

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Abstract

Background: The pathological features of severe cardiac injury induced by COVID-19 and relevant clinical features is unknown. This post-mortem study intended to determine the pathological findings of hearts from critically ill COVID-19 cases and explore the association of pathological changes and clinical characteristics.

Methods: This autopsy cohort study, including hearts from 26 deceased COVID-19 patients admitted in intensive care unit, was conducted at four sites in Wuhan, China. Pathological changes were evaluated using hematoxylin and eosin, and immunohistochemical staining. Cases were divided into neutrophil-infiltration group and no-neutrophil group according to histopathological identification of neutrophilic infiltrates or not.

Results: Among 26 cases, four cases had active myocarditis with histopathological examination. All cases with myocarditis accompanied with extensive neutrophil infiltration, while cases without myocarditis did not. Detection rates of interleukin-6 and tumor necrosis factor-α in neutrophil-infiltration group were significantly higher compared to no-neutrophil group. At admission, patients with neutrophil infiltration in myocardium had significantly higher baseline values of aspartate aminotransferase, D dimer and high-sensitivity C reactive protein compared to other 22 patients (P<0.05 for all). During hospitalization, patients with neutrophil infiltration had a significantly higher maximum of creatine kinase (CK)-MB than patients without neutrophil infiltration.

Conclusions: In hearts from deceased patients with severe COVID-19**,** active myocarditis was commonly infiltrated with neutrophils. Cases with neutrophil-infiltrated myocarditis had severe abnormal laboratory tests involving multiple organs at admission, and a high peak value of CK-MB during hospitalization. Role of neutrophil on severe heart injury and even systemic condition in COVID-19 should be emphasized.

Introduction

Coronavirus disease 2019 (COVID-19) outbreaks caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) still repeatedly and intermittently occur around the whole world. Although COVID-19 is mainly characterized by the infection of lung and respiratory failure, cardiac injury with troponin elevation was demonstrated to be associated with the increased mortality(1, 2). Several post-mortem autopsy studies showed that the injury of heart tissue and cardiomyocytes including myocardial necrosis were common and non-specific, but the rate of pathology-confirmed myocarditis was low(3, 4). In addition, despite found in heart tissue, the presence of SARS-CoV-2 via reverse transcription-polymerase chain reaction (RT-PCR) was rarely detected in cardiomyocytes, which failed to qualify virus direct invasion as a primary cause of cardiac injury(5, 6). To date, the definite mechanism of pathological changes in heart induced by COVID-19 is still unclear.

A proportion of patients with COVID-19 was progressed to critically ill cases, and had a significantly higher mortality(7, 8). Especially with the current rapid spread of Delta and Omicron variants, an

increasing trend of severe cases with worse prognosis emerges(9). Critically ill patients experienced a long stay in intensive care unit (ICU), and were more prone to develop multiple organ dysfunction syndrome including heart(10, 11), which may produce significant histological and immunological changes. Therefore, we intended to conduct a post-mortem pathological study among critically ill COVID-19 patients, to describe pathological features of hearts and explore the relationship between these changes and clinical characteristics.

Methods

Study population and specimen disposal

This autopsy cohort study included 26 deceased patients from Huoshenshan Hospital (n=8), Taikang Tongji Hospital (n=5), Zhongfaxincheng Hospital (n=5), and Wuhan Jinyintan Hospital (n=8), China during February 18th to April 4th, 2020. Patient hospitalization information has been described in our previous study(12). Briefly, all 26 patients were confirmed with COVID-19 by nasopharyngeal or pharyngeal PCR analyses of SARS-CoV-2 RNA and were hospitalized in ICU. Full autopsy was performed after patient death with the approval of the ethics committees and written consent of patient relatives in accordance with regulations issued by the National Health Commission of China and the Helsinki Declaration.

To minimize autolysis, decedents were promptly stored at 4℃ after death and the range of the postmortem interval (time of death to time of autopsy) was 4-24 hours. Autopsy materials were collected, fixed in 4% neutral formaldehyde for at least 24 hours and sampled as formalin-fixed, paraffin-embedded tissues for histopathological analyses.

Pathological Analysis

Autopsies of hearts were performed by two experienced pathologists and tissues at ventricles, atriums and epicardial coronary arteries were respectively collected for further analyses. A median of 25 full-thickness blocks of myocardium were examined histologically (range 11–40 blocks). The pathological changes of hearts were evaluated using hematoxylin and eosin (H&E) staining and immunohistochemical (IHC) staining. H&E staining was performed according to the standard procedure. IHC staining was performed on routine automated diagnostic immunohistochemical staining devices (Roche, BenchMark-ultra). Myocarditis was defined as microscopic findings of multiple foci of increased leukocyte infiltration associated with myocyte injury, which was not due to some other cause(3). Number of myocardium-infiltrating mononuclear cells per mm^2 in high-power field was counted in each sample with the most inflammation using IHC staining for CD4 (Zhongshan Jinqiao, #ZM-0418), CD8 (Zhongshan Jinqiao, #ZA-0508), CD20 (Zhongshan Jinqiao, #ZM-0039) and CD68 (Zhongshan Jinqiao, #ZM-0060). Primary antibodies used for IHC staining includes interleukin-6 (IL-6, Abcam, ab6672, 1:600) and tumor necrosis factor alpha (TNF-α, Cell Signal Technology, #8184, 1:20). Images were captured

using a digital camera (DP73, Olympus) under a light microscope (BX43, Olympus). The diluent without primary antibodies was used as negative control for IHC staining.

Statistical Analysis

Clinical characteristics, laboratory tests, echocardiography results, complications during hospitalization, medications and invasive procedures of each patient were abstracted from hospitalization records and other forms of information. Time from syndrome onset to hospitalization were also collected. For laboratory tests including cardiac markers and inflammatory indicators, the baseline values at admission and maximum values during hospitalization were recorded.

Continuous variables were presented as mean ± standard deviation (SD) or median with range if non-parametric. Categorical data were presented as count with percentages (%). To identify the correlation between pathological findings and clinical characteristics, the Kendall's tau-b index for bivariate correlation analysis was conducted. A 2-sided p value < 0.05 was considered to be statistically significant. All the statistical analyses were completed via IBM SPSS version 25 and R packages version 3.6.1.

Results

A total of 26 patients admitted in ICU due to COVID-19 were included in this pathological study. General patient characteristics and main death causes were published elsewhere(12). In brief, the median age of study cohort was 68 years (range 53-88), and 50.0% (13 patients) were male. Median duration in ICU until deaths was 20 days (range 3-61). 20 patients had at least one comorbidity, including 10 with chronic cardiovascular diseases (including three coronary artery disease, one dilated cardiomyopathy, two valvular heart disease, one arrhythmia and three cardiac dysfunction), nine with hypertension, four with diabetes, and six with chronic pulmonary diseases. Most of 26 patients died of pulmonary injuries related to COVID-19.

Heart failure (HF) occurred in 10 (38.5%) patients. Atrial fibrillation (AF) was documented among six (23.1%) patients, which was the main type of new-onset arrhythmias during hospitalization. Due to the serious illness, various complications emerged during hospitalization in ICU, including respiratory failure, pleural effusion, pneumothorax, anemia, renal dysfunction and disseminated intravascular coagulation (DIC). 18 patients received anticoagulation treatment, and two patients received antiplatelet therapy. Also, multiple invasive procedures including non-end-stage endotracheal intubation, ventilator, deep vein puncture, bronchoscopy, dialysis and extracorporeal membrane oxygenation were intermittently or continuously used in critical situations. Treatment information was listed in Supplemental Materials (Table S1).

Pathological findings

Active myocarditis with neutrophil infiltration

As described in our previous work, a series of common pathological changes of hearts were found in all 26 patients, including myocardial cell degeneration and scattered necrosis, mild interstitial oedema and infiltration of monocytes, and lymphocytes and/or neutrophils(13). There were also cardiomyocyte hypertrophy, atrophy, and interstitial fibrosis of varying degrees based on underlying diseases. After morphological analysis of more heart tissue blocks, active myocarditis was found in only four (15.4%) cases. Surprisingly, neutrophilic infiltrates were detected in all four cases of myocarditis: diffuse neutrophilic infiltrates associated with adjacent cardiomyocyte degeneration or necrosis, involving bilateral ventricles and atriums, was found in first two cases of myocarditis, the second case was accompanied with obvious myocardial interstitial oedema (Figure 1A and 1B); the third and fourth cases of active myocarditis showed multiple small discrete foci of mixed inflammatory cells with visible neutrophils and mild lymphocytes associated with single-cell necrosis of cardiomyocytes, involving the left ventricle and atrium (Figure 1C and 1D). Whereas the rest 22 cases without active myocarditis showed a small infiltration of scattered mononuclear cells rather than neutrophils in the myocardial interstitium. To further explore the severity and property of inflammation in myocardium, IHC staining was performed to detect the expression of TNF-α and IL-6 proteins in all cases. Positive expression of TNF-α and IL-6 in infiltrated inflammatory cells and myocardial interstitial cells was found in all four cases with neutrophil infiltration (Figure 1E-L), while cases without neutrophil infiltration showed negative or mild expression of these inflammation related factors (Figure 1M-P). Cases with neutrophil infiltration showed higher ratios of TNF-α (+) and IL-6 (+) than those in cases without neutrophil infiltration (TNF-α (+): 100% vs 31.8% (seven in 22 cases), $P = 0.022$; IL-6 (+): 100% vs 4.6% (one in 22 cases), $P < 0.001$, Table 1).

We further analyzed the immunologic characteristics of myocardium-infiltrating mononuclear cells using IHC staining for markers CD4 (marked helper T lymphocytes), CD8 (marked cytotoxic T lymphocytes), CD20 (marked B lymphocytes) and CD68 (marked monocytes and macrophages). All four cases with active myocarditis were dispersedly infiltrated of very mild CD4+, CD8+, CD20+ lymphocytes and single or small clusters of CD68+ macrophages. And this pattern was also seen in all other cases without active myocarditis (Figure 2). The number of each subtype cells per mm^2 in high-power field was counted and presented in Table 1. No significant difference of CD4+, CD8+, CD20+ or CD68+ cell density was found between cases with active myocarditis and cases without active myocarditis ($P > 0.05$ for all, Table 1). Kendall's tau-b index showed that no significant correlation was found between number of each subtype of cell density and positive detection of TNF-α or IL-6 ($P > 0.05$ for all). Other various types of pathologic findings were shown in Supplemental Materials (Figure S1). Two of the four cases with neutrophil infiltration were found to have neutrophil-predominant endocarditis. Dilated cardiomyopathy with tricuspid valve infective endocarditis occurred in one case without neutrophil infiltration. Epicarditis with focal infiltration of mixed inflammatory cells occurred in three cases with neutrophil infiltration and in seven cases without neutrophil infiltration. Mixed thrombi were found in four cases without neutrophil infiltration, including one in left atrium, one in right atrium and two in right ventricle. Epicardial coronary arteriosclerosis was also found in nine of 26 cases, including the one case with neutrophil infiltration. There was no thrombotic occlusion or endarteritis of epicardial coronary in all 26 cases. Intravascular

microthrombi in myocardial interstitial were observed under microscope in 12 (46.2%) cases, including all four cases with neutrophil infiltration and other eight cases without neutrophil infiltration. The detection rate of cardiac microthrombi in cases with neutrophil infiltration was significantly higher than that in cases without neutrophil infiltration (100% vs 36.4%, $P = 0.02$, Table 1).

Clinical characteristics of neutrophil infiltration group versus no-neutrophil infiltration group

In order to observe the dynamic changes of clinical characteristics in 26 deceased patients, baseline characteristics at admission, and the maximum values of a series of laboratory tests which reflected the severe medical conditions of patients, were both collected. Baseline characteristics including parameters of cardiac injury, inflammation, coagulation and liver function at admission were presented in Table 2. The median time from symptom onset to hospital admission in neutrophil infiltration cases was 20.5 days (range 13-26), which was significantly longer than that (10 days, range 1-24) cases without neutrophil infiltration ($P = 0.02$). In comparison with cases without neutrophil infiltration, cases with neutrophil infiltration in heart tissues had a significantly higher baseline value of aspartate aminotransferase (AST), D dimer or high-sensitivity C reactive protein (hsCRP) ($P = 0.05$ for all, Table 2). In terms of baseline cardiac markers, creatine kinase (CK) in cases with neutrophil infiltration was significantly higher than that in cases without neutrophil infiltration (median 277.5 (range 91.0-486.0) IU/L vs 36.0 (11.5-547.0) IU/L, p = 0.03), while CK-MB and brain natriuretic peptide (BNP) were similar between two groups ($P > 0.05$ for both, Table 2). However, baseline hypersensitive Troponin I (hsTnI) was available on only 16 (61.5%) of 26 patients. 10 (38.5%) patients were not examined for hsTnI until transferred into ICU (six patients) or presenting with symptoms indicating HF or AF (four patients).

Maximum values of laboratory tests during hospitalization were also compared between two groups (Table 2). Cases with neutrophil infiltration still had a significantly higher peak value of the inflammatory indicator hsCRP compared with cases without neutrophil infiltration (median 134.0 (range 98.2-211.0) mg/L vs 10.0 (9.5-163.5) mg/L, $P = 0.04$). A higher trend was also observed in the peak value of AST in cases with neutrophil infiltration in comparison with cases without neutrophil infiltration (median 687 (range 53-1568) IU/L vs 123 (26-800) IU/L, $P = 0.07$). Moreover, the maximum value of CK-MB during hospitalization in neutrophil-infiltrated cases was significantly higher than that in cases without neutrophil infiltration (median 280.0 (range 14.0-996.0) IU/L vs 38.7 (5.9-234.7) IU/L, $P = 0.04$). Cases with neutrophil infiltration had a quantitatively higher peak value of hsTnI during hospitalization compared to cases without neutrophil infiltration, although without statistical significance (median 1.112 (range 0.008-7.775) ng/ml vs 0.220 (0.008-8.749) ng/ml, $P = 0.56$). Other main laboratory parameters were comparable between two groups (Table 2). HF occurred in one of the four cases with neutrophil infiltration, and AF occurred only in six cases without neutrophil infiltration.

Discussion

Since the pandemic of COVID-19 around the world, a considerable proportion of patients with COVID-19 developed into critically ill cases, and often experienced multiple organ failure (MOF) including not only

lung but heart and other organs(14, 15). To explore the specific pathological changes of heart, we conducted this autopsy study of hearts from 26 critically ill patients who died of COVID-19 in Wuhan from February to April 2020 (whom could be deemed as the earliest series of severe COVID-19 cases all over the world). This study mainly reported that: 1) Although with a low rate, active myocarditis was commonly and specifically accompanied with neutrophil infiltration; 2) The positively IHC detection rates of cytokines including TNF-α and IL-6 were significantly higher in cases with neutrophil infiltration compared to cases without neutrophil infiltration, but was not correlated with the extent of lymphocyte and macrophage infiltration; 3) Clinical data showed that cases with neutrophil infiltration had a significantly prolonged time from syndrome onset to hospitalization, and higher baseline levels of a series of laboratory tests including CK, AST, hsCRP and D dimer compared to cases without neutrophil infiltration at hospital admission. While almost 50% of 26 patients did not examine cardiac biomarkers until the exacerbation of illness; 4) In terms of the peak values of laboratory tests during hospitalization, cases with neutrophil infiltration had a significantly higher level of CK-MB, and a quantitatively higher level of hsTnI than those in cases without neutrophil infiltration.

Role of neutrophil: a new notion of COVID-19 related myocarditis?

In comparison with the dramatic pathological changes in lung(16, 17), microscopic findings from heart in COVID-19 were not intensive and unspecific: previous post-mortem studies found various pathological manifestations, most of those focused on the necrosis, myocarditis, inflammatory infiltration and fibrin microthrombi(3, 4, 18). Compared with other autopsy studies, the scattered necrosis cardiomyocytes in our study were more common, probably because patients in our autopsy were all severe cases and had a significantly longer stay in ICU, which greatly increased risks of cardiac injury. Similar to already existed findings(19), a small proportion of cases was found to have active myocarditis which indicated more severe injury of hearts. Surprisingly, neutrophil infiltration was found in all the four cases with myocarditis in our study, but was rarely found in cases without myocarditis. A series of trials have demonstrated that neutrophil infiltration into pulmonary tissues prominently caused the deterioration of COVID-19(20-23). Infiltrated neutrophils could release neutrophil extracellular traps (NETs), which are extracellular networks of chromatin and microbicidal proteins in response to SARS-CoV-2 infection, while excessive activation of NETs simultaneously results in lung cell deaths in critically ill patients(24-27). NETs derived from neutrophils are responsible for multiple causes pathophysiological changes including microthrombi, angiotensin-converting enzyme 2 (ACE2) activity and oxidative stress(28, 29). Moreover, NETs are identified to be correlated with cytokine storms: various cytokines could medicate the migration of neutrophil to injury sites(30); reversely, the generation of NETs subsequently stimulates the aggravation of cytokine storms such as Il-6 via IL-1β(20, 31, 32). In the present post-mortem study, the positive detection rates of IL-6 and TNF-α were significantly higher in four cases with neutrophil infiltration than those in the other 22 cases without neutrophil infiltration by IHC staining. Although the identification of NETs was not conducted, results from our study indirectly confirmed precious findings, and to some extent identified the strong association of neutrophil and severe cardiac injury.

In all 26 cases, the presence of SARS-CoV-2 nucleic acids were found in only 5 heart tissues by real-time RT-PCR, which was reported previously(12). Interestingly, none of these 5 cases had pathologically diagnosed myocarditis. A pathological autopsy study from 39 deceased COVID-19 patients also reported the localization of virus infection was in interstitial cells or infiltrated macrophage in hearts, rather than cardiomyocytes(33). Also, other pathological studies from endomyocardial biopsy (EMB) or autopsy rarely reported direct invasion of SARS-CoV-2 into cardiomyocytes(34). Current evidences still fail to determine the key role of SARS-CoV-2 infection on cardiac injury, while inflammatory infiltration was now regarded as a preliminary cause of heart damage in severe COVID-19. The Dallas Criteria(35) only according to histological evidences has already been not fully suitable for diagnosis of myocarditis, and IHC analysis for inflammatory infiltration was particularly advocated(36). A comparison of CD3+ T cells and CD68+ macrophages between COVID-19 cases and noninfectious control cases were conducted in a state-of-art review(34). No significant difference of the total number of CD3+ and CD68+ cells was observed between two groups, while the number count of CD68+ cells was significantly higher in COVID-19 group than that in control group(34). Our study also found that the CD68+ macrophages were single or clustered in myocardium, but no difference of the number count of CD68 cell was found between cases with and without active myocarditis. Moreover, each case with myocarditis was infiltrated with neutrophils accompanied with distinct cytokines, which was not prevalent in cases without myocarditis. Therefore, based on previous findings on association of neutrophil infiltration and critically ill COVID-19, we reasonably reckon that neutrophil and relevant inflammatory infiltration may be an essential cause of devastating heart damage into myocarditis. Some researchers believed glucocorticoid could act an immunomodulatory role on inhibiting cytokine storm and excessive immune response for purpose of improving therapeutic effect on critically ill patients (37, 38). Besides, other specific cytokine inhibitors, including IL-6 receptor inhibitor tocilizumab is being explored(39, 40). Role of neutrophil infiltration on severe cardiac injury deserves more special attention in COVID-19.

Cardiac biomarkers: a warning sign of severe COVID-19?

Several studies evaluating risk factors of poor prognosis in COVID-19 identified a series of laboratory factors may be predictors of in-hospital mortality, including AST, D dimer and hsCRP(41, 42). Significant elevation of the baseline levels of these three parameters were also found in patients with neutrophil infiltration in hearts from our study. According to our findings, a link may exist between a relatively severe situation (involving liver function, coagulation and inflammation) and pathological changes of heart tissues, indicating that COVID-19 could simultaneously cause damage of multiple organs although initiated in respiratory system. From our data, however, nearly 50% patients were not tested for cardiac biomarkers at admission until they were transferred to ICU or presented with relevant symptoms. This result may reflect that, under an emergent situation, inspections on heart may be easily neglected by physicians who mainly focused on treatment strategy on respiratory system. Furthermore, we found that cases with neutrophil infiltration had a relatively worse situation both on admission and during ICU stay. The peak level of CK-MB among the whole duration of hospitalization was significantly higher in cases with neutrophil infiltration than that in cases without neutrophil infiltration. Peak value of hsTnI was also found to be quantitatively higher in cases with neutrophil infiltration, although without statistical

difference due to limited sample size. The dramatic elevation of cardiac biomarkers was consistent with severe pathological changes of hearts. In combination with earlier findings(1, 2), biomarkers of cardiac injury including CK-MB and troponin could fairly be not only the indicator of heart damage, but a predictor of the systemic inflammatory response of COVID-19. Cardiac biomarkers, as meaningful alerts of critical illness, should be particularly attended by clinical physicians in COVID-19.

This study had several limitations. Firstly, the sample size was low, and findings from 26 cases indispensably had potential bias. Secondly, immunohistochemical staining for NETs was not done, while the effect of NETs on COVID-19 have been explored by previous studies. Finally, we did not explore further about details of heart injury mechanisms due to COVID-19, and more researches are needed for this issue.

Conclusion

This autopsy study of heart tissue from critically ill patients died of COVID-19 showed active myocarditis was commonly infiltrated with neutrophils. Cases with neutrophil-infiltrated myocarditis had more severe abnormal baseline laboratory tests involving baseline AST, D dimer and hsCRP, and a higher peak value of CK-MB during hospitalization in comparison with cases without neutrophil-infiltrated myocarditis. Role of neutrophil on severe heart injury and even systemic condition in COVID-19 should be emphasized.

Abbreviations

COVID-19: coronavirus disease 2019; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; RT-PCR: reverse transcription-polymerase chain reaction: ICU: intensive care unit; H&E: hematoxylin and eosin; IHC: immunohistochemical; IL-6: interleukin-6; TNF-α: tumor necrosis factor alpha; SD: standard deviation; HF: heart failure; AF: atrial fibrillation; DIC: disseminated intravascular coagulation; AST: aspartate aminotransferase; hsCRP: high-sensitivity C reactive protein; CK: creatine kinase; CK-MB: creatine kinase MB; brain natriuretic peptide: BNP; hsTnI: hypersensitive Troponin I; NETs: neutrophil extracellular traps.

Declarations

Ethics approval and consent to participate: Full autopsy was performed after patient death with the approval of the ethics committees and written consent of patient relatives in accordance with regulations issued by the National Health Commission of China and the Helsinki Declaration.

Consent for publication: All patients' relatives provided the consent of individual data for publication.

Availability of data: Data in this study is available with reasonable requests by contacting the corresponding author.

Competing interests: The authors have declared that no competing interest exists.

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Author contributions: Among the authors, Quanyu Zhang collected the specimens and designed the conduction of study. Huarong Zhang and Xiaohong Yao was responsible for specimen disposal and pathological analysis of all cases. Xiaowei Yan collected and analyzed the clinical information of recruited patients. Sicong Ma was in charge of statistical analysis and manuscript writing. Quanyu Zhang and Sicong Ma had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Yu Shi, Yifang Ping, Mianfu Cao, Chengfei Peng, Shuai Wang, Min Luo and Chenghui Yan provided assistance of staining procedure and figure exhibition. The corresponding authors, Xiuwu Bian, Yaling Han and Shuyang Zhang, contributed to the leadership of the whole process of study conduction, and acted as the key role of initiating, designing, conducting, and concluding the study.

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Tables

Table 1. Pathologic findings of cases with versus without neutrophil infiltration

Pathologic findings	Cases with neutrophil infiltration (myocarditis) N=4	Cases without neutrophil infiltration (myocarditis) N=22	*P* value
TNF-α (+), No. (%)	4 (100%)	7 (32%)	0.02
IL-6 (+), No. (%)	4 (100%)	1 (5%)	< 0.001
Number of lymphocytes per mm^2, median (range)			
CD4+ cell	6.5 (range 4-13)	8.5 (range 3-17)	0.45
CD8+ cell	8 (range 5-15)	12 (range 3-50)	0.29
CD20+ cell	2 (range 1-5)	3 (range 1-5)	1.00
CD68+ cell	61 (range 34-89)	50 (range 24-154)	0.83
Microthrombi (+), No. (%)	4 (100%)	8 (36%)	0.02

TNF-α: tumor necrosis factor; IL-6: interleukin-6.

Table 2. Clinical characteristics of cases with versus without neutrophil infiltration

	Neutrophil (-) N=22	Neutrophil (+) N=4	*P* Value	Neutrophil (-) N=22	Neutrophil (+) N=4	*P* Value
Age, years, (median, range)	69 (53-88)	62.5 (56-77)	0.21			
Sex, male, No. (%)	11 (50.00%)	2 (50.00%)	1.00			
Time from symptom onset to hospital admission, days, (median, range)	10 (1-24)	20.5 (10-26)	0.01			
Laboratory test (median, range)	**Baseline characteristics at admission**			**Maximum value during hospitalization**		
CK-MB, IU/L	11.4 (5.5-45.0)	13.5 (7.20-128.0)	1.00	38.7 (5.9-234.7)	280.0 (14.0-996.0)	0.04
CK, IU/L	36.0 (11.5-547.0)	277.5 (91.0-486.0)	0.03	144.9 (36.0-1997.0)	2276.5 (91.0-7491.0)	0.17
hsTnI, ng/ml	NA	NA	NA	0.220 (0.008-8.749)	1.112 (0.008-7.775)	0.56
BNP, pg/ml	55.1 (10.0-1243.0)	56.7 (10.0-299.1)	0.80	387.0 (89.3-26000.0)	173.0 (56.0-328.0)	0.19
IL-6, pg/ml	6.89 (16.80-204.10)	16.54 (6.89-26.30)	0.48	63.44 (16.14-5000.00)	20.28 (11.79-455.00)	0.19
hsCRP, mg/L	10.0 (2.15-160)	96.6 (67.6-211.0)	0.02	10.0 (9.5-163.5)	134.0 (98.2-211.0)	0.04
D-Dimer, mg/L	2.97 (0.36-21.00)	11.92 (4.25-18.19)	0.04	6.45 (0.36-56.63)	14.43 (4.25-50.00)	0.13
Neutrophil, 10×10^9/L	6.34 (3.57-16.66)	12.12 (3.44-20.03)	0.62	17.14 (3.94-50.34)	17.55 (7.71-23.03)	0.67
PCT, ng/ml	0.29 (0.08-1.91)	1.01 (0.05-88.34)	0.89	2.58 (0.14-47.53)	3.37 (0.11-88.34)	0.92
ALT, IU/L	27 (4-110)	52 (34-1204)	0.20	107 (4-1000)	128 (40-1400)	0.46
AST, IU/L	38.4 (9.2-92.6)	55.9 (48.0-1487.0)	0.04	123 (26-800)	687 (53-1568)	0.07

CK-MB: creatine kinase-MB; CK: creatine kinase; hsTnI: hypersensitive troponin I; NA: not available; BNP: brain natriuretic peptide; IL-6: interleukin-6; hsCRP: hypersensitive C reaction protein; PCT: procalcitonin; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

Figures

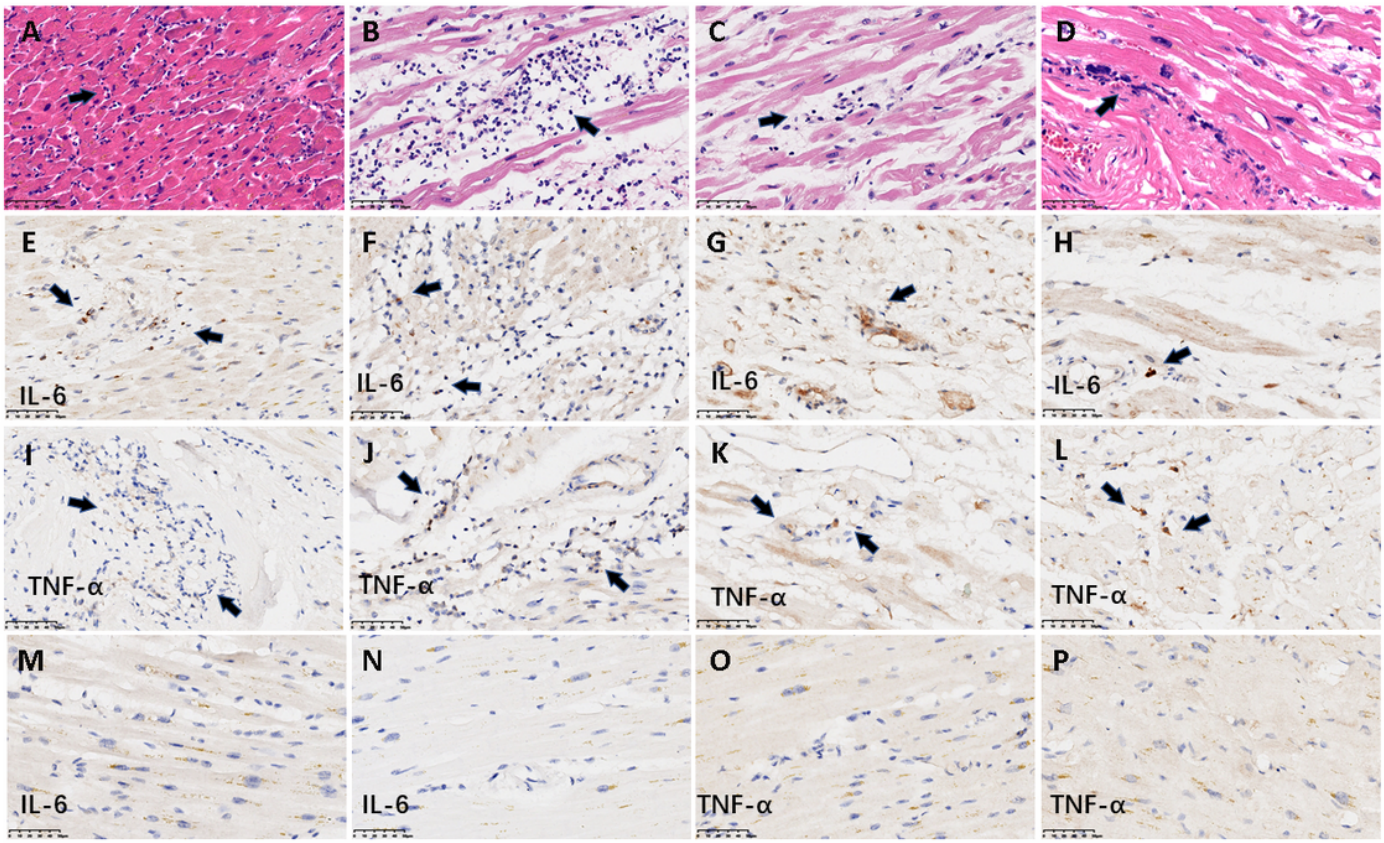

Figure 1

Representative histological and IHC findings from hearts

Figure 1 shows the histological and IHC findings from heart tissues. Figure A-D demonstrate active myocarditis in the four cases. A and B) the histology in the myocardium demonstrated diffuse neutrophilic infiltrates with myocyte injury in a 62-year-old man and a 56-year-old woman, respectively; C and D) multiple small discrete foci of mixed inflammatory cells with visible neutrophils associated with single-cell necrosis of cardiomyocytes in a 63-year-old woman and a 76-year-old man, respectively. The arrows denote the infiltrated neutrophils. Figure E-L denote the positive IHC staining of IL-6 and TNF-α from the four cases with active myocarditis. E-H) IL-6; I-L) TNF-α. The longitudinal images in the first three rows were derived from the same case (A, E, I from a 62-year-old man; B, F, J from a 56-year-old woman; C, G, K from a 63-year-old woman; D, H, L from a 76-year-old man). Arrows denote the positive signal of IHC staining. Figure M-P represent negative expression of IL-6 and TNF-α from two cases without neutrophil infiltration. M and O) IHC staining from an 81-year-old man; N and P) IHC staining from a 59-year-old woman. Scale bars represent 50μm. IHC: immunohistochemical.

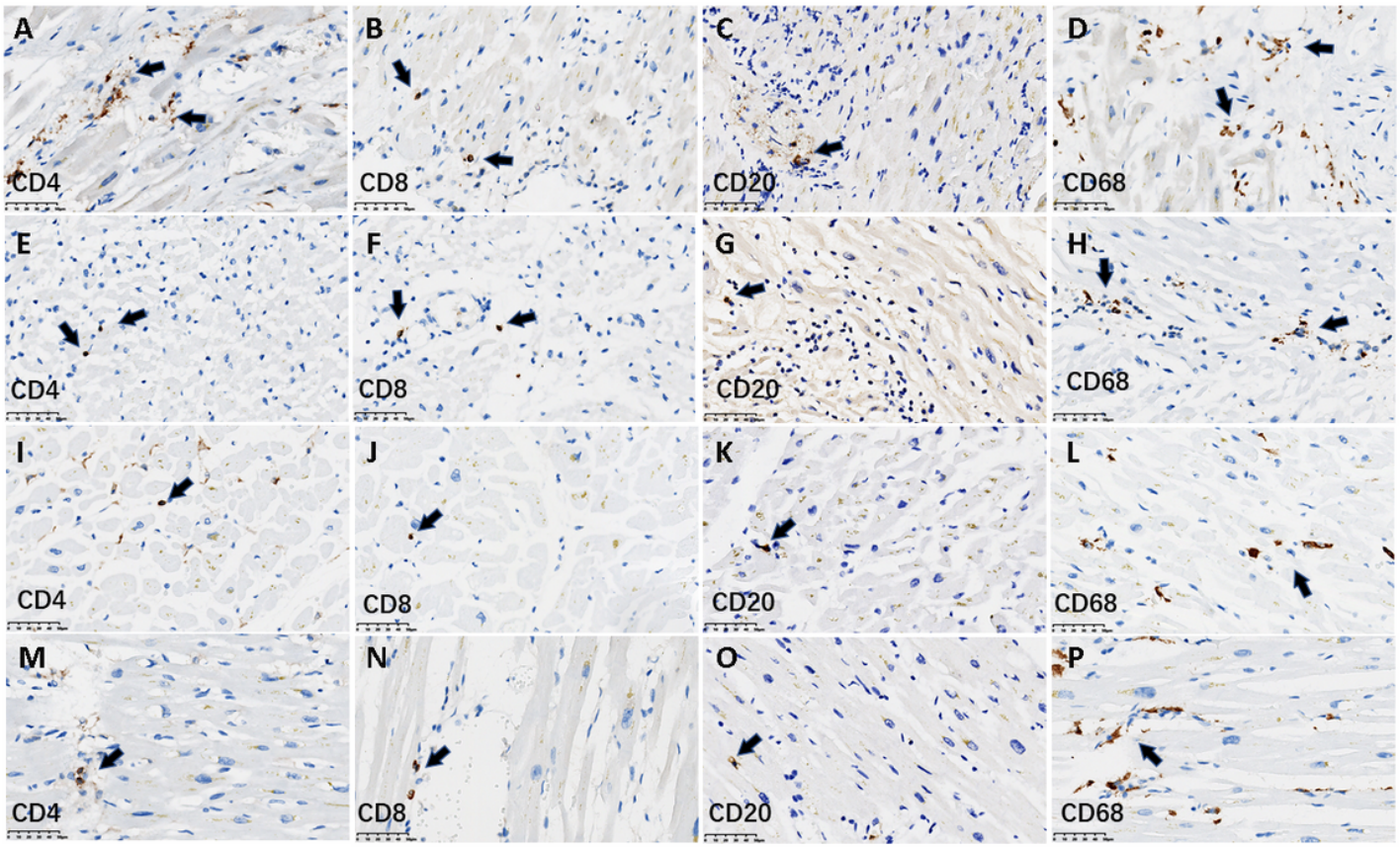

Figure 2

Lymphocyte infiltration using IHC staining within myocardium in representative cases

Figure 2 shows the infiltration of lymphocytes and macrophages stratified by CD4+, CD8+, CD20+ and CD68+ cells in myocardium of four cases. Figure A-H denote IHC staining for lymphocytes from two cases with neutrophil-infiltrated pathological myocarditis. A-D) 62-year-old man; E-H) a 56-year-old woman. Figure I-P denote IHC staining for lymphocytes from two cases without pathological myocarditis. I-L) an 81-year-old man; M-P) a 59-year-old woman. The immunostaining of two myocarditis cases showed there was a scattered infiltration of very mild CD4+, CD8+, CD20+ lymphocytes and single or small clusters of CD68+ macrophages. This pattern was also seen in other two cases without myocarditis. The arrows denote the lymphocytes or macrophages. Scale bars represent 50 μm. IHC: immunohistochemical.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.png
- SupplementalMaterials.docx