ISSN 1881-7815 Online ISSN 1881-7823

BST
BioScience Trends

Volume 14, Number 3
June, 2020

IRCA
BSSA

IACMHR

www.biosciencetrends.com

BST
BioScience Trends

ISSN: 1881-7815
Online ISSN: 1881-7823
CODEN: BTIRCZ
Issues/Year: 6
Language: English
Publisher: IACMHR Co., Ltd.

BioScience Trends is one of a series of peer-reviewed journals of the International Research and Cooperation Association for Bio & Socio-Sciences Advancement (IRCA-BSSA) Group and is published bimonthly by the International Advancement Center for Medicine & Health Research Co., Ltd. (IACMHR Co., Ltd.) and supported by the IRCA-BSSA and Shandong University China-Japan Cooperation Center for Drug Discovery & Screening (SDU-DDSC).

BioScience Trends devotes to publishing the latest and most exciting advances in scientific research. Articles cover fields of life science such as biochemistry, molecular biology, clinical research, public health, medical care system, and social science in order to encourage cooperation and exchange among scientists and clinical researchers.

BioScience Trends publishes Original Articles, Brief Reports, Reviews, Policy Forum articles, Case Reports, News, and Letters on all aspects of the field of life science. All contributions should seek to promote international collaboration.

Editorial Board

Editorial Office

Pearl City Koishikawa 603,
2-4-5 Kasuga, Bunkyo-ku, Tokyo 112-0003, Japan
E-mail: office@biosciencetrends.com

BioScience Trends

Editorial and Head Office

Pearl City Koishikawa 603, 2-4-5 Kasuga, Bunkyo-ku,
Tokyo 112-0003, Japan

E-mail: office@biosciencetrends.com
URL: www.biosciencetrends.com

Review

Original Article

Brief Report

Communication

Erratum

Mini-Review

DOI: 10.5582/bst.2020.03106

Potential for jaktinib hydrochloride to treat cytokine storms in patients with COVID-19

Xianmin Meng[1], Yun Ling[2], Li Zhang[1], Qian Zhang[1], Ping Dong[1], Tongyu Zhu[3], Hongzhou Lu[4,*]

[1] Department of Pharmacy, Shanghai Public Health Clinical Center, Fudan University, Shanghai, China;
[2] Department of Infection, Shanghai Public Health Clinical Center, Fudan University, Shanghai, China;
[3] Department of Urology, Shanghai Public Health Clinical Center, Fudan University, Shanghai, China;
[4] Department of Infection and Imuunity, Shanghai Public Health Clinical Center, Fudan University, Shanghai, China.

SUMMARY Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has become a serious public health threat to the whole world, and the number of infected is still rising dramatically at this moment. Several studies have confirmed that cytokine storms play a critical role in causing a case to worsen from mild to severe or critical. The current treatment for cytokine storms is limited, so the international medical community is focusing on a specific and effective remedy. Jaktinib hydrochloride is a broad spectrum JAK inhibitor. It can inhibit cytokine-induced immune activation by multiple mechanisms and also slow viral proliferation by inhibiting AAK1 without causing unacceptable toxicity. Jaktinib hydrochloride has potential for the treatment of patients with coronavirus disease 2019 (COVID-19).

Keywords severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), coronavirus disease 2019 (COVID-19), cytokines, jaktinib hydrochloride, feasibility

1. Introduction

The outbreak of novel coronavirus-related pneumonia (coronavirus disease 2019, or COVID-19) not only poses a serious threat to Chinese public health and economic and social development but has also become a global public health problem. Among all of the challenges for medical professionals, treatment of critical patients has always been the top priority (*1-4*). Studies indicate that a "cytokine storm" is an important signal that a patient's condition had changed from mild to severe or critical and even life-threatening. In fact, how to suppress that cytokine storm is one of the keys to hopefully curing those critical patients (*5-7*).

In the "Guidelines for the treatment of COVID-19, Version 7" issued by Chinese health authorities, an elevated level of human interleukin-6 (IL- 6) should be treated with tocilizumab in patients in relatively severe condition with extensive lung lesions (*8,9*). Suppressing cytokine storms prior to their occurrence or in their early stages has been crucial to reducing the severity of COVID-19 in patients and improving their prognosis. This implies that a medication to suppress cytokine storms would hold promise. Jaktinib hydrochloride is a broad spectrum novel JAK (Janus kinase, JAK) inhibitor that is expected to inhibit cytokine storms in patients with COVID-19 (*10,11*).

The current article describes the pathogenesis of and relevant treatments for cytokine storms induced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and how jaktinib hydrochloride may play a role in inhibiting those storms in clinical practice.

2. Pathogenesis of SARS-CoV-2 infection

SARS-CoV-2 belongs to a new type of coronavirus of the genus *Betacoronavirus*. It has an envelope, and the particles are round or oval, often polymorphic, with a diameter of 60-140 nm. SARS-CoV-2 and SRAS-CoV have similar pathogenic mechanisms and pathological processes. SARS-CoV-2 invades cells with S protein by receptor-mediated endocytosis of angiotensin-converting enzyme 2 (ACE2) on pulmonary alveolar II epithelial cells (AT2) (*12,13*). After entering the lung cells, the virus replicates in large quantities, triggering humoral and cellular immune responses, perhaps systematically. In the early stages of infection, the body deploys a large number of T cells to fight the virus while some patients will have a lower cellular immunity because of over consumption of T cells. Meanwhile, anti-virus-

specific antibodies will also begin to be produced. As the disease progresses, the destruction of lung cells by the virus increases, and the body's immune response and leukocyte-related cytokine release further kills and eliminates the virus along with necrotic lung cells. In addition, the excessive increase in inflammatory factors, such as various interleukins, can trigger a series of cytokine storms. These excessive inflammatory reactions backfire, resulting in injury and causing overwhelming pulmonary inflammation or secondary fibrotic lesions; they can also ultimately lead to respiratory failure or even death in severe cases.

At present, one of the direct clinical manifestations of COVID-19 in severe cases is the multiple organ damage caused by cytokine storms. Although the virus is the initiating factor, immune overexpression caused by cytokine storms is a direct cause of systematic injury. For instance, clinical evidence indicates that after reaching the turning point of cytokine storms, high-flow oxygen and invasive ventilation are ineffective, eventually resulting in the use of extracorporeal membrane oxygenation (ECMO) (*5*). One of the direct reasons for this is that patients with COVID-19 have scarce surfactants in the alveoli, impaired lung interstitia, impaired ventilation, and thusly as a consequence of unconvertible ventilation failure, ECMO is the only potential rescue technique. Although the factors that cause COVID-19 to worsen from mild to severe or critical are similar, the main problem has to be handled in a quite different manner. The major problem in a mild case is the viral infection, while the main problem in severe or exacerbated illness is the series of cytokine storms.

3. SARS-CoV-2-induced cytokine storm

Cytokine storm syndrome (CSS) is usually defined as abnormal activation of the immune system and uncontrolled release of cytokines, leading to systemic inflammation and multiple organ dysfunction. Several studies have indicated that CSS is also the main reason for death due to SARS and MERS (*14-17*). Cytokine storms can directly damage the pulmonary capillary mucosa, leading to alveolar edema and inactivation of surfactant proteins, which can further induce inflammatory factors to diffuse in the lungs, cause alveolar structures to damaged and degenerate, and result in pulmonary ventilation dysfunction (*18*). During a cytokine storm, the immune system produces pro-inflammatory cytokines that facilitate the progression of the disease while anti-inflammatory factors can accelerate pathogen removal and contribute to tissue repair. The extent of the immune response is correlated with prognosis to an extent.

As mentioned, the first step in the early stages of infection is a non-specific immune response; as the infection progresses, T or B lymphocytes begin to function as the main effector cells of specific immune responses (*14*). In the stage of a specific immune response, pulmonary endothelial cells produce a large volume of inflammatory factors (*19*). Tumor necrosis factor (TNF), IL-1, IL-8, and monocyte chemoattractant protein 1 (MCP-1) are the first to increase, followed by IL-6 and IL-10 (*20*). IL-6 is produced by stimulation caused by TNF and IL-1β, and the peripheral blood concentration can be used to evaluate the intensity of the systemic inflammatory response (*21*).

The mechanism by which SARS-CoV-2 causes cytokine storms is not fully understood. Early studies indicated that patients with COVID-19 have high levels of expression of IL-1B, IFN-γ, interferon-induced protein 10 (IP-10), and MCP-1, and these may activate a Th1 cell response (*22*). In comparison to patients with mild or moderate COVID-19 not necessitating treatment in the ICU, patients with severe COVID-19 in the ICU have higher serum levels of granulocyte colony-stimulating factor (GCSF), IP-10, MCP-1, and macrophage inflammatory protein 1A (MIP-1A) as well as higher levels of expression of TNF-α , suggesting a correlation between the severity of disease and the occurrence of a cytokine storm. In addition, serum levels of IL-2, IL-7, and IL-10 are also significantly elevated (*22*). An autopsy of patients with COVID-19 revealed diffuse alveolar injury with fibrous mucus-like exudates in both lungs, large numbers of shed cells and the formation of transparent membranes, and inflammatory infiltration of monocytes in the alveolar stroma (*23*). In peripheral blood samples from patients who died of COVID-19, $CD4^+$ and $CD8^+$ T lymphocyte counts were decreased, human leukocyte antigen DR (HLA-DR) and the CD38 double-positive T lymphocyte ratio significantly increased, implying that these immune T lymphocytes are excessively activated. In $CD4^+$ T lymphocytes, an incremental increase in the proportion of CC chemokine receptor 4 (CCR-4) and CCR6-positive highly proinflammatory Th17 cells was also detected. $CD8^+$ T lymphocytes contain high concentrations of cytotoxic particles, suggesting that during the occurrence of cytokine storms in COVID-19, excessive activation of T lymphocytes may be accompanied by serious immune impairment (*24*). Based on the above findings, Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways may exist in patients with COVID-19, resulted in immune overreaction and inducing a cytokine storm, eventually causing an excessive immune inflammatory response and/or secondary lung injury/fibrosis, possibly leading to respiratory failure.

4. Current treatment options for SARS CoV-2-induced cytokine storm

Previous versions of the "Guidelines for the treatment of COVID-19" issued by Chinese health authorities

have displayed a gradual increase in the recognition of cytokine storms in COVID-19, and recommended therapeutic strategies are being updated accordingly. The fifth edition mentions that a cytokine test is necessary if available for the first time, and corticosteroid treatment in an appropriate dosage that does not exceed the equivalent of methylprednisolone 1-2 mg/kg/day (*25*) can be administered to patients with inflammatory overactivation for a brief period (3-5 days). The seventh edition indicates that severe and critical cases usually involve complex inflammatory factors, consistently reduced peripheral blood lymphocytes, and conversely increased levels of inflammatory cytokines such as IL-6 and C-reactive protein. After the seventh edition, immunotherapy has been recommended for the management of severe and critical patients, tocilizumab is indicated for patients with severe disease, extensive lung injury, and elevated IL-6 levels according to laboratory results (*8*). The following medications and therapies are listed to inhibit cytokine storms in patients with COVID-19:

i) Glucocorticoids. For critical cases or patients with immune activation, the benefits and risks should be promptly assessed, and caution should always be exercised regarding duration and dosage. Usually, the principle is to start with small doses (≤ 0.5 to 1.0 mg/kg·d methylprednisolone or equivalent) and short-term treatment (≤ 7d) (*8,26*).

ii) IL-6 antagonist (tocilizumab), a recombinant humanized anti- IL-6 receptor monoclonal antibody, is most frequently used in clinical practice for treatment of general infection and the ensued inflammation caused by a cytokine storm (*8,27*). Recent studies have found tocilizumab to be an effective therapeutic strategy, and especially in patients with severe COVID-19 (*9,28*). Therefore, if laboratory results reveal elevated IL-6 in a systematic infection, tocilizumab is indicated.

iii) Chloroquine and hydroxychloroquine. By inhibiting the production and release of TNF and IL-6, these two drugs suppress the occurrence of a cytokine storm. Nearly 300 clinical studies are currently examining different dosages of chloroquine, hydroxychloroquine, or both to treat COVID-19 worldwide, and some of them actually involve severe and critical patients. However, there has been some controversy regarding the safety and efficacy of the two drugs in the treatment of COVID-19 (*29,30*).

iv) Vitamin C in high doses. Vitamin C is administered intravenously at a dose of 100 to 200 mg/kg daily. Some evidence has potentially indicated significant improvement in the oxygenation index (*31*).

v) Ulinastatin. Ulinastatin is a broad-spectrum hydrolase inhibitor, 1.6 million units every 8 h. Under mechanical ventilation, when the oxygenation index is > 300 mmHg, it can be reduced to 1 million units/d (*31*).

vi) Unfractionated heparin. Anticoagulation therapy protects endothelial cells and reduces cytokine release, alleviating the immune response. Unfractionated heparin (3 to 15 IU/kg per hour) is recommended when FDP ≥ 10 μg/mL and/or D-dimer ≥ 5 μg/mL (*31*).

vii) Continuous renal replacement therapy (CRRT). Inflammatory factors can be continuously removed, including cytokines and complement activation products by extracorporeal blood purification, thereby suppressing the systemic inflammatory response (*32,33*).

5. Jaktinib hydrochloride as a drug to treat SARS-CoV-2-induced cytokine storm?

Jaktinib hydrochloride is a broad-spectrum JAK inhibitor with an innovative structure. It is a class 1 new drug according to the Chinese classification, and its development has been sponsored by the National Thirteenth Five-Year Plan. Currently, this compound has passed the milestone of phase I, II, and III clinical trials for five indications, including idiopathic pulmonary fibrosis and primary myelofibrosis.

5.1. Rationale to treat SARS CoV-2- induced cytokine storm by jaktinib hydrochloride

The JAK family of non-receptor protein-tyrosine kinases consists of JAKl, JAK2, JAK3, and tyrosine kinase 2 (TYK2). JAK transmits intracellular signals from cell surface receptors that act on various cytokines and growth factors involved in inflammation and immune function, thus affecting the immune process. Different receptors can activate different subtypes of JAK, thus exhibiting differentiated biological functions (*34*). JAK-STAT signaling pathways are associated with the pathogenesis of a variety of inflammatory diseases, such as rheumatoid arthritis (*35*). Over the past 30 years, JAK has become a very important target, and several JAK inhibitors such as baricitinib, ruxolitinib, and tofacitinib have been successfully developed and marketed for the treatment of primary myelofibrosis, bone marrow fibrosis, and rheumatoid arthritis (*36,37*). As a new JAK inhibitor, jaktinib hydrochloride can suppress JAKl, JAK2, JAK3, and TYK2 at the cell level, block the JAK-STAT signaling pathways to stop the release of cytokines by multiple mechanisms including IL-2, of IL-4, of IL-6, IL-7, and IL-10, and significantly relieve the inflammation due to immune reactions.

The pathophysiological mechanism of SARS-CoV and MERS-CoV has yet to be fully clarified. Earlier studies found that increased levels of pro-inflammatory factors (such as IL-1B, IL-6, IL-12, IFN-γ, IP-10, and MCP-1) in plasma of SARS patients are associated with pneumonia and severe lung injury (38,39). Patients with a MERS-CoV infection were also found to have elevated levels of pro-inflammatory factors (such as IFN-γ, TNF-α, IL-15, and IL-17) in plasma (*17*). Patients with SARS-CoV-2 have been

found to have elevated levels of Th2 cytokines (*e.g.* IL-4 and IL-10) besides elevated levels of cytokines. This inhibits the inflammatory response and differs in a SARS-CoV infection. JAK inhibitors can inhibit the JAK-STAT signaling pathway, reducing the level of IP-10 expression induced by S protein in mouse lungs and repairing the damage caused by a virus on the immune system. JAK inhibitors do not completely inhibit the antiviral action of IFN-γ. Therefore, in addition to antiviral therapy, JAK inhibitors can provide a new therapeutic strategy for virus-mediated immune damage, and especially in the treatment of excessive immune inflammation and/or secondary lung injury or fibrosis caused by SARS-CoV-2 (*10,40*). A prospective, multicenter, single-blind, randomized controlled phase II trial has indicated that recipients of ruxolitinib had numerically faster clinical improvement, significant improvement according to chest CT, faster recovery from lymphopenia, and less severe adverse reactions compared to a group receiving a placebo (*11*). By inhibiting JAK1, JAK2, JAK3, *etc*., jaktinib hydrochloride can prevent abnormal activation of the JAK-STAT pathway and inhibit leukocyte-mediated inflammation *via* multiple channels. It is expected to significantly counteract the inflammation/cytokine storm caused by the immune response in patients with COVID-19 (Figure 1). Jaktinib hydrochloride inhibits the development of pneumonia caused by cytokine storms *via* a similar mechanism and thus prevents the development of pulmonary fibrosis.

Studies have found that SARS-COV-2 invades cells by ACE2 receptors on AT2 alveolar epithelial cells in the lungs (*12*). AP2- associated protein kinase 1 (AAK1) is an endocytosis regulator. Inhibition of AAK1 may block virus transmission in cells and the intracellular assembly of virus particles. Blocking this process of infection may help to slow disease progression (*41*). Jaktinib hydrochloride can inhibit the activity of AAK1 and may block endocytosis whereby the virus enters a cell, interrupting the process of virus assembly, reducing the proliferation of the virus *in vivo*, and having direct antiviral action (Figure 1).

In animal experiments, jaktinib hydrochloride significantly inhibited inflammation and reduced tissue damage. In a bleomycin-induced pulmonary fibrosis model, jaktinib hydrochloride significantly alleviated the damage and inflammation of the terminal bronchioles and accompanying small pulmonary arteries in areas of fibrotic lesions and surrounding areas. Moreover, jaktinib hydrochloride had a significant result on lung injury and inflammation in fibrotic lesions in comparison to the positive control drug pirfenidone. A study of lung samples from autopsied patients with confirmed COVID-19 revealed characteristic interstitial lung disease, thereby indicating the fundamental potential for use of jaktinib hydrochloride.

5.2. Registered clinical trials on JAK inhibitors including jaktinib hydrochloride to treat COVID-19

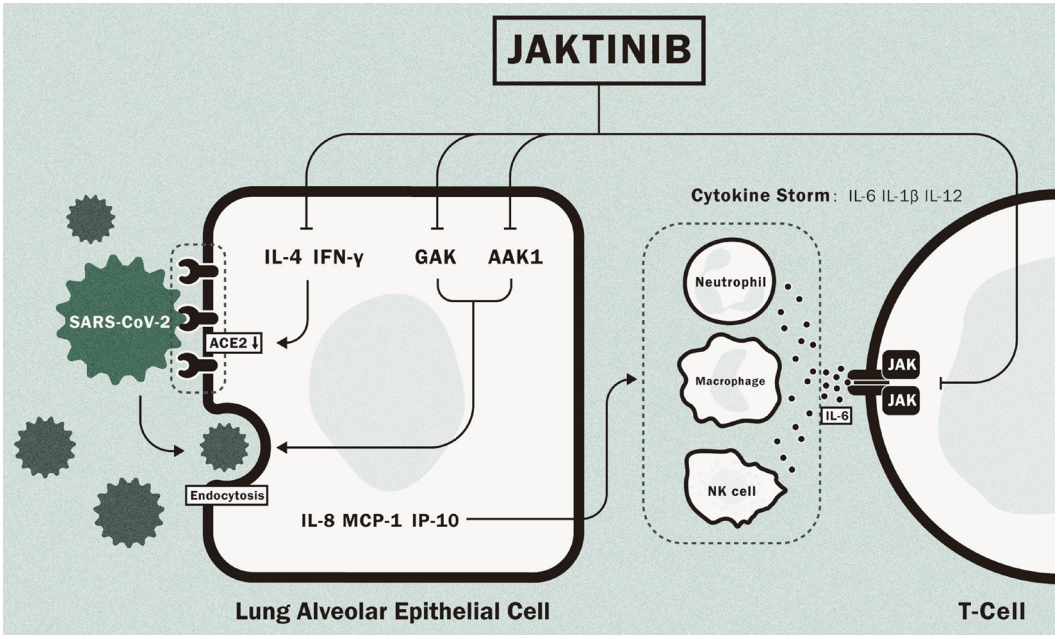


Figure 1. Mechanism of jaktinib in inhibition of viral entry and initiation of a cytokine storm. (1) Via its spike protein, SARS-CoV-2 binds to ACE2 on the surface of pulmonary alveolar II epithelial cells, inducing activation of clathrin-mediated endocytosis. AAK1- and GAK-mediated phosphorylation of clathrin adapter proteins starts the assembly of the clathrin cage around the enclosed virus. Inhibition of AAK1 and GAK by jaktinib thereby inhibits viral entry. (2) Jaktinib stops the release of cytokines such as, IL-1β, IL-12, and IL-6, avoiding the occurrence of a cytokine storm by suppressing JAK. (SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ACE2, angiotensin-converting enzyme 2; AAK1, AP2-associated protein kinase 1; GAK, cyclin G-associated kinase; JAK, Janus kinase)

In view of the pathogenesis of SARS-CoV-2 and the role of cytokine storms in the progression of COVID-19 in severe cases, JAK inhibitors may be an effective option for treatment of COVID-19. In light of the worldwide pandemic, JAK inhibitors are now being used clinically to treat COVID-19. To survey clinical trials on JAK inhibitors as an intervention strategy in COVID-19, a search of the databases of *ClinicalTrials.gov* was conducted using COVID-19, ruxolitinib, baricitinib, and tofacitinib as keywords. Prior to May 28, 2020, 28 studies related to JAK inhibitors and COVID-19 were identified (Table 1). Those studies included 24 interventional studies and 4 observational studies. The estimated study start date was mainly from March to May 2020 and the estimated completion date was mainly from May to October 2020. Thirteen studies had an estimated enrollment of 100 patients or more, 5 had an estimated enrollment of 100 to 200, and 10 had an estimated enrollment of more than 200. Notably, there were only 4 multicenter randomized controlled trials, 3 with baricitinib and 1 with ruxolitinib.

Because many patients with severe respiratory disease due to COVID-19 have features consistent with cytokine release syndrome and increased activation of the JAK/STAT pathway, JAK inhibitors might play a useful role in treating those patients. As shown by Table 1, this view is shared by numerous researchers in many countries and regions around the world. A clinical trial on jaktinib hydrochloride by the current authors is still in the recruitment stage (Registration NO.: ChiCTR2000030170). This study will follow the principles of the Declaration of Helsinki and it has been approved by the ethics committee of the Shanghai Public Health Clinical Center.

6. Conclusion

Humans are now facing SARS-COV-2 globally. There is no established specific treatment available to inhibit cytokine storms in patients with COVID-19, so effective treatments should have top priority. New drugs or vaccines are not likely to be developed soon, but nevertheless the potential of existing innovative medicines could be explored as a plausible alternative (*42*). As a broad-spectrum JAK inhibitor, jaktinib hydrochloride can mitigate the cytokine storms by inhibiting the immune activation induced by IL-6, IL-2, IL-1, granulocyte-macrophage colony-stimulating factor, IFN-γ, *etc*. In addition, jaktinib hydrochloride has the potential to inhibit the proliferation of SARS-CoV-2 *in vivo* by inhibiting AAK1 activity. For patients whose condition has worsened from mild to severe or potentially life-threatening due to cytokine storms, jaktinib hydrochloride may play a dual role in inhibiting cytokine storms and virus replication. This could potentially delay or reverse disease progression, thereby reducing the mortality of COVID-19.

Table 1. Registered clinical trials on baricitinib, ruxolitinib, and tofacitinib for COVID-19

Drugs	No. of studies	Listed Countries	Recruitment Status			Study Phase			Centers		Allocation		Intervention Model			Masking		
			Not yet recruiting	Recruiting	Completed	Phase 2	Phase 3	Other	Multicenter	Single -center	Randomized	Non -randomized	Parallel Assignment	Single Group Assignment	Other	Open Label	Double Blind	Other
Baricitinib	14	9	5	8	1	5	5	4	6	10	6	8	8	2	4	8	2	4
Ruxolitinib	12	9	6	6	0	7	3	2	2	8	4	8	5	5	2	9	2	1
Tofacitinib	2	1	2	0	0	2	0	0	1	1	1	1	1	1	0	2	0	0
total	28	-	13	14	1	14	8	6	9	19	11	17	14	8	6	19	4	5

Conflict of Interest: The authors declare that they have no competing interests. This study was Approved by the ethics committee of Shanghai Public Health Clinical Center (Ethics number YJ-2020-S024-01).

References

1. World Health Organization. Public Health Emergency of International Concern declared. *https://www.who.int/emergencies/diseases/novel-coronavirus-2019/events-as-they-happen* (accessed February 26, 2020).
2. Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. Int J Antimicrob Agents. 2020; 55:105924.
3. Yang X, Yu Y, Xu J, *et al*. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: A single-centered, retrospective, observational study. Lancet Respir. Med. 2020; 8:475-481.
4. Guan W-j, Ni Z-y, Hu Y, *et al*. Clinical characteristics of 2019 novel coronavirus infection in China. medRxiv. doi: https://doi.org/10.1101/2020.02.06.20020974
5. Wan S, Yi Q, Fan S, *et al*. Characteristics of lymphocyte subsets and cytokines in peripheral blood of 123 hospitalized patients with 2019 novel coronavirus pneumonia (NCP). medRxiv. 2020; https://doi.org/10.1101/2020.02.10.20021832
6. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020; 395:1033-1034.
7. Chen C, Zhang X, Ju Z, He W. Advances in the study of the mechanism of cytokine storms induced by COVID-19 and corresponding immunotherapies. Zhonghua Shao Shang Za Zhi. 2020; 36:E005. (in Chinese)
8. General Office of the National Health and Health Commission. Plan for Diagnosis and Treatment of Novel Coronavirus-related Pneumonia (Draft Version 6). *http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989/files/ce3e6945832a438eaae415350a8ce964.pdf* (accessed March 3, 2020). (in Chinese)
9. Xu X, Han M, Li T, Sun W, Wang D, Fu B, Zhou Y, Zheng X, Yang Y, Li X, Zhang X, Pan A, Wei H. Effective treatment of severe COVID-19 patients with tocilizumab. Proc Natl Acad Sci U S A. 2020; 117:10970-10975
10. Richardson P, Griffin I, Tucker C, Smith D, Oechsle O, Phelan A, Stebbing J. Baricitinib as potential treatment for 2019-nCoV acute respiratory disease. Lancet. 2020; 395:e30-e31.
11. Cao Y, Wei J, Zou L, *et al*. Ruxolitinib in treatment of severe coronavirus disease 2019 (COVID-19): A multicenter, single-blind, randomized controlled trial. J Allergy Clin Immunol. 2020; S0091-6749(20)30738-7. doi: 10.1016/j.jaci.2020.05.019.
12. Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, Zhong W, Hao P. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci. 2020; 63:457-460.
13. Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y, Zuo W. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov. bioRxiv. 2020; doi: https://doi.org/10.1101/2020.01.26.919985.
14. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: Causes and consequences of cytokine storm and immunopathology. Semin Immunopathol. 2017; 39:529-539.
15. Hui DSC, Zumla A. Severe acute respiratory syndrome: Historical, epidemiologic, and clinical features. Infect Dis Clin North Am. 2019; 33:869-889.
16. Reichsoellner M, Raggam RB, Wagner J, Krause R, Hoenigl M. Clinical evaluation of multiple inflammation biomarkers for diagnosis and prognosis for patients with systemic inflammatory response syndrome. J Clin Microbiol. 2014; 52:4063-4066.
17. Al-Jasser FS, Nouh RM, Youssef RM. Epidemiology and predictors of survival of MERS-CoV infections in Riyadh region, 2014-2015. J Infect Public Health. 2019; 12:171-177.
18. Zhang B, Swamy S, Balijepalli S, Panicker S, Mooliyil J, Sherman MA, Parkkinen J, Raghavendran K, Suresh MV. Direct pulmonary delivery of solubilized curcumin reduces severity of lethal pneumonia. FASEB J. 2019; 33:13294-13309.
19. Krischuns T, Gunl F, Henschel L, Binder M, Willemsen J, Schloer S, Rescher U, Gerlt V, Zimmer G, Nordhoff C, Ludwig S, Brunotte L. Phosphorylation of TRIM28 enhances the expression of IFN-beta and proinflammatory cytokines during HPAIV infection of human lung epithelial cells. Front Immunol. 2018; 9:2229.
20. Kuldanek SA, Kelher M, Silliman CC. Risk factors, management and prevention of transfusion-related acute lung injury: A comprehensive update. Expert Rev Hematol. 2019; 12:773-785.
21. Tanaka T, Narazaki M, Masuda K, Kishimoto T. Regulation of IL-6 in immunity and diseases. Adv Exp Med Biol. 2016; 941:79-88.
22. Huang C, Wang Y, Li X, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395:497-506.
23. Wu JH, Li X, Huang B, Su H, Li Y, Luo DJ, Chen S, Ma L, Wang SH, Nie X, Peng L. Pathological Changes of Fatal Coronavirus Disease 2019 (COVID-19) in the Lungs: Report of 10 Cases by Postmortem Needle Autopsy. Zhonghua Bing Li Xue Za Zhi. 2020; 6:568-575. (in Chinese)
24. Xu Z, Shi L, Wang Y, *et al*. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med. 2020; 8:420-422.
25. General Office of the National Health and Health Commission. Diagnosis and treatment of pneumonia caused by a novel coronavirus (Draft version 5). *http://www.gov.cn/zhengce/zhengceku/2020-02/05/5474791/files/de44557832ad4be1929091dcbcfca891.pdf* (accessed February 7, 2020). (in Chinese)
26. Shang L, Zhao J, Hu Y, Du R, Cao B. On the use of corticosteroids for 2019-nCoV pneumonia. Lancet. 2020; 395:683-684.
27. Faguer S, Vergez F, Peres M, Ferrandiz I, Casemayou A, Belliere J, Cointault O, Lavayssiere L, Nogier MB, Prevot G, Huart A, Recher C, Rostaing L. Tocilizumab added to conventional therapy reverses both the cytokine profile and $CD8^{+}Granzyme^{+}$ T-cells/NK cells expansion in refractory hemophagocytic lymphohistiocytosis. Hematol Oncol. 2016; 34:55-57.
28. Cellina M, Orsi M, Bombaci F, Sala M, Marino P, Oliva

G. Favorable changes of CT findings in a patient with COVID-19 pneumonia after treatment with tocilizumab. Diagn. Interv. Imaging. 2020; 101:323-324.
29. Gao J, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends. 2020; 14:72-73.
30. Guastalegname M, Vallone A. Could chloroquine/hydroxychloroquine be harmful in coronavirus disease 2019 (COVID-19) treatment? Clin Infect Dis. 2020; ciaa321. doi: 10.1093/cid/ciaa321.
31. Shanghai Expert Group on Clinical Treatment of Novel Coronavirus Disease. Comprehensive treatment and management of corona virus disease 2019: Expert consensus statement from Shanghai. Chinese Journal of Infectious Diseases. 2020; 38. doi: 10.3760/cma.j.issn.1000- 6680.2020.0016.
32. Ning HB, Li K, Peng Z, Wang YG, Gu Y, Shang J. Continuous renal replacement therapy for a severe case of novel coronavirus-related pneumonia. Chinese Journal of Infectious Diseases. 2020; 38. doi: 10.3760/cma.j.cn311365-20200218-00079. (in Chinese)
33. Chinese Medical Association Expert Group on Kidney Disease. Recommendations for prevention and control of novel coronavirus infection in pediatric blood purification center. Chinese Journal of Nephrology, .2020; 36:82-84. (in Chinese)
34. Schwartz DM, Bonelli M, Gadina M, O'Shea JJ. Type I/II cytokines, JAKs, and new strategies for treating autoimmune diseases. Nat Rev Rheumatol. 2015; 12:25-36.
35. Banerjee S, Biehl A, Gadina M, Hasni S, Schwartz DM. JAK-STAT signaling as a target for inflammatory and autoimmune disease: Current and future prospects. Drugs. 2017; 77:521-546.
36. Roskoski, Robert. Janus kinase (JAK) inhibitors in the treatment of inflammatory and neoplastic diseases. Pharmacol Res.2016; 111:784-803.
37. Schwartz DM, Kanno Y, Villarino A, Ward M, Gadina M, O'Shea JJ. JAK inhibition as a therapeutic strategy for immune and inflammatory diseases. Nat Rev Drug Discov.2017;16:843-862.
38. Chien JY, Hsueh PR, Cheng WC, Yu CJ, Yang PC. Temporal changes in cytokine/chemokine profiles and pulmonary involvement in severe acute respiratory syndrome. Respirology. 2006; 11:715-722.
39. Kong SL, Chui P, Lim B, Salto-Tellez M. Elucidating the molecular physiopathology of acute respiratory distress syndrome in severe acute respiratory syndrome patients. Virus Res. 2009; 145:260-269.
40. Cantini F, Niccoli L, Matarrese D, Nicastri E, Stobbione P, Goletti D. Baricitinib therapy in COVID-19: A pilot study on safety and clinical impact. J. Infect. 2020.
41. Stebbing J, Phelan A, Griffin I, Tucker C, Oechsle O, Smith D, Richardson P. COVID-19: Combining antiviral and anti-inflammatory treatments. Lancet Infect. Dis. 2020; 20 :400-402.
42. Lurie N, Saville M, Hatchett R, Halton J. Developing Covid-19 vaccines at pandemic speed. N Engl J Med. 2020; 382:1969-1973.

Received April 22, 2020; Revised June 2, 2020; Accepted June 9, 2020.

*Address correspondence to:
Hongzhou Lu, Department of Infection and Immunity, Shanghai Public Health Clinical Center, Fudan University, Shanghai 201508, China.
E-mail: luhongzhou@fudan.edu.cn

Released online in J-STAGE as advance publication June 12, 2020.

Mini-Review

DOI: 10.5582/bst.2020.03010

Current status of laparoscopic radical hilar cholangiocarcinoma in Mainland China

Yizhen Chen[1,2,§], Youyao Xu[1,2,§], Yuhua Zhang[1,*]

[1] Division of Hepatobiliary and Pancreatic Surgery and Minimally Invasive Surgery, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, Zhejiang, China;
[2] Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China.

SUMMARY Our purpose was to explore the status of laparoscopic radical resection of hilar cholangiocarcinoma (LRRHcca) in Mainland China. Studies published before February 2020 were retrieved from CNKI database, Pubmed database and Wanfang database. Search terms included "hilar cholangiocarcinoma", "Klatskin tumor", "laparoscopy", "radical operation". Relevant articles regarding LRRHcca in Mainland China were also retrieved. 13 articles were included in this study, with a total of 189 cases. The operation time was 354 min (weighed average, WA), and the mean intraoperative blood loss was 324 mL (WA). The rate of negative margin (R0 rate) was 95.2%, and the number of lymph nodes received was 9.5 (WA). 2.6% of cases were converted to laparotomy. The incidence of postoperative complications was 21.2%, with 3.2% for those classified as Clavien-Dindo ≥ 3, 12.2% for bile leakage, 1.6% for postoperative abdominal hemorrhage, 1.6% for liver insufficiency, and 1.1% for abdominal infection. In-hospital mortality was 0.5%, with mean postoperative hospital stay of 15 days (WA), and the rate of reoperation was 1.1%. The mean postoperative follow-up time was 16 months (WA), and 1-year overall survival rate was 84.5%. In conclusions, laparoscopic radical hilar cholangiocarcinoma is safe and feasible in experienced hands after careful selection of HCCA cases.

Keywords hilar cholangiocarcinoma, Klatskin tumor, laparoscopy, radical operation

1. Introduction

Hilar cholangiocarcinoma (HCCA), also known as Klatskin tumor, accounts for 60 -70% of the total incidence of cholangiocarcinoma. Radical surgical resection is the only chance for long-term survival of HCCA patients (*1,2*). Common bile duct resection, major liver resection, radical lymph node dissection, biliary reconstruction, and even vein or artery resection are required for R0 resection of HCCA. Laparoscopic radical resection of HCCA (LRRHcca) is rarely reported due to its technically demanding. Laparoscopy was originally used for HCCA exploration and tumor staging (*3-5*). Compared with laparoscopic surgery for liver or pancreatic cancer, LRRHcca is still in the exploratory stage. However, experience gained in other complex abdominal laparoscopic procedures and improvements of laparoscopic instruments have motivated surgeons to try LRRHcca. Since the first report of LRRHcca in Mainland China in 2003 (*6*), more and more hepatobiliary surgery centers in Mainland China have begun to perform this operation. Total laparoscopic resection was performed even for HCCA of Bismuth-IV in the experienced center. Current limited clinical data suggest that LRRHcca was associated with minor injury, less pain, faster recovery, and fewer complications. These advantages have prompted more hepatobiliary surgeons to explore LRRHcca. This article reviewed the relevant articles of LRRHcca in Mainland China, and performed data extraction and analysis on China Mainland cases. This article reviewed the current status of LRRHcca and mainly focuses on the safety and feasibility of this operation.

2. Articles search strategies and analysis methods

2.1. Articles search strategies

Studies published before February 2020 were retrieved from CNKI database, PubMed database and Wanfang database. Search terms included "hilar cholangiocarcinoma", " Klatskin tumor", "laparoscopy", "radical operation". This study collected perioperative and prognostic data of LRRHcca from the retrieved articles. These data were subjected to statistical analysis. We screened out the following articles: (*i*) Chinese

articles without English abstracts. (*ii*) Articles with data loss. (*iii*) Articles using the same hospital cases, Articles with fewer cases (Figure 1).

2.2. Data extraction

(*i*) Basic information of the article: first author, year of publication, number of cases. (*ii*) Related conditions during the operation: surgical method, Bismuth classification, operation time, intraoperative blood loss, R0 resection rate, lymph node positive rate, and number of lymph node dissections during operation. (*iii*) Relevant postoperative conditions: morbidity, clavien-Dindo ≥ 3, mortality, length of hospital stay, reoperation rate, follow-up after discharge, tumor metastasis or recurrence after discharge, and 1-year overall survival rate.

2.3. Statistical analysis

Weighted average (WA) is used to represent the statistical weighted average of different variables (operation time, intraoperative blood loss, *etc*.):

$$WA = (w1x1 + w2x2 + ... + wnxn) / (w1 + w2 + ... + wn).$$

Where w is the number of cases in related articles, and x is the average value provided in the article or calculated after our statistics. For data presented as a median x value or missing data, x and its corresponding w is excluded.

3. Results

This article systematically reviewed 13 articles of LRRHcca in Mainland China (*7-19*). The total number of included cases was 189 (Table 1). Surgical method was described for 73.5% (139/189) of total cases. Among these cases, 43.2% (60/139) underwent liver resection. 54 patients underwent liver resection combined with caudate lobectomy, 2 patients did not receive caudate lobectomy, and 4 patients received caudate lobectomy alone. The distribution rates for Bismuth type I, II, III and IV were 41.8% (79/189), 22.8% (43/189), 24.3% (46/189), and 11.1% (21/189), respectively. The number of articles reported of laparoscopic radical

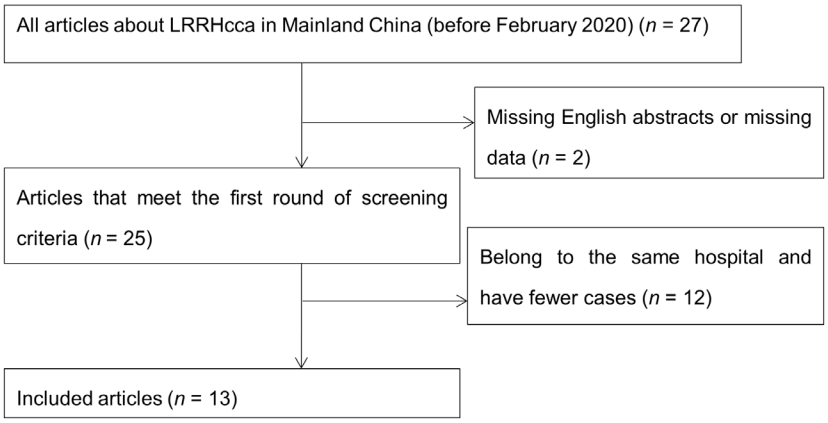


Figure 1. Screening process of LRRHcca related articles.

Table 1. Research Status of LRRHcca in Mainland China

First author (*Ref.*)	Publication year	Cases	Operation method (Hepatectomy, caudate lobectomy)	Bismuth-type classification (I:II:III:IV)
Jun Xu (*7*)	2010	4	1,0	1:1:1:1
Andong Zhu (*8*)	2012	38	NA,NA	13:18:7:0
Jianjun Li (*9*)	2012	3	1,0	2:0:1:0
Jun Li (*10*)	2017	9	2,4	1:3:2:3
Hongchao Zhu (*11*)	2018	7	0,0	7:0:0:0
Xueqing Liu (*12*)	2019	32	8,8	12:2:7:11
Yuhua Zhang (*13*)	2019	14	9,9	5:0:8:1
Xinyu Liu (*14*)	2019	6	6,6	0:0:3:3
Ruofan Wang (*15*)	2019	15	10,12	2:3:8:2
Yuxiao Zhu (*16*)	2019	14	2,2[#]	7:5:2:0
Hua Luo (*17*)	2019	4	3,3	1:1:2:0
Chang Duan (*18*)	2019	13	0,0	13:0:0:0
Junjian Yuan (*19*)	2019	30	14,14	15:10:5:0
Total/mean	-	189	56,58	79:43:46:21

NA, not applicable. [#], This article only reported the surgical methods for 2 cases.

hilar cholangiocarcinoma in Mainland China increased significantly in 2019 (Figure 2), accounting for 61.5% (8/13), and only 38.5% during the nine years from 2010 to 2018 (5/13). The number of cases reported in laparoscopic radical hilar cholangiocarcinoma in Mainland China in 2019 accounted for 67.7% (128/189) and 32.3% (61/189) between 2010 and 2018.

3.1. Intraoperative situation

The intraoperative data of LRRHcca are demonstrated in Table 2, including operation time, intraoperative blood loss, negative rate of surgical margin (R0 rate) and so on. Mean operative time was reported in 92.6% (175/189) of cases, with WA value of 354 min. The mean intraoperative blood loss was reported in 75.7% (143/189) of cases, with WA value of 324 mL. The intraoperative margin negative rate (R0 rate) was reported in 100% (189/189) of cases, with a total rate of 95.2% (180/189). Lymph node-positive rates were reported in 58.7% (111/189) of cases, with a total rate of 14.4% (16/111). The number of lymph nodes received was reported in 61.9% (117/189) of cases, with WA value of 9.5. Of the 189 patients, 5 patients were converted to laparotomy (2.6%), with 3 patients having tumor invasion of the portal vein, 1 patient having severe abdominal adhesions, and 1 patient having difficulty in separating tumor and bile duct under laparoscopy.

3.2. Postoperative situation

The postoperative conditions of LRRHcca are presented in Tables 3, 4 and 5. The postoperative complication rate was reported in 100% (189/189) of cases, and the overall complication rate was 21.2% (40/189); serious complications (Clavien-Dindo ≥ 3) occurred in 3.2% (6/189) of patients. The reoperation rate was 1.1% (2/189). In-hospital mortality was 0.5% (1/189). The average postoperative hospital stay was reported in 92.6% (175/189) of patients, ranging from 9.6 days to 27.6 days, and WA was 15 days.

Bile leakage is the most common complication

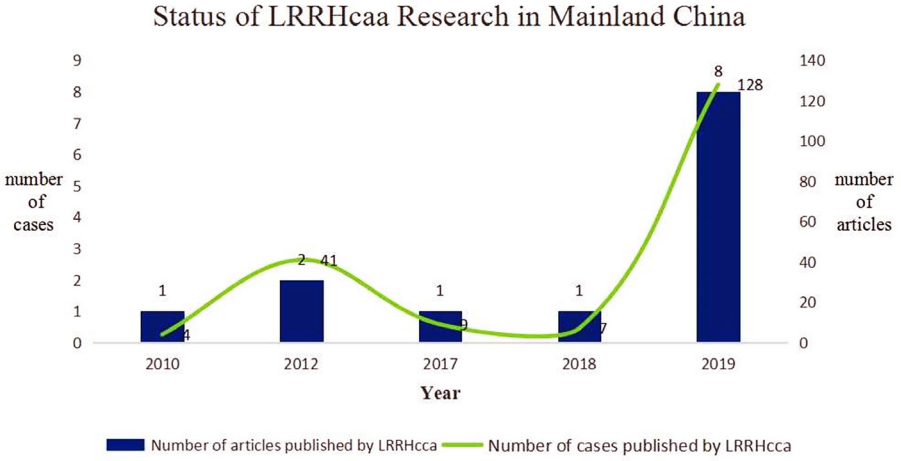


Figure 2. The number of cases and articles of LRRHcca in Mainland China. The line chart shows the number of articles published by LRRHcca over the years, and the bar chart shows the number of cases published by LRRHcca over the years.

Table 2. Intraoperative situation of LRRHcca in Mainland China

First author (*Ref.*)	Operation time (min)	Blood loss (mL)	R0, *n* (%)	lymph node positive rate, *n* (%)	Harvested lymph node, *n*	Conversion to laparotomy, *n* (%)
Jun Xu (*7*)	Mean 384	Mean 440	4 (100%)	NA	NA	0 (0%)
Andong Zhu (*8*)	Mean 267	Mean 83	38 (100%)	2 (5.3%)	Mean 9.4	0 (0%)
Jianjun Li (*9*)	Mean 520	Mean 333	3 (100%)	0 (0%)	NA	0 (0%)
Jun Li (*10*)	Mean 438	Mean 503	9 (100%)	NA	NA	0 (0%)
Hongchao Zhu (*11*)	Mean 248	Mean 156	7 (100%)	0 (0%)	Mean 9.3	0 (0%)
Xueqing Liu (*12*)	Mean 366	300 (75, 450)	24 (75%)	NA	NA	1 (3.1%)
Yuhua Zhang (*13*)	Mean 519	Mean 821	14 (100%)	NA	Mean 9.7	2 (14.3%)
Xinyu Liu (*14*)	Mean 590	Mean 400	5 (83.3%)	NA	NA	0 (0%)
Ruofan Wang (*15*)	Mean 404	Mean 502	15 (100%)	1 (6.7%)	Mean 9.5	2 (13.3%)
Yuxiao Zhu (*16*)	285 (190, 400)	175 (100, 500)	14 (100%)	3 (21.4%)	8 (6,15)	0 (0%)
Hua Luo (*17*)	Mean 616	Mean 468	4 (100%)	0 (0%)	NA	0 (0%)
Chang Duan (*18*)	Mean 260	Mean 177	13 (100%)	NA	Mean 9.6	0 (0%)
Junjian Yuan (*19*)	Mean 287	Mean 140	30 (100%)	10 (33.3%)	Mean 9.4	0 (0%)
Total/mean	35 4 (WA)	32 4 (WA)	180 (95.2%)	16 (14.4%)	9.5 (WA)	5 (2.6%)

NA, not applicable.

Table 3. Postoperative situation of LRRHcca in Mainland China

First author (*Ref.*)	Morbidity (%)	Mortality *n* (%)	LOS (days)	Clavien-Dindo ≥ 3 complications *n* (%)	Reoperation *n* (%)
Jun Xu (*7*)	0%	0 (0%)	Mean 15	0 (0%)	0 (0%)
Andong Zhu (*8*)	13.2%	0 (0%)	Mean 12	1 (2.6%)	1 (2.6%)
Jianjun Li (*9*)	66.7%	0 (0%)	Mean 16.7	0 (0%)	0 (0%)
Jun Li (*10*)	44.4%	0 (0%)	Mean 15.7	0 (0%)	0 (0%)
Hongchao Zhu (*11*)	0%	0 (0%)	Mean 10.7	0 (0%)	0 (0%)
Xueqing Liu (*12*)	15.6%	0 (0%)	Mean 27.6	0 (0%)	0 (0%)
Yuhua Zhang (*13*)	50%	1 (7.1%)	Mean 17.8	5 (35.7%)	1 (7.1%)
Xinyu Liu (*14*)	16.7%	0 (0%)	Mean 16.5	0 (0%)	0 (0%)
Ruofan Wang (*15*)	20%	0 (0%)	Mean 9.6	0 (0%)	0 (0%)
Yuxiao Zhu (*16*)	14.3%	0 (0%)	NA	0 (0%)	0 (0%)
Hua Luo (*17*)	25%	0 (0%)	Mean 14.3	0 (0%)	0 (0%)
Chang Duan (*18*)	23.1%	0 (0%)	Mean 10.6	0 (0%)	0 (0%)
Junjian Yuan (*19*)	23.3%	0 (0%)	Mean 8.9	0 (0%)	0 (0%)
Total/mean	40 (21.2%)	1 (0.5%)	15 (WA)	6 (3.2%)	2 (1.1%)

LOS, length of hospital stay. NA, not applicable.

Table 4. Details of postoperative complications of LRRHcca in Mainland China

First author (*Ref.*)	Abdominal Hemorrhage, *n* (%)	Abdominal infection, *n* (%)	Bile leak, *n* (%)	Hepatic insufficienc, *n* (%)	Others, *n* (%)
Jun Xu (*7*)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Andong Zhu (*8*)	1 (2.6%)	0 (0%)	3 (7.9%)	0 (0%)	1 (2.6%)
Jianjun Li (*9*)	0 (0%)	0 (0%)	1 (33.3%)	0 (0%)	1 (33.3%)
Jun Li (*10*)	0 (0%)	0 (0%)	2 (22.2%)	0 (0%)	2 (22.2%)
Hongchao Zhu (*11*)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Xueqing Liu (*12*)	0 (0%)	0 (0%)	1 (3.1%)	2 (6.3%)	2 (6.3%)
Yuhua Zhang (*13*)	1 (7.1%)	0 (0%)	5 (35.7%)	0 (0%)	1 (7.1%)
Xinyu Liu (*14*)	0 (0%)	0 (0%)	1 (16.7%)	0 (0%)	0 (0%)
Ruofan Wang (*15*)	0 (0%)	0 (0%)	1 (6.7%)	0 (0%)	2 (13.3%)
Yuxiao Zhu (*16*)	0 (0%)	0 (0%)	2 (14%)	0 (0%)	0 (0%)
Hua Luo (*17*)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)
Chang Duan (*18*)	0 (0%)	2 (15.4%)	1 (7.8%)	0 (0%)	0 (0%)
Junjian Yuan (*19*)	1 (3.3%)	0 (0%)	5 (16.7%)	0 (0%)	1 (3.3%)
Total/mean	3 (1.6%)	2 (1.1%)	23 (12.2%)	3 (1.6%)	9 (4.8%)

Table 5. Short-term effect of LRRHcca in Mainland China

First author (*Ref.*)	follow-up time (months)	Metastasis or Recurrence, *n* (%)	mortality during the follow-up, *n* (%)	1-year overall Survival, (%)
Jun Xu (*7*)	NA	NA	NA	NA
Andong Zhu (*8*)	Mean 12.5	1 (2.6%)	2 (5.3%)	97.4%
Jianjun Li (*9*)	Mean 57	0 (0%)	0 (0%)	100%
Jun Li (*10*)	Mean 17	NA	2 (22.2%)	77.8%
Hongchao Zhu (*11*)	12-18	0 (0%)	0 (0%)	100%
Xueqing Liu (*12*)	NA	NA	NA	80%
Yuhua Zhang (*13*)	NA	NA	NA	62.5%
Xinyu Liu (*14*)	7 (1,42)	0	0 (0%)	NA
Ruofan Wang (*15*)	6-60	3 (20%)	4 (26.7%)	NA
Yuxiao Zhu (*16*)	16 (3,24)	1 (7.1%)	1 (7.1%)	NA
Hua Luo (*17*)	1-4	0 (0%)	0 (0%)	NA
Chang Duan (*18*)	36-60	NA	NA	74.4%
Junjian Yuan (*19*)	6-12	3 (10%)	3 (10%)	NA
Total/mean	16 (WA)	8 (6.8%)	12 (8.6%)	84.5%

NA, not applicable.

after laparoscopic hilar cholangiocarcinoma radical resection, with a result of 12.2% (23/189). The rates of ascites fluid, pulmonary infections, stress ulcers, anastomotic edema, wound infection, liquefaction of the incision fat were 1.1% (2/189), 1.1% (2/189), 1.1% (2/189), 0.5% (1/189), 0.5% (1/189), and 0.5% (1/189), respectively. The incidence of postoperative abdominal bleeding was 1.6% (3/189). Liver dysfunction happened at the rate of 1.6% (3/189) and the incidence of abdominal infection was 1.1% (2/189).

The average postoperative follow-up time was reported in 26.5% (50/189) of cases, the median postoperative follow-up time was reported in 47% (89/189) cases, and data was lost in 26.5% (50/189) cases, the mean follow-up time was 16 months (WA). Detailed follow-up results were obtained in 61.9% (117/189) of the cases, disease free survival rate was 93.2% (109/117). Since the follow-up time corresponding to disease free survival is median or missing, the time for disease free survival is not summarized. 74.1% (140/189) of cases reported the survival situation in detail, 8.6% (12/140) of cases died during follow-up. Death due to tumor recurrence and metastasis, cardiovascular and cerebrovascular disease, liver failure were 5.7% (8/140), 2.1% (3/140) and 0.7% (1/140), respectively. The 1-year overall survival rate was 84.5%.

4. Discussion

4.1. Development history

The first case of LRRHcca in Mainland China was reported by Professor Liu (*6*) in 2003. Since this article lacks English abstract, it is not included in this study. This article reported a case of hilar cholangiocarcinoma, who underwent hemi-hepatectomy without caudate lobe resection and extrahepatic bile duct resection. A negative margin of bile duct incision was achieved. In 2008, Chen *et al.* (*20*) conducted LRRHcca for 4 patients. All 4 patients underwent extrahepatic bile duct resection combined with partial hepatic hilar resection, but did not undergo extensive hepatectomy with caudate lobe resection. These indicate that laparoscopic techniques were feasible for the resection of hilar bile duct tumors, hilar lymph node dissection, and biliary reconstruction. After 2012, the series of LRRHcca articles gradually increased (*9,11,21*). In recent years, LRRHcca has developed very rapidly in Mainland China. The number of articles in 2019 was 1.6 times the total from 2010 to 2018, and the number of cases reported in 2019 was 2.1 times the number from 2010 to 2018. Although this failed to represent the annual LRRHcca operation volume, it suggested that LRRHcca has gradually become a hot spot for hepatobiliary surgeons. More and more minimally invasive hepatobiliary centers have begun to try this operation.

4.2. Technical aspects

Radical surgery for hilar cholangiocarcinoma requires extensive hepatectomy including combined caudate lobe resection, thorough lymph node dissection, and difficult bile-enteric anastomosis techniques to ensure adequate margins. This is technically difficult even in traditional open surgery. The development of LRRHcca represents the development of laparoscopic techniques. In recent years, the accumulated experience of laparoscopic liver resection, laparoscopic radical gastrectomy, laparoscopic pancreaticoduodenectomy, and other complex operations laid a solid foundation in technical aspects for exploring LRRHcca. Currently, laparoscopic caudate lobe resection, high bile-gut anastomosis, and thorough lymph node dissection have become routine and mature techniques in many China Mainland laparoscopic centers. This will inevitably lead to the exploration and development of LRRHcca. However, for cases requiring vascular resection and reconstruction, laparoscopy is not recommended. Even if successful cases of portal vein resection and reconstruction under laparoscopy were reported, open surgery is still recommended to ensure the safety of the operation. Only 3 (1.6%) of the 189 patients underwent portal vein resection and reconstruction (*12,14*), and none underwent hepatic artery resection and reconstruction. Preoperative CT, MRCP and 3D imaging results were used to determine the location, size and invasion range of the tumors. Preliminary judgment of resectability can be achieved by preoperative examination while final judgment can only be confirmed through intraoperative exploration. Although vascular resection and reconstruction is feasible during laparoscopic pancreatoduodenectomy (LPD), which has good operating space and stump angle, it is extremely difficult and risky for LRRHcca, which has limited vascular angle and operating space. Among 189 cases, Bismuth-I type accounted for 41.8%, Bismuth-II type accounted for 22.8%, Bismuth-III type accounted for 24.3%, and Bismuth-IV type accounted for 11.1%. Since difficulty of operation is dramatically increased for cases of Bismuth III and IV, surgeons are recommended to explore LRRHcca starting from cases of Bismuth-I and II.

4.3. Perioperative safety

Although the data regarding the average operation time, average blood loss and postoperative complication rate in this article suggest that LLRHcca in Mainland China is safe and reliable, these retrospective studies probably have selection bias of data reporting. Based on the recently published data from our center (*13*), the average postoperative hospital stay was 23 days, and the Clavien-Dindo ≥ 3 rate was 35%. More clinical research is needed to further confirm safety and prognosis of LRRHcca.

4.4. Oncology efficacy

The statistical data in this study showed that the R0 rate of LRRHcca in Mainland China was 95.2%, suggesting that laparoscopy is associated with a better R0 rate. The positive rate of resection margin directly affects the prognosis of patients (*22*). Hepatectomy was performed in 43.2% (60/139) of the patients that described the specific surgical method, and less than half of the patients

underwent liver resection to achieve a rate of nearly 100% R0. Since Bismuth-I type and Bismuth-II type accounted for a total of 64.6% of the patients, most of these two types of patients can achieve curative resection after bile duct resection. In the initial stage of LRRHcca, the surgeon will tend to choose cases of Bismuth-I and Bismuth-II. Compared with the 1-year overall survival data of open radical hilar cholangiocarcinoma in Mainland China (*23*), the postoperative efficacy of LLRHcca is not significantly different from that of open surgery (71.1%).

5. Conclusions

The data in this paper indicates that laparoscopic radical hilar cholangiocarcinoma is safe and feasible in experienced hands after careful selection of HCCA cases. Surgeons with enough experience in laparoscopic operations and sophisticated skills are encouraged to explore this challenging operation. The clinical value of this operation will be verified by high-quality clinical trials in the future.

References

1. Zhang W, Yan LN. Perihilar cholangiocarcinoma: Current therapy. World J Gastrointest Pathophysiol. 2014; 5:344-354.
2. Rizvi S, Khan SA, Hallemeier CL, Kelley RK, Gores GJ. Cholangiocarcinoma – evolving concepts and therapeutic strategies. Nat Rev Clin Oncol. 2018; 15:95-111.
3. Ruys AT, Busch OR, Gouma DJ, van Gulik TM. Staging Laparoscopy for Hilar Cholangiocarcinoma: is it Still Worthwhile? Indian J Surg Oncol. 2012; 3:147-153.
4. Barlow AD, Garcea G, Berry DP, Rajesh A, Patel R, Metcalfe MS, Dennison AR. Staging laparoscopy for hilar cholangiocarcinoma in 100 patients. Langenbecks Arch Surg. 2013; 398:983-988.
5. Rotellar F, Pardo F. Laparoscopic staging in hilar cholangiocarcinoma: is it still justified? World J Gastrointest Oncol. 2013; 5:127-131.
6. Liu R, Wang Y, Zhou N. A case of laparoscopic left hepatectomy for hilar cholangiocarcinoma. Chin J Hepatobiliary Surg. 2003; 09:384. (in Chinese)
7. Xu J, Wang ZF, Liu C, Guan Y. Application of laparoscopic resection of hilar cholangiocarcinoma. Chin J Laparoscopic Surgery (Electronic Edition). 2010; 3:241-243. (in Chinese)
8. Zhu A, Liu Q, Chen D. Laparoscopic-assisted Radical Resection of Hilar Cholangiocarcinoma: Report of 38 Cases. Chin J Minim Inva Surg. 2012; 12:783-786. (in Chinese)
9. Li J, Lu B, Cai X, Huang Y, Huang F, Jin X, Yan Y. Laparoscopic radical resection for hilar cholangiocarcinoma: attached 3 cases report. Chin J Endosc. 2012;111-114. (in Chinese)
10. Li J, Zhao L, Zhang J, Li Z, Li A, Wei Y, Xu J. Application of the laparoscopic technique in perihilar cholangiocarcinoma surgery. Int J Surg. 2017; 44:104-109.
11. Zhu H, Kuang Z, Zhang F, Hu C, Zzhou L, Huang W. Total laparoscopic Bismuth Type I radical resection of hilar cholangiocarcinoma: a report of 7 cases. Chin J Minim Inva Surg. 2018; 18:274-276+288. (in Chinese)
12. Liu X, Feng F, Wang W, Qin J, Wang Z, Xing Z, Duan J, Dong Z, Li S, Liu J. Laparoscopic radical resection of hilar cholangiocarcinoma: a report of 32 patients. Chin J Hepatobiliary Surg. 2019; 23:200-206. (in Chinese)
13. Zhang Y, Dou C, Wu W, Liu J, Jin L, Hu Z, Zhang C. Total laparoscopic versus open radical resection for hilar cholangiocarcinoma. Surg Endosc. 2019.
14. Liu X, Peng C, Peng S, Li O, Yi W, Yang P, Guo C, Cheng W, SL. L. Laparoscopic radical resection of type III and IV hilar cholangiocarcinoma: experience of six patients. Chin J Hepatobiliary Surg. 2019; 25:45-48. (in Chinese)
15. Wang R, Xu J, Li Q, Yang G, Li W, Zhang L, Xie M, Li J. Clinical application of laparoscopic radical resection of hilar cholangiocarcinoma: An analysis of 15 cases. Chin J Prac Surg. 2019; 39:350-354. (in Chinese)
16. Zhu Y, Wang J, Ma H, Liu L, Zhao W. Experience of toatal laparoscopic radical resection of hilar cholangiocarcinoma: a report of 14 cases. Chin J Bas Clin Gen Surg. 2019; 26:56-60. (in Chinese)
17. Luo H, Kuang M, Deng L, Chen X, Hu CH, Yang P, Hu JC, Zeng X, Chen X. Radical resection of laparoscopic hilar cholangiocarcinoma:report of 4 cases. J Abdom Surg. 2019; 32:31-34+32. (in Chinese)
18. Duan C, Zhu X, Wang M, Niu Y, Zhang W, Li S. Clinical investigation of two surgical methods in radical resection of Bismuth I hilar cholangiocarcinoma. Chin J Oper Proc Gen Surg (Electronic Edition) 2019; 13:552-555. (in Chinese)
19. Yuan J, Wang Z, Li F, Liu R. Total laparoscopic radical resection for hilar cholangiocarcinoma in 30 cases. Chin J Gen Surg. 2019; 34:523-526. (in Chinese)
20. Chen D, Cao C, Xu G, Li J, Li J, Wang W, Zhu G. Laparoscopic Cholangiotomy for Radical Excision of Upper Cholangiocarcinoma. Chin J Minim Inva Surg. 2008; 8:31-34. (in Chinese)
21. Du Y, Zhao W, Ma J, Ma H. Total laparoscopic radical resection in treatment of hilar cholangiocarcinoma (report of 6 cases). Chin J Bas Clin Gen Surg. 2017; 24:1487-1491. (in Chinese)
22. Buettner S, Margonis GA, Kim Y, *et al*. Conditional probability of long-term survival after resection of hilar cholangiocarcinoma. HPB (Oxford). 2016; 18:510-517.
23. Duan X, Liu X, Chen W, Cai J, Chen D, Zhang Q, Lai J, Liang L. Long-term outcomes and prognostic factors of surgical resection of hilar cholangiocarcinoma. Chin J Dig Surg. 2016; 15:329-334. (in Chinese)

Received February 15, 2020; Revised April 8, 2020; Accepted May 8, 2020.

[§]These authors contributed equally to this work.

**Address correspondence to:*
Yuhua Zhang, Division of Hepatobiliary and Pancreatic Surgery and Minimally Invasive Surgery, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, No. 158 of Shangtang Road, Hangzhou 310014, China.
E-mail: zhangyuhua1013@126.com

Released online in J-STAGE as advance publication May 9, 2020.

Original Article

DOI: 10.5582/bst.2020.03133

Predicting intervention effect for COVID-19 in Japan: state space modeling approach

Genya Kobayashi[1,*], Shonosuke Sugasawa[2], Hiromasa Tamae[3], Takayuki Ozu[3]

[1] Graduate School of Social Sciences, Chiba University. Chiba, Japan;
[2] Center for Spatial Information Science, The University of Tokyo. Chiba, Japan;
[3] Nospare Inc. Tokyo, Japan.

SUMMARY Japan has observed a surge in the number of confirmed cases of the coronavirus disease (COVID-19) that has caused a serious impact on the society especially after the declaration of the state of emergency on April 7, 2020. This study analyzes the real time data from March 1 to April 22, 2020 by adopting a sophisticated statistical modeling based on the state space model combined with the well-known susceptible-infected-recovered (SIR) model. The model estimation and forecasting are conducted using the Bayesian methodology. The present study provides the parameter estimates of the unknown parameters that critically determine the epidemic process derived from the SIR model and prediction of the future transition of the infectious proportion including the size and timing of the epidemic peak with the prediction intervals that naturally accounts for the uncertainty. Even though the epidemic appears to be settling down during this intervention period, the prediction results under various scenarios using the data up to May 18 reveal that the temporary reduction in the infection rate would still result in a delayed the epidemic peak unless the long-term reproduction number is controlled.

Keywords COVID-19, epidemic peak, SIR model

1. Introduction

Since the first case of the coronavirus disease 2019 (COVID-19) in Japan was confirmed on January 15, 2020, the number of confirmed cases has been increasing day by day. Although the Japanese government declared a state of emergency on April 7, it does not have a legal force to regulate individual activities and remains at only requesting the avoidance of outings. Although the spread of the epidemic seems to have slowed down, one still needs to remain cautious. Needless to say, Japanese economy has been seriously shocked and the public interest mainly lies on how the number of infected persons transits in the future and when the outbreak will converge. Although there already exists a rapidly increasing number of statistical analyses of the epidemic, the statistical evidence focusing on the situations in Japan is still limited except for (*1-3*). Therefore, the purpose of this study is to provide a statistical evidence regarding the future transition of the infectious proportion in Japan, including the intensity and timing of the epidemic peak, based on the real-time data on the cumulative number of confirmed, recovered and deceased persons, shown in Figure 1. Specifically, the observations up to about two weeks after the state was declared (left panel of Figure 1) is first used to prediction under the various scenarios for the effect and length of the intervention. Then using the extended data that includes more recent observations (right panel of Figure 1), the prediction results are validated.

We consider the famous susceptible-infected-recovered (SIR) model (*4*) for modeling the epidemic process as widely adopted in the existing literature on COVID-19. However, this deterministic model is not necessarily sufficient to explain the variability of the transition since the observed number is subject to nonignorable randomness. To handle such randomness in the data, we employ the state spate models combined with the SIR model (SS-SIR model) developed by (*5,6*). The model was originally proposed for statistical modeling of the seasonal trend of influenza. The advantages of the SS-SIR model are mainly three points; (*i*) the unknown parameters in the SIR model can be estimated with little knowledge about the true values by adequately using the data information; (*ii*) future prediction of a variety of quantities such as the number of infections or the epidemic peak as well as uncertainty quantification of the prediction can be carried out easily;

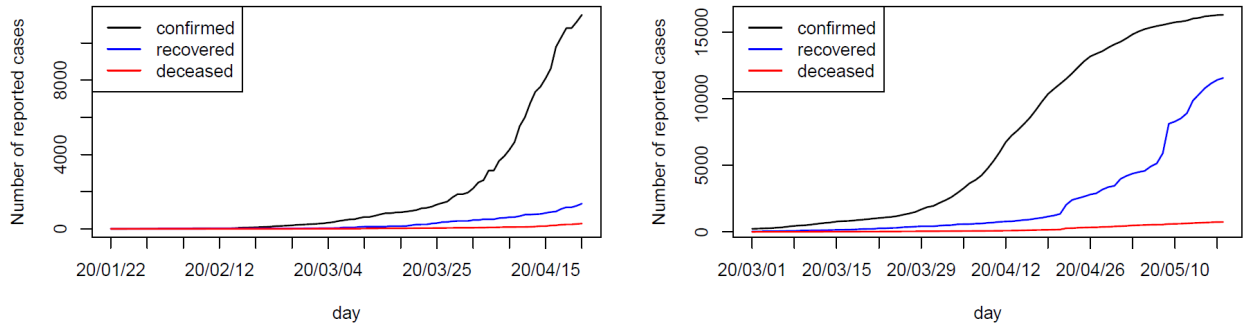


Figure 1. The cumulative numbers of confirmed, recovered and deceased persons in Japan from March 1, 2020 to April 22 (left) and to May 18 (right).

(*iii*) whether the real-time data follows the assumed SIR model or not can be assessed through the parameter estimate; (*iv*) the effect of the intervention can be estimated by appropriately extending the model. These advantages are quite essential because (*i*) information required for modeling the epidemic trend of a new virus is scarce, (*ii*) it is important to compute not only point prediction but also interval prediction to understand the possible worst and best scenarios of future transition, and (*iii*) understanding if the real-time data actually follows the SIR model is critical for the reliability of future simulations based on the SIR model.

2. Methods

2.1. Data

We use the numbers of confirmed, recovered and deceased persons collected on an open source platform (*https://www.kaggle.com/lisphilar/covid19-dataset-in-japan*). Although the original data starts from February 6, the numbers before the end of February are treated collectively. This is because the confirmed numbers in this period are relatively small and using the data from March 2020 would be useful to reliably predict the future numbers of infectious persons after May 2020. Hence, the period of the data used in our analysis consists of $T = 53$ days from March 1 to April 22. We use the difference between the cumulative numbers of confirmed persons and recovered plus deceased persons, denoted by $Z(t)$ for $t = 1, \dots, T$, which can be interpreted as the number of confirmed persons being infectious. It is further assumed that only p $(0 < p \leq 1)$ fraction of infectious individuals can be identified by diagnosis, which is called *identification* rate hereafter. Then we define $Y(t)$ as $Z(t) = Np \times Y(t)$ where $N = 1.265 \times 10^8$ is the population of Japan, thereby $Y(t)$ is the proportion of the infectious population at time t. Regarding the specific values of p, we follow the discussion in (*1*). Since (*7*) reported that 77 persons were confirmed among the possible 940 infected population, the 95% confidence interval of p is (0.059, 0.105). Based on this argument, the results under the following three scenarios $p = 0.05$, 0.1 and 0.2 are compared.

2.2. Statistical model

Here the model proposed by (*6*) is described. Let $S(t)$, $I(t)$ and $R(t)$ denote the proportions of individuals being susceptible, infected and recovered population at the time t, respectively, satisfying $S(t) + I(t) + R(t) = 1$. The SIR model describes the epidemic over time via the nonlinear ordinary differential equations (ODE) given by

$$\begin{aligned} S'(t) &= -\beta S(t)I(t), \\ I'(t) &= \beta S(t)I(t) - \gamma I(t), \\ R'(t) &= \gamma I(t), \end{aligned} \qquad (1)$$

where the unknown infection rate $\beta > 0$ and removal rate $\gamma > 0$ control the transition from one compartment to the next and jointly determine the epidemic process. Let $\theta(t) = (S(t), I(t), R(t))$ define the three-dimensional vector of the unobserved true proportion at the time t. To allow randomness in the evolution of $\theta(t)$, the following model is considered:

$$\theta(t)|\theta(t\text{-}1) \sim \mathrm{Dir}(\kappa f(\theta(t\text{-}1); \beta, \gamma)), \quad t = 1, \dots, T, \qquad (2)$$

where Dir(·) denotes the Dirichlet distribution, $f(\theta(t\text{-}1); \beta, \gamma)$ is the solution of the deterministic SIR model Eq.(1) starting the ODE at $\theta(t\text{-}1)$ and $\kappa > 0$ is the unknown parameter controlling the randomness in the evolution. In the above model, the conditional expectation of $\theta(t)$ given the previous state $\theta(t\text{-}1)$ is $f(\theta(t\text{-}1); \beta, \gamma)$, so the distribution of $\theta(t)$ is centered around the deterministic model Eq.(1). It is noted that the conditional variance of $\theta(t)$ decreases as κ increases, thus the validity of the assumption of the deterministic model Eq.(1) can be verified through the estimate of κ.

Let $Y(t)$ be the observed value of $I(t)$. Since $Y(t)$ is not necessarily equal to the true $I(t)$, $Y(t)$ is observed based on the following probabilistic model:

$$Y(t)|I(t) \sim Beta(\lambda I(t), \lambda(1\text{-}I(t))), \quad t = 1, \dots, T, \qquad (3)$$

where Beta(·,·) denotes the Beta distribution and λ

> 0 is an unknown parameter having a similar role to κ in Eq.(2). The statistical model for $Y(t)$ with the combination of Eq.(2) and (3) is seen as a *state space model*.

The unknown parameters in the model are the two parameters β and γ in the SIR model and two scale parameters κ and λ that control the randomness in the two equations Eq.(2) and (3). The estimation of these parameters and future prediction is conducted within the Bayesian framework in which we assign prior distributions for these parameters and compute the posterior distribution via the *Bayes rule*. Due to the complexity of the model, the analytical derivation of the posterior distribution is not feasible. Instead, we rely on the simulation-based method known as *Markov Chain Monte Carlo* (MCMC) algorithm (*8*) to generate random numbers from the posterior distribution. Then the parameter estimates are calculated and future prediction is carried out based on the output of the MCMC algorithm.

Regarding the prior distributions, we assign slightly non-informative priors to reflect the uncertainty about the new epidemic and let the data tell the truth adequately. The details of the settings of the prior distributions and algorithm are provided in Supplementary Material (*http://www.biosciencetrends.com/action/getSupplementalData.php?ID=67*).

3. Results

3.1. Prediction of epidemic peak

The SS-SIR model is applied to the Japanese data with the three identification rates p. First, we found that the estimates of the precision parameters κ and λ are very large. For example, the point estimates are $\widehat{\lambda} = 1.76 \times 10^5$ and $\widehat{\kappa} = 3.00 \times 10^5$ for $p = 0.1$ indicating that the deterministic SIR model explains the transition of the real-time data well. Table 1 reports the estimates and 95% credible intervals of the representative parameters. Under the three settings for p, the point estimates of β are between 0.20 and 0.25 and those of γ are between 0.13 and 0.17. The estimates of the basic reproduction number R_0 are between 1.42 and 1.49. For $p = 0.1$, for example, the 95% credible interval of R_0 is (1.20-1.65). The estimates of PI and PT appear to vary depending on the identification rate. Figure 2 reports the future predictions of the proportion of the infectious proportion under the three identification rates. The figure allows us to easily understand the degree of uncertainty in prediction, and worst and best scenarios for the future epidemic process through the interval prediction. It is seen that the predicted timing of the epidemic peak and peak intensity depend on the identification rate through the differences in the estimates of PT and PI. Specifically, the point predictions of the trajectory of the infectious proportion have the timing of the peak on July 19, July 21 and August 1 with the intensities and 95% prediction intervals of 3.84% (1.23%-7.37%), 2.60% (0.42%-6.40%) and 2.25% (0.19%-6.14%) for $p = 0.05$, 0.1 and 0.2, respectively. The sensitivity of prediction results with respect to p was also found in (*1*), but that under our setting of p is far less dramatic. Moreover, all the scenarios predict that the epidemic

Table 1. Estimates and 95% credible intervals of parameters of the SS-SIR model under the three identification rates p.

		Estimate (95% interval)		
Parameter	Description	$p = 0.05$	$p = 0.1$	$p = 0.2$
β	Infection rate	0.20 (0.12- 0.32)	0.25 (0.13 - 0.47)	0.25 (0.14 - 0.53)
γ	Removal rate	0.13 (0.07 - 0.24)	0.17 (0.09 - 0.39)	0.17 (0.09 - 0.44)
R_0 (= β/γ)	Basic reproduction number	1.49 (1.30 - 1.70)	1.42 (1.20 - 1.65)	1.42 (1.19 - 1.65)
PT	Peak timing	156 (105 - 249)	151 (96 - 255)	160 (97 - 261)
PI (%)	Peak intensity	4.55 (1.86 - 7.92)	3.46 (0.77 - 7.20)	3.34 (0.68 - 7.22)

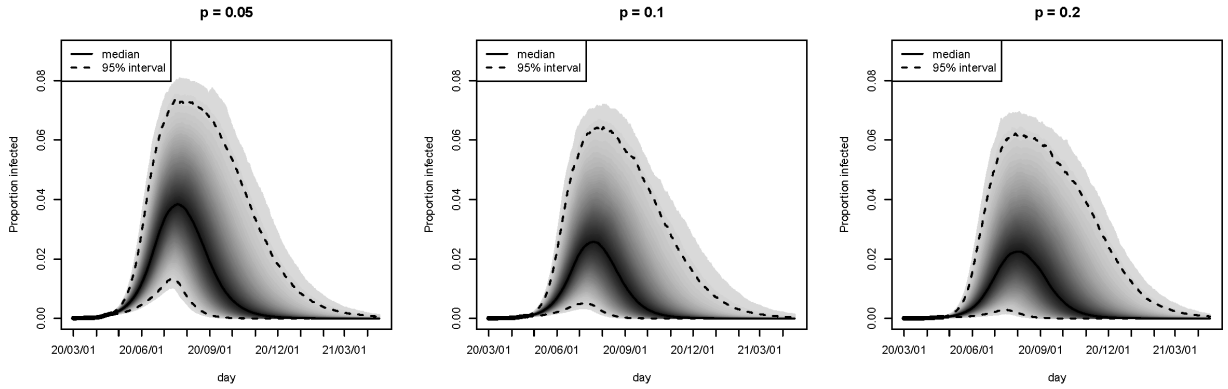


Figure 2. Results of the prediction of the proportion of the infectious population with $p = 0.05$ (left), 0.1 (center) and 0.2 (right). The observed data points $\{Y(t), t = 1, \ldots, T\}$ are shown by the black circles.

peak comes during the summer 2020. This result is also consistent with (*1*).

3.2. Effect of intervention

On April 7, 2020, the Japanese government declared a state of emergency aiming at reducing human contacts by 80%, which is considered to be sufficient to terminate the epidemic. However, the government reports that the actual reduction is still limited to around 60% or 70% (*https://corona.go.jp*), mainly because the state does not have a legal force to regulate individual activities. Also, the Japanese government was initially planning to lift the state on May 6, but the public concern lay on whether such a short period of the state of emergency is sufficient or not.

Through simulation, we here assess the efficacy of further intervention and public awareness on mitigating the infection risk under various scenarios. Specifically, we consider various settings for the degrees of reduction in human contacts that are achieved by the government during the intervention and by the public awareness after the intervention, and the period of intervention denoted by T^*, under the state of emergency and predict the future epidemic transitions. Here, we focus on $p = 0.1$. The results under $p = 0.05$ and 0.2 are found in Supplementary Material (*http://www.biosciencetrends.com/action/getSupplementalData.php?ID=67*). It is recognized that the realization of the effect of reducing human contacts takes about two weeks since the incubation period of COVID-19 is at most 2 weeks as reported by World Health Organization. Since April 22, the last date in the real-time data, is almost two weeks after the declaration of the state of emergency, we assume that the infection rate changes from β to $c\beta$ from April 23. For the degree of reduction in human contacts, the following six scenarios are considered: $c = 0.6$, 0.5, 0.4, 0.3, 0.2 and 0.1 If 80% reduction of human contacts was achieved, the reality would have corresponded to $c = 0.2$ or 0.1. In view of the current situation, however, $c = 0.4$ or 0.3 would be closer to the reality. We also suppose that the intervention will continue for T^* days from April 23 with the three scenarios, $T^* = 14$, 28 and 45. Note that $T^* = 14$ corresponds to May 6 on which the government was initially planning to lift the state. The other two dates to respectively correspond to the two-week and one-month extension of the intervention that continue until May 20 and June 6, respectively. We further suppose that the infection rate becomes c^* after the intervention period with the three scenarios: $c^* = 1$, 0.9 and 0.8. The first scenario implies that the level of human mobility after the intervention returns to the original level before the intervention. The latter two scenario can reflect the remaining strain in the public awareness on mitigating the spread of infection through, for example, voluntary avoidance of outings and social distancing.

Figure 3 presents the nine panels on the future prediction under the combinations of the three scenarios of each T^* and c^*. Comparing the different scenarios of T^*, the figure reveals that setting c to smaller values is effective only when it is combined with larger T^*. For example, the left upper panel of Figure 3 exhibits little differences among the six choices of c when $c^* = 1$ and $T^* = 14$. Contrary, the small values of c such as $c = 0.2$ with $T^* = 28$ and 45 can lead to a convergence of the epidemic. Under $c = 0.2$ and $c^* = 1$, the epidemic can be terminated in terms of point prediction when $T^* = 45$, while the epidemic peak belatedly comes on September 3, 2020 with 2.2% of the peak intensity when $T^* = 14$. The result suggests that the termination of the intervention due to the initial plan of lifting the state of emergency on May 6 would have been too early and only resulted in a slight delay in the epidemic peak and a slight reduction in the peak intensity.

The degree of reduction in β after the intervention, c^*, also has a dramatic effect on the consequence of the epidemic. The upper panels of Figure 3 show that the efficacy of the temporary reduction in β under the intervention can be quite limited if β returns to the original level after the intervention. In contrast, if at least 20% reduction in β can be achieved for a sufficiently long period of time after the intervention, the epidemic can be effectively suppressed. In the case of $c^* = 0.9$, for example, the peak intensity is more than halved to 1.11% with the peak on September 13 even under the mild degree of intervention for a short period of time ($c = 0.6$ and $T^* = 14$). When a longer intervention $T^* = 45$ is carried out, the peak is further delayed to November 11 with 0.81% of the peak intensity. Furthermore, in the case of $c^* = 0.8$, the figure shows the epidemic is almost completely suppressed in terms of point prediction regardless of the degree of intervention and length of intervention period. This would be because that the infectious proportion decreases during the intervention and the reproduction number under $c^* = 0.8$ remains close to one even after the intervention. To summarize, our results show that not only the degree of reduction in β during the intervention but also and more importantly the length of intervention and the long-term level of β after the intervention is critical to control the spread of the epidemic.

Figure 4 presents a more detailed picture of the prediction results around the dates indicated by T^*. The figure shows that there are scenarios under which the proportion of the infectious population comes to increase again as the infection rate changes from $c\beta$ to c^*. Whether there will be another wave generally depends on the combination of c, T^* and c^*, but there most likely would have been to see a rise in the infectious proportion in the case of $T^* = 14$. In the figure, the more recent observations between April 23 and May 18 are also plotted. The figure shows that the predicted trajectories do not seem to follow well the stationary and reduction in the infectious proportion due to the sudden change

in the infection rate under the scenarios. It seems that the decline in the trajectories in the case of $c = 0.6$ appear to resemble the actual rate at which the observed infectious proportion decreased. In the following section, a modified model is estimated using an extended dataset to validate our prediction method.

3.3. Validating the prediction results

As requested by one of the reviewers, the dataset is updated to include more recent observations. Given that the unsettled epidemic situation, the Japanese government has decided to postpone the lifting of the state until the end of May ($T = 79$). On May 14, the state was partially lifted for the 39 prefectures among out of where the situation is somewhat milder. For the remaining 8 prefectures, the government is planning to lift the state by the end of May. Using the updated dataset, the effect of the intervention can be estimated based on a modified version of the model Eq.(2) and the foregoing prediction results can be validated. Based on the current plan of the government, the infection rate β is assumed to become $c\beta$, $0 < c < 1$, from April 23 as in Section 3.2, but now c is estimated from the data. The estimate of c stands for the effect of the intervention under the state of emergency.

For $p = 0.1$, the point estimate of c is 0.583 with the 95% credible interval (0.307-0.787). Therefore, this

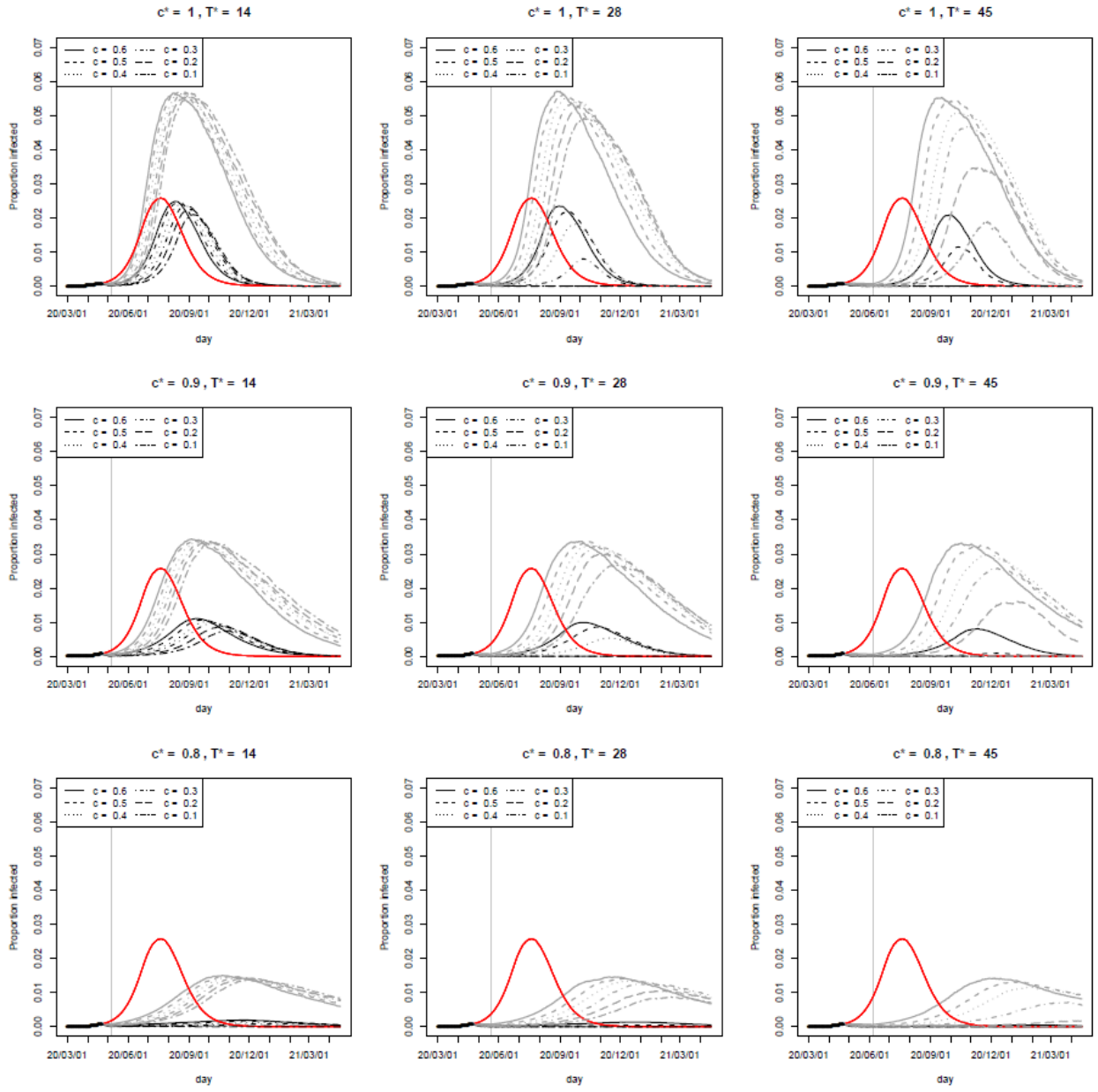


Figure 3. Future prediction under the nine combinations of T^* (the period of the intervention) and c^* (the multiplier for β after the intervention) for $p = 0.1$. The red, black and grey curves respectively represent the future point prediction without intervention shown in Figure 2, point prediction under each scenario and upper bounds of the 95% prediction intervals. The black circles represent the observed data points. The grey vertical lines indicate the dates on which there is a change in the infection rate represented by c^*.

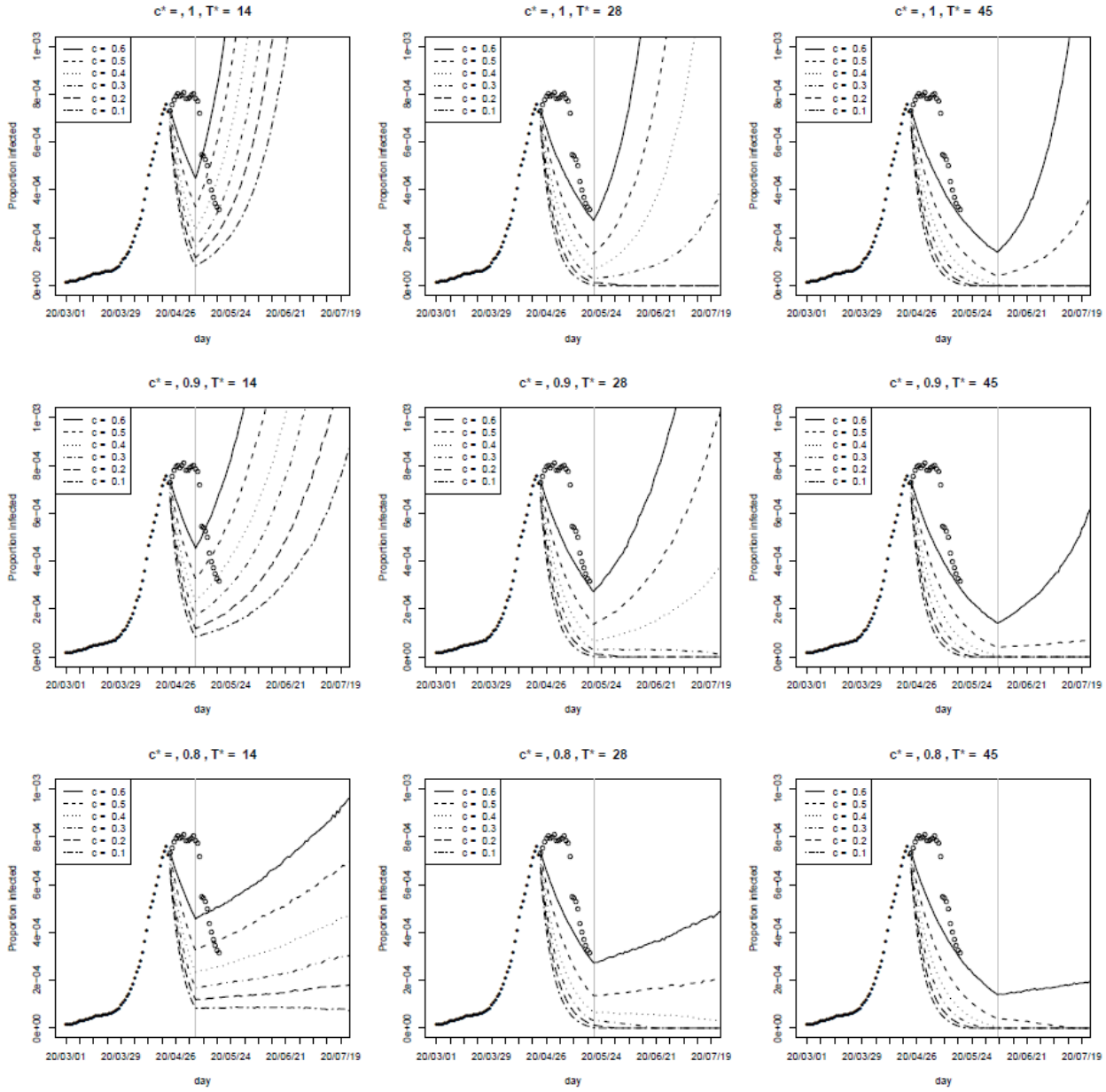


Figure 4. Details Figure 3. around the dates on which the infection rate changes according to c^*. The curves represent the future point prediction. The black circles are the observed data points up to April 22 and white circles are the observed data points additionally obtained after April 22 up to May 18. The grey vertical lines indicate the dates on which there is a change in the infection rate represented by c^*.

estimate is consistent with the conjecture in Section 3.2 that the actual reduction in human contact under the intervention is by about 40%. The estimate for R_0 is 1.482 (1.273-1.723). Our estimate for R_0 remains unchanged even when the data include more recent observations. The estimate of the effective reproduction number cR_0 during the intervention period is 0.854 (0.495-1.091). Although the upper bound of the 95% credible interval of cR_0 slightly exceeds one, the posterior distribution is concentrated mainly below one, implying that the epidemic would progress towards convergence if the value of c continues to remain around our estimate. Some more details are found in Supplementary Material (*http://www.biosciencetrends.com/action/getSupplementalData.php?ID=67*).

As in Section 3.2, the future trajectories of the infectious proportion are simulated under the following scenarios. The infection rate remains to be $c\beta$ until T^* days from May 18 where T^* = 13, 27, 43 and 74, corresponding to May 31, June 14, June 30 and July 31. Then it becomes $c^*\beta$ with c^* = 1,0.9 and 0.8 after the intervention period. Figure 5 presents the predicted infectious proportions under the various scenarios. Similar to Figure 3, a smaller c^* combined with a larger T^* will suppress the epidemic. While the estimate of c^* implies that the intervention is successful to some extent, the prediction results suggest the possibilities of a second wave in the case of, for example, c^* = 1 and T^* = 13. Finally, Figure 6 provides a more detailed picture of Figure 5 around the dates indicated by T^*. It

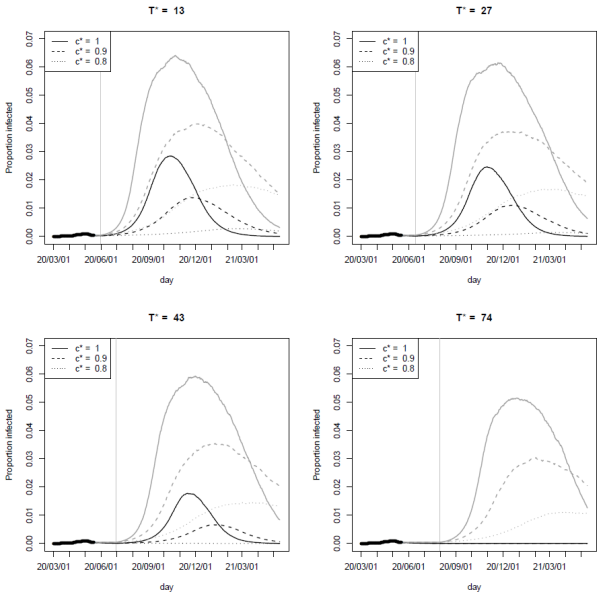


Figure 5. Future prediction under the combinations of T^* (the period of the intervention) and c^* (the multiplier for β after the intervention) for $p = 0.1$ using the extended dataset. The black and grey curves respectively represent the future point prediction under each scenario and upper bounds of the 95% prediction intervals. The grey vertical lines indicate the dates on which there is a change in the infection rate represented by c^*.

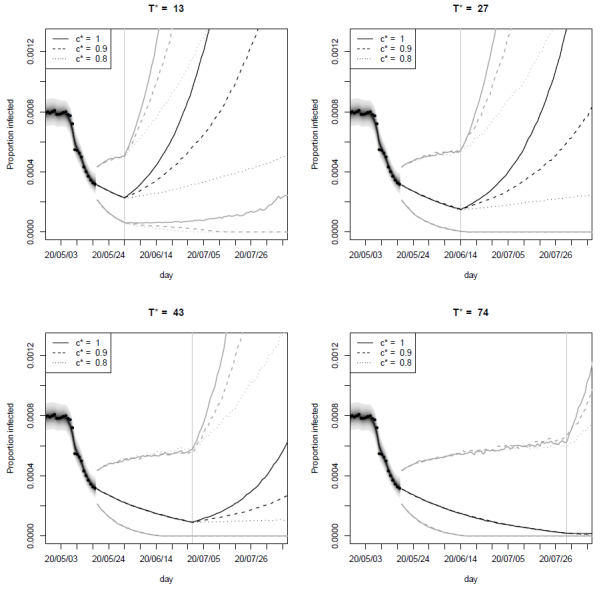


Figure 6. Details Figure 5 around the dates on which the infection rate changes according to c^*. The black and grey curves respectively represent the future point prediction and 95% prediction intervals. The black circles represent the observed data points along with the 95% credible intervals. The grey vertical lines indicate the dates on which there is a change in the infection rate represented by c^*.

is seen that the infectious proportions are successfully suppressed until the day on which c changes to c^*. The rate at which the infectious proportions decline naturally follows that at which the observed proportions decline. As in the previous analysis, the infectious proportions are most likely predicted to rise again after those change points. Since a further extension of the intervention does not seem very feasible under the recent decline in the newly confirmed cases and the public pressure caused by the economic crisis, controlling the post-intervention infection rate represented by c^* is critical as in the previous prediction results.

4. Discussion

In this research, we have employed the probabilistic version of the famous SIR model, called SS-SIR model, to model the real-time data on the infectious population of COVID-19 in Japan. The advantage of the SS-SIR model is that we can obtain not only future point prediction but also uncertainty quantification through, for example, the future prediction intervals. The basic reproduction number R_0 is estimated to be approximately between 1.4 and 1.5 in this study. This is smaller than the estimate of 2.6 in (*1*) obtained from the SEIR model applied to the early stage data in Japan. Note, however, that (*1*) did not estimate the removal rate and onset rate but fixed their values to those found in the existing studies. We also confirmed that the estimate of R_0 remains unchanged even when the model is estimated by using the extended dataset. Moreover, our estimate in the case of Japan is also smaller than those reported from the case studies in China (*9-14*). Our result may have reflected the fact that the number of cases in Japan does not increase as rapidly as other countries (*15*).

Through the future prediction under the various scenarios on the possible reduction in the infection rate β and the length of the intervention period, we have obtained the following epidemiological insights:

• Even if a large reduction in the infection rate could be achieved during the intervention period (*e.g.* the state of emergency), the convergence of the epidemic can still depend on the long-term value of the infection rate $c^*\beta$ after the intervention.

• As long as the value of $c^*\beta$ can be maintained to be slightly smaller even after the intervention period than the value of β before the intervention ($c^* < 1$), there is a possibility that the epidemic terminates with a significantly smaller epidemic size than the case without intervention.

• Using the extended dataset, the effect of intervention under the state is about 40% reduction in the infection rate.

These findings confirm that the intervention under the state of emergency and its extension has been successful to suppress the epidemic to some extent. Under the public pressure, the government will most likely lift the state for all prefectures by the end of May. Our findings suggest that a long-term effort to control the infection as indicated by the parameter c^* is indispensable.

Acknowledgements

This research was supported by Japan Society for the Promotion of Science (KAKENHI) Grant Numbers 18K12754 and 18K12757. This problem in the present paper was provided by Nospare Inc..

Author Contributions: H.T. and T.O. collected the data. G.K. and S.S. analyzed the empirical data using the models. All the authors participated in the study design, drafted the manuscript, and gave comments on the current version of the manuscript.

References

1. Kuniya T. Prediction of the Epidemic Peak of Coronavirus Disease in Japan, 2020. J Clin Med. 2020; 9:789.
2. Karako K, Song P, Chen Y, Tang W. Analysis of COVID-19 infection spread in Japan based on stochastic transition model. BioSci Trends. 2020; 14:134-138.
3. Mizumoto K, Kagaya K, Zarebski A, Chowell G. Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. Euro Surveill. 2020; 25:2000180
4. Kermack W, McKendrick A. Contribution to the Mathematical Theory of Epidemics. Proc R Soc Lond A Math Phys Sci. 1927; 115:700-721.
5. Dukic V, Lopes HF, Polson NG. Tracking Epidemics with Google Flu Trends Data and a State-Space SEIR Model. J Am Stat Assoc. 2012; 107:1410-1426.
6. Osthus D, Hickmann K, Caragea PC, Higdon D, Del Valle SY. Forecasting seasonal influenza with a state-space SIR model. Ann Appl Stat. 201; 11:202-204.
7. Bloomberg. Japan's Hokkaido may Have 940 Infected, Researcher Says. *https://www.bloomberg.com/news/articles/2020-03-03/japan-s-hokkaido-could-have-up-to-940-infected-researcher-says* (accessed March 3, 2020).
8. Gamerman D, Lopes HF. Markov Chain Monte Carlo: Stochastic Simulation for Bayesian Inference, Second Edition. Chapman and Hall/CRC. CRC Press, 2006.
9. Sun H, Qiu Y, Yan H, Huang Y, Zhu Y, Chen S. Tracking and predicting COVID-19 epidemic in China mainland. medRxiv. 2020; doi: https://doi.org/10.1101/2020.02.17.20024257
10. Imai N, Cori A, Dorigatti I, Baguelin M, Connelly C, Riley S, Ferguson N. Report 3: Transmissibility of 2019-nCoV. Imperial College London: London, UK. 2020.
11. Liu Y, Gayle AA, Wilder-Smith A, Rocklov J. The reproductive number of COVID-19 is higher compared to SARS coronavirus. J Travel Med. 2020; 27:taaa021.
12. Liu T, Hu J, Hang M, *et al*. Transmission dynamics of 2019 novel coronavirus (2019-nCoV). bioRxiv. 2020; doi: https://doi.org/10.1101/2020.01.25.919787
13. Tang B, Wang X, Li Q, Bragazzi N, Tang S, Xiao Y, Wu J. Estimation of the Transmission Risk of the 2019-nCoV and Its Implication for Public Health Interventions. J Clin Med. 2020; 9:462.
14. Wu J, Leung K, Leung G. Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: A modelling study. Lancet 2020; 395:689-697.
15. Nippon.com. Coronavirus Cases by Country. *https://www.nippon.com/en/japan-data/h00673/coronavirus-cases-by-country.html* (Accessed April 24, 2020).

Received May 1, 2020; Revised May 21, 2020; Accepted May 26, 2020.

**Address correspondence to:*
Genya Kobayashi, Graduate School of Social Sciences, Chiba University. 1-33, Yayoi-cho, Inage-ku, Chiba 263-8522, Japan.
E-mail: gkobayashi@chiba-u.jp

Released online in J-STAGE as advance publication May 28, 2020.

Original Article

DOI: 10.5582/bst.2020.03110

The impact of social capital on civil society organizations delivering voluntary counseling and testing HIV/AIDS service: a cross-sectional study in China

Liu Liu[1], Danni Wang[2], Xia Qin[3], Zhi Hu[1,3,*], Ren Chen[3,*]

[1] Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Hefei, Anhui, China;
[2] Central Laboratory of Preventive Medicine, School of Public Health, Anhui Medical University, Hefei, Anhui, China;
[3] School of Health Services Management, Anhui Medical University, Hefei, Anhui, China.

SUMMARY In China, Voluntary HIV Counseling and Testing (VCT) services are mostly provided by Civil Society Organizations (CSOs). This cross-sectional study investigated the association between CSOs' social capital and VCT service availability in eight Chinese provinces during July–December 2015. Data on CSOs' characteristics were collected through questionnaire-based interviews. Social capital was measured using a purpose-developed questionnaire. Logistic regression models tested the association between social capital and the scale of VCT services. A total of 103 CSOs that provided VCT to MSM (MSM-focused CSOs), and 109 CSOs that provided such service to non-MSM population (other CSOs) were included. Overall, 144 (67.9%) CSOs were not registered with local governments, while 106 (50%) received RMB 50,000 ($7,670) funding in 2014. Multivariate logistic regression analysis indicated that the CSOs with a higher level of shared vision were more likely to provide a large-scale of VCT service than those with a lower level of shared vision (AOR = 1.95). Moreover, intra-networks were positively associated with the VCT service (AOR = 2.87) among other CSOs, while the level of shared vision was positively associated with the VCT service (AOR = 3.08) among other-MSM-focused CSOs. There was no significant association between social capital and total service scale. Our findings suggest that increasing social capital can potentially enhance VCT service and play an important role in AIDS prevention.

Keywords HIV/AIDS, Civil Society Organizations, Social Capital, Voluntary Counseling and Testing service, China

1. Introduction

Voluntary HIV counseling and testing (VCT) are important diagnostic services for early detection and treatment for people living with HIV (PLWH) and for implementing HIV/AIDS prevention and control strategies (*1*). Over the past decade, VCT services have played an important role in the management of the HIV/AIDS epidemic (*2*). Since the detection strategy expansion in 2004, the detection rate and number of new diagnoses has been increasing substantially. However, in China, approximately one third of HIV infected people have not yet been diagnosed (*3-7*). These undiagnosed individuals are not only the main source of infection for the spread of the disease, but owing to the lack of diagnosis, they may miss the appropriate time at which treatment should be administered. Previous studies have focused on individual factors associated with willingness or acceptance rates of HIV interventions (*8, 9*), while only a few studies have addressed the VCT service utilization from the perspective of social capital, especially social capital of Civil Society Organizations (CSOs). From a public health perspective, this study hopes to provide information on other diseases or subsequent evaluation through a retrospective analysis of data.

Currently, the mode through which AIDS spreads in China is not evident. With the rapid development of Internet technology and wide use of social software, the risk factors of HIV transmission are increasing. The latest report on AIDS epidemic in China showed that 71.1% and 22.7% of the 41,351 new HIV cases occurred through heterosexual and homosexual transmission, respectively, in the third quarter of 2018 (*10*). These data indicate that the VCT services in China need to target men who have sex with men (MSM), commercial sex workers (CSWs), and other hard-to-reach populations (*11*). Often these groups are not socially accepted

and VCT services do not reach them. Aware of this problem, the Chinese authorities have launched CSOs that promote HIV/AIDS intervention strategies. Non-governmental, not-for-profit, voluntary entities formed by volunteers work independently from the state and market, representing a separate set of interests and ties. These CSOs are a conglomerate of community-based organizations (CBOs), scientific research institutions or universities, governmental and non-governmental organizations, and grassroots organizations. The number of CSOs specifically dedicated to HIV/AIDS has increased from 0 in 1988 to more than 700 in 2019. CSOs provide all advantages of easy access to high-risk behavior groups and those infected with HIV/AIDS. The currently existing CSOs are involved in nearly all aspects of HIV/AIDS prevention, treatment, and care, attracting attention on research focused on capacity building and effective management (*12-14*). They also improve their working ability constantly and play an important auxiliary role. Therefore, CSOs are an indispensable force and an important complement to the AIDS prevention and control team. However, few previous studies have examined the organizational social capital, such as VCT service, which may improve the success rate of the HIV/AIDS management strategy.

In recent years, social capital has gained a great amount of attention in public health, although there has been very little consensus on its definition. Understood as "features of social organization, such as networks, norms, and trust, which facilitate coordination and cooperation for mutual benefit" (*15*), social capital can be an individual or a collective asset stored within relationships among individuals, groups, or organizations. Furthermore, social capital might be an important factor in eradicating HIV/AIDS. Studies have explored and discussed the association between social capital and individual knowledge, attitude, or behavior (*16-19*). However, few investigations on the effect of social capital of CSOs on HIV/AIDS have been conducted.

In this study, we adopted a perspective of structural, relational, and cognitive on organizational social capital described by Ghoshal as our operational definition (*20,21*). Questionnaires were designed to measure the intra-organizational social capital, which present members' interactions and relationships within the CSOs. Structural social capital within CSOs refers to social interaction ties; relational social capital includes assets that are rooted in these relationships, such as trust and support, and cognitive social capital is embodied in attributes like a shared code or a shared paradigm that facilitates a common understanding of collective goals. Within the context of this definition, we hypothesized that social capital is positively associated with the VCT service offered by the CSOs.

2. Materials and Methods

2.1. Ethics statement

Ethical approval for the study was obtained from the Biomedical Ethics Committee, Anhui Medical University (Approval No. 20131235).

2.2. Study sites, participants, and procedure

According to the Classification Criteria of AIDS Epidemic Level in China (*22*), the AIDS epidemic is currently distributed in 12 I Type provinces, 14 II Type province, and 5 III Type provinces, representing 38.71%, 45.16%, and 16.13% of the country, respectively. Based on the epidemic status and geographic distribution of HIV/AIDS, the sample size is allocated according to the ratio. We selected eight provinces: Hunan, Sichuan, and Yunnan (high epidemic areas); Anhui, Hubei, Shandong, and Jilin (medium epidemic areas); Gansu (low epidemic areas). Inclusion criteria for the CSOs were (*i*) organizations listed as social organizations working on HIV/AIDS, and (*ii*) they have existed for 1 year or longer. Following the methodology described by Tsai and Ghoshal, we collected data through a questionnaire-based survey. We sampled 1-3 members of each CSO, who were key informants for this CSO. To be eligible for inclusion, these members had to meet the following criteria: (*i*) occupy director/manager-level post, and (*ii*) having worked at that organization for 1 year or longer.

Cross-sectional surveys were conducted in the selected provinces between July and December 2015. Respondents were asked to sign consent forms ahead of the interview. Information on the survey was explained to the interviewee before trained investigators from the Anhui Medical University collected survey responses through a face-to-face interview. Data were collected on the basic information, VCT service scale, total service scale, and social capital of the CSO. The collective level of the variable was defined by respondent who is the director of the CSO or the mean value of 2-3 respondents accordingly.

2.3. CSOs' basic information

Based on literature review (*12*), we collected basic information regarding the participating CSOs, which includes factors that might be related to the VCT service. These factors are shown in Table 1.

2.4. VCT service and total service scale

The VCT services offered by the CSOs were measured according to the following criteria: (*i*) VCT sites were government-based, for example, established within municipal Centers for Disease Prevention and Control (CDC), and the service users chose to get tested introduced and encouraged by the CSOs, and (*ii*) the CSOs provided VCT services independently, for

Table 1. Basic characteristics and intra-organizational social capital level among study CSOs *n* (%)

Items	Overall (*n* = 212)	MSM-focused CSOs (*n* = 103)	Other CSOs (*n* = 109)	X^2	*P*
Number of core staff				11.30	< 0.001**
< 3	47 (22.2)	14 (13.6)	33 (30.3)		
3-5	113 (53.3)	56 (54.4)	57 (52.3)		
>5	52 (24.5)	33 (32.0)	19 (17.4)		
Funding (Dollar)				1.39	0.50
< 3,068	53 (25.0)	23 (22.3)	30 (27.5)		
3,068-18,408	106 (50.0)	51 (49.5)	55 (50.5)		
> 18,408	53 (25.0)	29 (28.2)	24 (22.0)		
HIV/AIDS service year				7.44	0.02*
< 5	70 (33.0)	26 (25.2)	44 (40.4)		
5-9	98 (46.2)	57 (55.3)	41 (37.6)		
> 9	44 (20.8)	20 (19.4)	24 (22.0)		
Registration				5.50	0.02*
No	136 (64.2)	59 (57.3)	77 (70.6)		
Yes	76 (35.8)	44 (42.7)	32 (29.4)		
AIDS epidemic situation				14.02	< 0.001**
Low	24 (11.3)	8 (7.8)	16 (14.7)		
Middle	84 (39.6)	54 (52.4)	30 (27.5)		
High	104 (49.8)	41 (39.8)	63 (57.8)		
Net-works				1.59	0.21
Low	100 (47.2)	44 (42.7)	56 (51.4)		
High	112 (52.8)	59 (57.3)	53 (48.6)		
Trust				1.09	0.30
Low	87 (41.0)	46 (44.7)	41 (37.6)		
High	125 (59.0)	57 (55.3)	68 (62.4)		
Support				0.22	0.64
Low	92 (43.4)	43 (41.7)	49 (45.0)		
High	120 (56.6)	60 (58.3)	60 (55.0)		
Shared vision				0.53	0.47
Low	96 (45.3)	44 (42.7)	52 (47.7)		
High	116 (54.7)	59 (57.3)	57 (52.3)		

*$p < 0.05$; **$p < 0.001$.

example, setting up a small room to provide counseling and testing with strict adherence to guidelines. The scale of the VCT services provided by CSOs was measured with a question: How many people among your service users have received VCT during the past year? The total service scale was measured with a question: How many people from the target population did your organization provide HIV/AIDS service to in the past year?

2.5. Key elements of social capital

Based on classic research on organizational social capital within firms (*21*) and our previous research (*23-25*), we considered four dimensions of social capital in this study: networks, trust, support, and shared vision. The networks dimension assessed the members' interaction within the organization. The trust dimension referred to members' trust and trustworthiness in the organization. The support dimension represented how well the members could help each other. Shared vision captured the common understanding of collective goals and proper ways of acting within the organization. Key members were asked to describe the level to which they agreed with the description of each item on a 5-point response scale. The social capital questionnaire is reported in an appendix.

2.6. Data analysis

The continuous variables constituting CSO basic information, including the number of core staff, amount of funding, and duration of HIV/AIDS service were transformed into ordinal variables, using the upper and lower quartiles as cutoff points. Quantitative data about the VCT service and number of service users were collected from the key informants. To evaluate the association between VCT service characteristics and social capital, the level of VCT service and number of service users were converted into binary variables. The median was used as the cutoff point to define a high level of VCT service (≥ 200) *vs.* low level of VCT service (< 200), as well as high number of service users (≥ 600) *vs.* low number of service users (< 600).

Cronbach's α values were calculated to estimate the reliability of the social capital scale. The social capital of CSOs was measured by a component score of each dimension, using factor analysis. It was grouped into a binary variable and the mean component score was used as the cutoff point between high social capital (component score ≥ 0) *vs.* low social capital (component score < 0) (*24*).

All data were manually entered into Epidata 3.0 software, crosschecked, and verified by trained

staff. Separate analyses were performed on MSM-focused CSOs and other CSOs. A descriptive analysis was performed, and the results were expressed as frequencies and percentages; differences between groups were tested with the X^2 test and Fisher's exact test. Multivariate logistic regression models were used to calculate odds ratios (ORs) and corresponding 95% confidence intervals (CIs), which measured the association between VCT service scale (high level of VCT service *vs.* low level of VCT service) and total service scale (high number of service users *vs.* low number of service users), adjusted for the following potential confounders: core staff size, funding, HIV/AIDS service year, registration state, and district status of the HIV/AIDS epidemic. IBM SPSS Statistics 23.0 was used for all statistical analyses (SPSS Inc., Chicago, IL).

3. Results

3.1. Descriptive statistics

A convenience sample of 327 individuals (key informants) from 212 organizations was selected for this study, including 61 individuals from 28 organizations in Sichuan, 103 individuals from 63 organizations in Yunnan, 39 individuals from 30 organizations in Anhui, 22 individuals from 13 organizations in Hubei, 21 individuals from 16 organizations in Shandong, 34 individuals from 25 organizations in Jilin, and 34 individuals from 24 organizations in Gansu. According to the National HIV/syphilis/HCV sentinel surveillance report in 2018 (*26*), sexual transmission accounted for 95% of the annual newly reported infections, and sexual contact between men and men was the main mode of transmission. According to the different service objects of CSOs, is divided into two orientations: included MSM and excluded MSM. The total of 212 CSOs included 103 (48.6%) MSM-focused and 109 (51.4%) other organizations (Table 1). The median received funding was RMB 50,000 (equivalent to $7,670) (interquartile range RMB20,000-120,000 [equivalent to $3,068-18,408]). Nearly half of the included CSOs had two or fewer core staff. Overall, 44 (20.8%) CSOs had participated in HIV/AIDS prevention and control for more than 9 years. Additionally, 144 (67.9%) CSOs were unregistered and 104 (49.8%) CSOs were based in districts classified as high HIV/AIDS epidemic. There were significant differences in core staff size, HIV/AIDS service year, registration state, and districts' HIV/AIDS epidemic level between MSM-focused CSOs and non-MSM-focused CSOs. However, the differences between MSM-focused CSOs and non-MSM-focused CSOs in scores on each dimension of social capital were not statistically significant (Table 1).

Participants at the director level of the organization were asked to report the number of total service users and objects who adopted VCT services in their organization during last year. The total service scale and VCT service scale differed between MSM-focused CSOs and other CSOs (Table 2).

3.2. Factor analysis and social capital characteristics

Four factors were extracted with eigenvalues above 1.0. After running a varimax orthogonal rotation, the four factors explained 73.1% of the total variance. The overall Cronbach's α coefficient for social capital was 0.98, with dimension-specific coefficients of 0.91 (networks), 0.89 (trust), 0.86 (support), and 0.68 (shared vision).

3.3. Correlates of organizational social capital and total service scale

As shown in Table 3, CSOs participated in HIV/AIDS prevention and intervention over 9 years than below 5 years (AOR = 5.09), registered CSOs (AOR = 2.25) were likely to provide service to larger target population than not registered CSOs. Compared to CSOs located in low-epidemic areas, CSOs based in middle- and high-epidemic areas were more likely to provide services to a smaller target population (AOR = 0.23/0.30). Among the MSM-focused CSOs, the organizations in receipt of funding between RMB 20,000 ($3,068) and RMB 120,000 ($18, 408), which also had participated in HIV/AIDS prevention and control for 9 years or more, were more likely to provide service to a larger target population (AOR = 4.53 and AOR = 6.68, respectively) compared to organizations in receipt of funding < RMB 20,000 ($3,068) that had participated in the HIV/AIDS prevention and control < 5 years. Among other

Table 2. Distribution of service scale among MSM-focused CSOs and non-MSM-focused CSOs *n* (%)

Items	Overall (*n* = 212)	MSM-focused CSOs (*n* = 103)	Other CSOs (*n* = 109)	X^2	*P*
Number of service people				4.76	0.03*
< 600	111 (52.4)	46 (44.7)	65 (59.6)		
≥ 600	101 (47.6)	57 (55.3)	44 (40.4)		
Number of VCT people				6.68	< 0.001**
< 200	116 (54.7)	47 (45.6)	69 (63.3)		
≥ 200	96 (45.3)	56 (54.4)	40 (36.7)		

*$p < 0.05$; **$p < 0.001$.

Table 3. Multivariate logistic regression analysis of organizational social capital associated with total service scale (total service people < 600 *vs.* total service people ≥ 600)

Items	Adjusted OR (95 % CI)		
	Overall CSOs	MSM-focused CSOs	Other CSOs
Number of core staff			
< 3	1.00	1.00	1.00
3-5	1.99 (0.82-4.82)	0.96 (0.21-4.34)	3.56 (1.06-11.98)*
> 5	2.75 (0.97-7.76)	0.75 (0.15-3.70)	8.60 (1.70-43.66)*
Funding (Dollar)			
< 3,068	1.00	1.00	1.00
3,068-18,408	1.63 (0.72-3.73)	4.53 (1.25-16.38)*	0.54 (0.16-1.87)
> 18,408	1.38 (0.50-3.84)	3.07 (0.61-15.41)	0.41 (0.08-2.04)
HIV/AIDS service year			
< 5	1.00	1.00	1.00
5-9	1.70 (0.81-3.57)	2.58 (0.78-8.54)	1.12 (0.38-3.25)
> 9	5.09 (1.97-13.17)*	6.68 (1.32-33.97)*	4.29 (1.16-15.89)*
Registration			
No	1.00	1.00	1.00
Yes	2.25 (1.23-4.50)*	1.57 (0.57-4.35)	2.56 (0.89-7.34)
AIDS epidemic situation			
Low	1.00	1.00	1.00
Middle	0.23 (0.07-0.71)*	0.11 (0.01-1.16)	0.19 (0.04-0.88)*
High	0.30 (0.10-0.94)*	0.13 (0.01-1.21)	0.70 (0.15-3.23)
Net-works			
Low	1.00	1.00	1.00
High	1.62 (0.85-3.06)	1.07 (0.41-2.81)	2.01 (0.75-5.38)
Trust			
Low	1.00	1.00	1.00
High	1.17 (0.62-2.01)	1.29 (0.51-3.28)	1.25 (0.45-3.48)
Support			
Low	1.00	1.00	1.00
High	1.88 (0.98-3.59)	2.37 (0.84-6.69)	1.54 (0.56-4.19)
Shared vision			
Low	1.00	1.00	1.00
High	0.81 (0.43-1.53)	0.46 (0.16-1.31)	0.88 (0.33-2.33)
Service object			
MSM-focused CSOs	1.00		
Other CSOs	1.91 (0.99-3.68)		

*$p < 0.05$.

CSOs, organizations with more core staff and longer service tenure were more likely to serve a larger target population (AOR = 3.56, AOR = 8.60, and AOR = 4.29). Concurrently, CSOs located in the middle- and low-level epidemic areas were more likely to provide services to a smaller target population (AOR = 0.19). The multivariate logistic regression analysis did not reveal any association between the social capital level and the total service scale.

3.4. Correlates of organizational social capital and VCT service scale

The number of core staff (3-5 core staff, AOR = 3.65; > 5 core staff, AOR = 5.59) was correlated with a larger VCT service scale among all participating CSOs (Table 4). More importantly, the level of shared vision was positively associated with the number of VCT service users (AOR = 1.95). Among the MSM-focused CSOs, the level of networks was positively associated with the number of VCT service users (AOR = 2.87). Among other CSOs, the number of core staff (3-5 core staff, AOR = 6.34; > 5 core staff, AOR = 12.77), and the HIV/AIDS epidemic level in the district (high epidemic district, AOR = 8.50) were correlated with a larger scale of the VCT service. Finally, high support level component of social capital was positively associated with the number of VCT service users (AOR = 3.08).

4. Discussion

4.1. CSOs working on HIV/AIDS in China

Over the past 20 years, CSOs in China have significantly increased their involvement in the AIDS response (*12*). Consistent with previous studies, our analyses have shown that CSOs working on HIV/AIDS had played a valuable role in all aspects of HIV/AIDS-related prevention and control efforts (*27,28*). In our study, 103 (48.6%) CSOs focused on MSM, hard-to-reach populations such as IDUs, and CSWs still took a small proportion, which reflected the political and

Table 4. Multivariate logistic regression analysis of organizational social capital associated with VCT service scale (VCT service people < 200 *vs.* VCT service people ≥ 200)

	Adjusted OR (95 % CI)		
	Overall CSOs	MSM-focused CSOs	Other CSOs
Number of core staff			
< 3	1.00	1.00	1.00
3-5	3.65 (1.43-9.35)*	1.96 (0.43-8.91)	6.34 (1.57-26.32)*
> 5	5.59 (1.88-16.64)*	3.01 (0.59-15.29)	12.77 (2.14-76.38)*
Funding (Dollar)			
< 3,068	1.00	1.00	1.00
3,068-18,408	1.71 (0.75-3.90)	1.03 (0.32-3.37)	3.18 (0.75-13.61)
> 18,408	1.95 (0.71-5.33)	1.28 (0.27-6.00)	1.93 (0.37-10.13)
HIV/AIDS service year			
< 5	1.00	1.00	1.00
5-9	1.21 (0.57-2.56)	2.13 (0.65-6.96)	0.53 (0.15-1.91)
> 9	1.98 (0.79-4.97)	4.31 (0.91-20.35)	1.66 (0.44-6.34)
Registration			
No	1.00	1.00	1.00
Yes	1.41 (0.71-2.77)	0.69 (0.05-9.54)	0.34 (0.04-2.79)
AIDS epidemic situation			
Low	1.00	1.00	1.00
Middle	1.38 (0.44-4.36)	0.38 (0.06-2.61)	3.11 (0.44-21.78)
High	2.27 (0.71-7.28)	0.77 (0.12-5.19)	8.50 (1.12-64.60)*
Net-works			
Low	1.00	1.00	1.00
High	1.67 (0.89-3.14)	2.87 (1.08-7.63)*	0.81 (0.29-2.27)
Trust			
Low	1.00	1.00	1.00
High	1.03 (0.54-1.93)	1.73 (0.69-4.35)	0.68 (0.23-1.99)
Support			
Low	1.00	1.00	1.00
High	1.09 (0.58-2.06)	1.38 (0.51-3.78)	1.27 (0.45-3.63)
Shared vision			
Low	1.00	1.00	1.00
High	1.95 (1.02-3.72)*	1.57 (0.58-4.22)	3.08 (1.03-9.21)*
Service object			
MSM-focused CSOs	1.00		
Other CSOs	1.79 (0.93-3.44)		

*$p < 0.05$.

cultural context of CSOs in China (*14,29*). Moreover, 67.9% of the CSOs included in the present study were unregistered, higher than in previous studies, which reported that 55-65% of China's CSOs working on HIV/AIDS were unregistered (*12,30*). It is widely acknowledged that the legal status of a CSO is paramount to its performance (*31,32*). For example, unregistered CSOs are typically unable to manage their own finances and bank accounts, making it difficult for them to mobilize large-scale resources (*33*). In the last few years, registration restrictions on CSOs have been loosened in China (*14*). However, without reforms to the registration process, most CSOs continue to face difficulties as professional organizations, which prevent them from expanding the services they provide (*12*).

According to previous studies, CSOs working on HIV/AIDS usually receive financial support from three sources: *i*) government investment, *ii*) international programs, and *iii*) corporate and individual donations. An examination into Chinese CSOs working on HIV/AIDS has revealed that these organizations typically have annual budgets of $US 7,000 (*34*). However, in our study, we found that a majority of the included organizations lacked funding. In 2014, the median amount of funding available to these organizations was RMB 50,000 ($US 7,670), while 22.6% of the included CSOs had not received any grants in the preceding year. Kaufman noted that despite large amounts donated to AIDS in China, little funding reaches the local NGOs (*28*). In June, 2014, the Global Fund to fight AIDS had reached its financial goal for operations in China, and a "Fund for Social Organizations Participating in HIV/AIDS Prevention and Control" was initiated by the Chinese government in June 2015 (*35*). These efforts notwithstanding, as Minghui *et al.* commented, China must make a parallel commitment to supporting at-risk groups and the civil society organizations that represent them (*36*).

4.2. VCT service offered by the CSOs

The VCT service is a delivery point for prevention and

treatment of AIDS/HIV. The Chinese government had launched the VCT service through local health agencies in major cities in 2003 (*37*). By 2012, 9016 VCT service sites had been established, and over 2,300,000 people received voluntary counseling and testing (*38*). Despite these developments, the rate of VCT access among MSM, IDUs, CSWs, and other high-risk populations remained low.

Our study indicated that over 104,000 individuals received a VCT service provided by one of the 212 CSOs, suggesting that more attention should be paid to service feasibility. The leader of China's National Centre for AIDS/STD Control and Prevention (NCAIDS/STD) stated that stigma and discrimination were significant obstacles to HIV VCT (*39*). However, CSOs have advantages to effectively offer and expand the VCT service even to under-served populations. For example, Chen (*37*) reported on VCT outreach among IDUs, in which potential service users were recruited by peer workers with a history of injection drug use, and knowledge of relevant behaviors and locations to effectively recognize and approach members of the target population. As commented by Van de Perre (*40*), the challenge is no longer to show the efficacy of the VCT service but to make it accessible to people who need it and to render it acceptable, accessible, and affordable.

4.3. Social capital and its measurement

In our study, careful attention was given to the design and validation of the social capital measurement questionnaire. As noted above, the analytical units of social capital can be on the level of an individual, organization, or society. Nevertheless, the research available on social capital specifically in the context of CSOs' is rare. We obtained better internal reliability values for the collective-social capital questionnaire used in our survey (0.68-0.91) than the values obtained by previous studies conducted in mainland China (*41*).

4.4. Social capital and VCT service

Our study is the first to explore the potential association between the VCT service and collective-social capital of CSOs dedicated to HIV/AIDS management in China. The results indicated that social capital, in particular, shared vision, was significantly associated with the level of VCT among all CSOs, while networks and shared vision were significantly associated with VCT service scale among MSM-focused CSOs and other CSOs, respectively. The multivariate logistic regression analysis did not reveal any association between social capital level and total service scale. These findings indicate that VCT service scale, rather than total service scale, can be used as a performance indicator for CSOs working on HIV/AIDS management. Capacity building and policy-making efforts should be separate and specific for MSM-focused and other CSOs.

In this study, networks could facilitate the VCT activities among MSM-focused CSOs. As Tsai and Ghoshal noted, frequent and close social interactions permit actors to know one another, share important information, and create a common point of view (*21*). This suggests that meetings and discussions between core and general members of CSOs staff could identify goals and strategies relevant to the organizations. They could also help implement suitable activities, including health and peer education, professional training, and communication and behavioral interventions more efficiently. Moreover, active interactions within organizations could help recruit new members and spread to them knowledge of HIV through conversations and discussions. In fact, a previous study conducted in China revealed that influence of acquaintances and peers is associated with the willingness to attend a VCT (*42*). Concurrently, other studies have suggested that knowledge about AIDS and perceptions of HIV/AIDS-associated risks also impact on VCT utilization (*16, 43-45*).

Previous research has defined cognitive social capital as a common understanding between social actors through shared language and narratives (*15,21*). It is embodied in attributes such as shared vision or shared values that facilitate individual and collective action. As cognitive social capital increases, the more likely a common perception and interpretation of events is to develop (*46*). When a shared vision was present within a CSO, the organization was dynamic and cohesive. The staff and members were more likely to unite and fulfill the collective aim and duty. Moreover, shared values indicate how the group should do things and enable individuals to be more committed to interpersonal relationships (*47*). Together, these factors contribute to the utilization of VCT.

As for the high-risk populations, examples from China and other Asian countries have shown that criminalization and stigma remain as obstacles to rights protection and disease prevention(*14,48,49*). As such, the goal for the CSOs should be to identify, understand, and reach the most vulnerable groups.

It was not surprised to find that CSOs focused on high-risk populations use a set of symbols and codes in line with the service population in daily communication, actually, many core members just came from the service population (*29*). When people within organizations use commonly-known symbols and codes, they might gain credibility and acceptance from service users, which, in turn, might help reduce fears of AIDS, making a higher level of VCT use achievable (*50*).

In the present study, the CSO registration status was positively associated with the level of VCT service. In China, official registration for CSOs takes place with the Ministry of Civil Affairs (MOCA) and criteria

for registration are high (*12*). At the same time, only the registered CSOs are legally recognized, leaving unregistered CSOs struggling with personnel recruitment and participation in open-bidding for domestic or international AIDS programs, which might be obstacles to reaching service users.

No association was found between trust or support and the level of VCT. Some scholars have proposed that trust measures only capture psychological aspects that might be relevant but are not necessarily indicative of social capital (*51*). This framework might account for some of our findings. Another possible factor is that trust and support were mainly rooted in organizations, while in consideration of VCT modality already defined above, VCT service could be offered by CDC and other governmental institutions, thus despite the high level of trust and support obtained from CSOs, the service objects still hesitated to have counseling and testing for HIV because of stigma and discrimination (*52*). Further research is needed to elucidate the association between support, trust, and the level of the VCT service. Additionally, a shift from the present clinic-based approach to a more routine and widespread public health model should be established (*53*), and Chinese CSOs have the capacity to fill this need (*37*). The current structure and number of AIDS prevention and control professionals are not sufficient to provide intervention to all vulnerable AIDS groups, and cannot meet the needs associated with the rapid increase in prevention and control tasks every year. After standardized training and daily guidance, CSOs involved in the prevention and control work will implement the advantages of a flexible working mode, will find it easier to work with the target population, and will be competent in the prevention and control work. CSOs in the field of AIDS prevention and treatment can compensate for the lack of professionals, provide more convenient testing and consulting services, and the communication between companions makes it easier to achieve the humanistic care needs of the infected people. Such communication can help relieve mention trauma and burdens.

Chinese governments at all levels coordinate and promote cooperation between CSOs, the Chinese Center for Disease Control and Prevention in the field of AIDS prevention and control (*54*). With the coordinated effort, they can gradually establish an information disclosure mechanism for CSOs, improve credit management systems, and ensure healthy and orderly development of CSOs participating in the prevention and control of AIDS. CSOs should clearly indicate the work orientation and development direction in AIDS prevention and control, and strengthen the organizational construction of rules and regulations, personnel team, working conditions, supervision and management, social reputation, the working advantages, working capacity, work performance and cooperation level, in AIDS prevention and control. They should also focus on stimulating their own vitality and actively participate in AIDS prevention and control activities. The Chinese Center for Disease Control and Prevention should establish a work information communication mechanism with CSOs, coordinate and solve the technical difficulties and problems related to CSO work, strengthen the training of professional skills and management ability regarding prevention and control, and assist CSOs in purchasing prevention and control services offered by the government.

There are some limitations associated with our study. First, the CSOs included in the present survey were a convenient sample, which might not represent all Chinese CSOs. Second, because of the nature of a cross-sectional study design, the reported relationship is an association, and no conclusions can be drawn regarding causality. Third, because social capital, as defined in this study, was measured at a collective level, the individual- and social-level impact of social capital were not considered. Fourth, due to the limitation in data source and data integrity, some important indicators are still lacking. The rate of VCT use, social discrimination, stigma, and other important factors are all significant variables of CSOs. These variables will be assessed in future studies. Regardless, our study provides a base upon which future surveys examining the impact of CSOs' social capital on the use of VCT service in the Chinese context can be built and applied to low and middle-income countries.

In conclusion, our study provides an initial exploration of correlations between aspects of social capital and the level of VCT service promoted by CSOs dedicated to HIV/AIDS management in China. To our knowledge, this is the first China-based study to investigate such associations. With further research, our findings can be used to develop evidence-based policy to improve the level of VCT service.

Acknowledgements

The authors thank the participations and all who were involved in the surveys. Specially thanks to Hunan, Sichuan, Yunnan, Anhui, Hubei, Shandong, Jilin and Gansu CDC and local civil society organizations for assisting the field work. This study was funded by grants 71303007 and 71874002 from the National Natural Science Foundation of China (Dr Chen), and the Faculty Doctoral Fellowship of Anhui Medical University (Grant No. XJ201702, Dr wang).

References

1. Schwartz CE, Zhang J, Stucky BD, Michael W, Rapkin BD. Is the link between socioeconomic status and resilience mediated by reserve-building activities: mediation analysis of web-based cross-sectional data

from chronic medical illness patient panels. BMJ open. 2019; 9:e025602.
2. Hong NT, Wolfe MI, Dat TT, McFarland DA, Kamb ML, Thang NT, Thai HN, Del Rio C. Utilization of HIV voluntary counseling and testing in Vietnam: an evaluation of 5 years of routine program data for national response. AIDS Educ Prev. 2011; 23:30-48.
3. Zhang T, Tian X, Ma F, Yang Y, Yu F, Zhao Y, Gao M, Ding Y, Jiang Q, He N. Community based promotion on VCT acceptance among rural migrants in Shanghai, China. PLoS One. 2013; 8:e60106.
4. Tucker JD, Wong FY, Nehl EJ, Zhang F. HIV testing and care systems focused on sexually transmitted HIV in China. Sex Transm Infect. 2012; 88:116-119.
5. Zhang TP, Liu C, Han L, Tang W, Mao J, Wong T, Zhang Y, Tang S, Yang B, Wei C, Tucker JD. Community engagement in sexual health and uptake of HIV testing and syphilis testing among MSM in China: a cross-sectional online survey. J Int AIDS Soc. 2017; 20:21372.
6. Wu ZY. HIV/AIDS prevention strategy with Chinese characteristics. Chinese Journal of Disease Control & Prevention. 2019; 23:885-889. (in Chinese)
7. Wu ZY. Achievement of HIV/AIDS program in the past 30 years and challenges in China. Chin J Epidemiol. 2015; 36:1329-1331. (in Chinese)
8. Denison JA, O'eilly KR, Schmid GP, Kennedy CE, Sweat MD. HIV voluntary counseling and testing and behavioral risk reduction in developing countries: a meta-analysis, 1990--2005. Aids Behav. 2008; 12:363-373.
9. Musheke M, Ntalasha H, Gari S, McKenzie O, Bond V, Martin-Hilber A, Merten S. A systematic review of qualitative findings on factors enabling and deterring uptake of HIV testing in Sub-Saharan Africa. BMC Public Health. 2013; 13:220.
10. National Center for AIDS/STD Control and Prevention, Chinese Center for Disease Control and Prevention. Update on the AIDS/STD epidemic in China and main response in control and prevention in the third quarter of 2015. Chinese Journal of AIDS & STD. 2015; 21:915. (in Chinese)
11. Zhang Q, Fu YS, Liu XM, Ding ZQ, Li MQ, Fan YG. HIV Prevalence and Factors Influencing the Uptake of Voluntary HIV Counseling and Testing among Older Clients of Female Sex Workers in Liuzhou and Fuyang Cities, China, 2016-2017: A Cross-Sectional Study. Biomed Res Int. 2020;9634328.
12. Li H, Kuo NT, Liu H, Korhonen C, Pond E, Guo H, Smith L, Xue H, Sun J. From spectators to implementers: civil society organizations involved in AIDS programmes in China. Int J Epidemiol. 2010; 39:ii65-71.
13. Xu H, Zeng Y, Anderson AF. Chinese NGOs in action against HIV/AIDS. Cell Res. 2005; 15:914-918.
14. Kaufman J. HIV, sex work, and civil society in China. J Infect Dis. 2011; 204:S1218-1222.
15. Sun X, Rehnberg C, Meng Q. How are individual-level social capital and poverty associated with health equity? A study from two Chinese cities. Int J Equity Health. 2009; 8:2.
16. Frumence G, Eriksson M, Nystrom L, Killewo J, Emmelin M. Exploring the role of cognitive and structural forms of social capital in HIV/AIDS trends in the Kagera region of Tanzania - a grounded theory study. Afr J AIDS Res. 2011; 10:1-13.
17. Cené CW, Akers AY, Lloyd SW, Albritton T, Powell Hammond W, Corbie-Smith G. Understanding social capital and HIV risk in rural African American communities. J Gen Intern Med. 2011; 26:737-744.
18. Campbell C, Scott K, Nhamo M, Nyamukapa C, Madanhire C, Skovdal M, Sherr L, Gregson S. Social capital and HIV competent communities: the role of community groups in managing HIV/AIDS in rural Zimbabwe. AIDS care. 2013; 25:S114-S122.
19. Sivaram S, Zelaya C, Srikrishnan AK, Latkin C, Go VF, Solomon S, Celentano D. Associations between social capital and HIV stigma in Chennai, India: considerations for prevention intervention design. AIDS Educ Prev. 2009; 21:233-250.
20. Helliwell JF, Putnam RD. The social context of well-being. Philos Trans R Soc Lond B Biol Sci. 2004; 359:1435-1446.
21. Murayama H, Fujiwara Y, Kawachi I. Social capital and health: a review of prospective multilevel studies. J Epidemiol. 2012; 22:179-187.
22. Chinese Center for Disease Control and Prevention. Classification Criteria of AIDS Epidemic Level in China. *http:// www.chinacdc.cn/jkzt/crb/zl/azb/jszl_ 2219/201306/t20130617_81691.html* (accessed June 1, 2013). (in Chinese)
23. Hu F, Niu L, Chen R, Ma Y, Qin X, Hu Z. The association between social capital and quality of life among type 2 diabetes patients in Anhui province, China: a cross-sectional study. BMC Public Health. 2015; 15:786.
24. Ma Y, Qin X, Chen R, Li N, Chen R, Hu Z. Impact of individual-level social capital on quality of life among AIDS patients in China. PLoS One. 2012; 7:e48888.
25. Wang D, Mei G, Xu X, Zhao R, Ma Y, Chen R, Qin X, Hu Z. Chinese non-governmental organizations involved in HIV/AIDS prevention and control: Intra-organizational social capital as a new analytical perspective. Biosci Trends. 2016; 10:418-423.
26. National Center for AIDS/STD Control and Prevention China CDC. National HIV/syphilis/HCV sentinel surveillance report in 2018. Beijing: 2019. (in Chinese)
27. Chen X. Situation analyses for civil society involving in HIV/AIDS prevention and control in China. Chinese J AIDS& STD. 2006; 12:580-582. (in Chinese)
28. Wang D, Xu X, Mei G, Ma Y, Chen R, Qin X, Hu Z. The Relationship Between Core Members' Social Capital and Perceived and Externally Evaluated Prestige and Cooperation Among HIV/AIDS-Related Civil Society Organizations in China. Eval Health Prof. 2017; 40:61-78.
29. Hussen SA, Jones M, Moore S, Hood J, Smith JC, Camacho-Gonzalez A, Del Rio C, Harper GW. Brothers Building Brothers by Breaking Barriers: development of a resilience-building social capital intervention for young black gay and bisexual men living with HIV. AIDS care. 2018; 30:51-58.
30. Hui L, Mei L, Jinfeng L. Situation analysis for NGOs and civil societies involved in HIV/ AIDSprevention and control programs in some provinces of China. Chinese J AIDS& STD. 2008; 14:246-248. (in Chinese)
31. Smith J, Mallouris C, Lee K, Alfvén T. The Role of Civil Society Organizations in Monitoring the Global AIDS Response. AIDS Behav. 2017; 21:44-50.
32. Lau JTF, Wang Z, Kim Y, Li J, Gu J, Mo PKH, Wang X. Low sustainability, poor governance, and other challenges encountered by grassroots non-governmental organizations targeting HIV prevention for men who have

sex with men in China - a nation-wide study. AIDS care. 2017; 29:1480-1490.
33. Niu C. A Survey of Grassroots NGOs involved in China's HIV/AIDS Prevention and Control. The Chinese Journal of Human Sextuality. 2005; 14:8-17. (in Chinese)
34. Rong G, Ke L, Jing L, Mengjie H. An analysis of involvement of China's social organizations in HIV/AIDS prevention and control. Chinese J AIDS& STD. 2011; 17:506-508, 518. (in Chinese)
35. Chinese Center for Disease Control and Prevention. Notifications on Establishing Fund of Social Organizaitons Participate in HIV/AIDS Prevention and Control Commission NHaFP, ed. *http://www.moh.gov.cn/jkj/s3585/201507/f6a7e1b9918c4416a9a4aeda0eaa4091.shtml* (accessed Dec 25, 2015).
36. Minghui R, Scano F, Sozi C, Schwartlander B. The Global Fund in China: success beyond the numbers. Lancet Glob Health. 2015; 3:e75-77.
37. Chen HT, Liang S, Liao Q, Wang S, Schumacher JE, Creger TN, Wilson CM, Dong B, Vermund SH. HIV voluntary counseling and testing among injection drug users in south China: a study of a non-government organization based program. AIDS Behav. 2007; 11:778-788.
38. Chenxi L, Jiefang L, Shujun L, Ning W. Current Research Situation of HIV Voluntary Counseling and Testing in China. Chinese J AIDS& STD 2014; 20:624-627. (in Chinese)
39. WU Z, Sun X, Sullivan S, Detels R. HIV Testing in China. Sience. 2006; 312:1475-1476.
40. Van de Perre P. HIV voluntary counselling and testing in community health services. Lancet. 2000; 356:86-87.
41. Jianglin K, Jintao S, Jianmin S. Dimensions'developing and structure's testing of team social capital. Studies in Science of Science. 2007; 25:935-940. (in Chinese)
42. Wang Y, Li B, Pan J, Sengupta S, Emrick CB, Cohen MS, Henderson GE. Factors associated with utilization of a free HIV VCT clinic by female sex workers in Jinan City, Northern China. AIDS Behav. 2011; 15:702-710.
43. Gage AJ, Ali D. Factors associated with self-reported HIV testing among men in Uganda. AIDS care. 2005; 17:153-165.
44. Feleke SA, Koye DN, Demssie AF, Mengesha ZB. Reproductive health service utilization and associated factors among adolescents (15-19 years old) in Gondar town, Northwest Ethiopia. BMC Health Serv Res. 2013; 13:294.
45. Tsegay G, Edris M, Meseret S. Assessment of voluntary counseling and testing service utilization and associated factors among Debre Markos University Students, North West Ethiopia: a cross-sectional survey in 2011. BMC Public Health. 2013; 13:243.
46. Grover E, Grosso A, Ketende S, Kennedy C, Fonner V, Adams D, Sithole B, Mnisi Z, Maziya SL, Baral S. Social cohesion, social participation and HIV testing among men who have sex with men in Swaziland. AIDS care. 2016; 28:795-804.
47. Knight L, Hosegood V, Timaeus IM. Obligation to family during times of transition: care, support and the response to HIV and AIDS in rural South Africa. AIDS care. 2016; 28:18-29.
48. Nall A, Chenneville T, Rodriguez LM, O'Brien JL. Factors Affecting HIV Testing among Youth in Kenya. Int J Environ Res Public Health. 2019; 16: E1450.
49. Watson J, TangW, Pan S, Wu D, Zhao P, Cao B, Liu C, Bien C, Huang W, Luo Z, Tucker JD. Out of the closet, into the clinic: opportunities for expanding men who have sex with men-competent Services in China. Sex Transm Dis. 2018; 45:527-533.
50. Muriisa RK, Jamil I. Addressing HIV/AIDS challenges in Uganda: does social capital generation by NGOs matter? SAHARA J. 2011; 8:2-12.
51. Carpiano RM, Fitterer LM. Questions of trust in health research on social capital: what aspects of personal network social capital do they measure? Soc Sci Med. 2014; 116:225-234.
52. Fu R, Kutner BA, Wu Y, Xie L, Meng S, Hou J, Gu Y, Xu H, Zheng H, He N, Meyers K. Do gay and bisexual men who conceal their same-sex behavior prefer different kinds of health services? Findings across four cities to inform client-centered HIV prevention in China. BMC Public Health 2020; 20:4.
53. Ikechebelu IJ, Udigwe GO, Ikechebelu N, Imoh LC. The knowledge, attitude and practice of voluntary counselling and testing (VCT) for HIV/AIDS among undergraduates in a polytechnic in southeast, Nigeria. Niger J Med. 2006; 15:245-249.
54. Lo CY. Securitizing HIV/AIDS: a game changer in state-societal relations in China? Global Health. 2018; 14:50.

Received March 28, 2020; Revised April 24, 2020; Accepted May 11, 2020.

**Address correspondence to:*
Zhi Hu, Ren Chen, School of Health Services Management, Anhui Medical University. No. 81, Meishan Road, Hefei 230032, Anhui, China.
E-mail: aywghz@126.com (ZH), chenren2006@hotmail.com (RC)

Released online in J-STAGE as advance publication May 16, 2020.

Original Article

DOI: 10.5582/bst.2020.01051

Design, synthesis, and biological evaluation of 4-phenoxybenzenesulfonyl pyrrolidine derivatives as matrix metalloproteinase inhibitors

Jing Mao[1], Hao Zhang[1], Xuejian Wang[1], Jianjun Gao[2], Jinbao Tang[1,*], Jian Zhang[1,*]

[1] Department of Medical Chemistry, School of Pharmacy, Weifang Medical University, Weifang, Shandong, China;
[2] Department of Pharmacology, School of Pharmacy, Qingdao University, Qingdao, Shandong, China.

SUMMARY A series of 4-phenoxybenzenesulfonyl pyrrolidine derivatives were designed, synthesized, and evaluated as matrix metalloproteinases (MMPs) inhibitors. All of the synthesized compounds displayed inhibitory activity against MMP-2 and MMP-9. Compounds **4a**, **4e**, and **4i** displayed more potent activity than the other compounds. While the three compounds mildly or moderately inhibited the proliferation of cancer cells, they significantly suppressed the migration and invasion of cancer cells at relatively low concentrations as determined by a wound healing assay and transwell assay. In addition, compound **4e** suppressed vascular endothelial cell tube formation and sprouting of microvessels from aortic rings *in vitro* in a dose-dependent manner. Compound **4e** markedly suppressed the pulmonary metastasis of H22 cells in mice. These findings along with molecular docking results suggested that compound **4e** might be a promising candidate for further structural optimization to develop MMP inhibitors as potential anticancer agents.

Keywords MMP inhibitor, pyrrolidine derivatives, anticancer agent

1. Introduction

Enzymatic proteolysis of cell surface proteins and extracellular matrix (ECM) is critical for tissue homeostasis and cell signaling (*1*). These proteolytic activities are mediated predominantly by a family of structurally and functionally related zinc-dependent endoproteinases termed matrix metalloproteinases (MMPs) (*2*). MMPs are involved in many aspects of physiological processes, as well as in pathologies, such as arthritis, cardiovascular, inflammation, tumor growth, and metastasis (*3-5*). Among more than 25 MMPs, MMP-2 and MMP-9 play an important role in virtually all aspects of cancer progression, making them important therapeutic targets for drug development (*6*).

A number of laboratories have endeavored in recent decades to discover many potent and orally active broad-spectrum MMP inhibitors. Some have reached the stage of an advanced clinical trial, as exemplified by CGS 27023A, prinomastat, and ilomastat (*7,8*). Colleagues of the current authors have previously reported several kinds of synthetic MMP inhibitors, such as caffeoyl pyrrolidine derivatives (*9*), sulfonyl pyrrolidine derivatives (*10*), quinoxalinone peptidomimetic derivatives (*11*), Pro-Gly peptidomimetic derivatives (*12*), and phosphonic 1,4-dithia-7-azaspiro[4,4] nonane derivatives (*13*).

In a previous work, the current authors' group thoroughly studied L-hydroxyproline scaffold-based derivatives as effective MMP inhibitors. Compound LY52 in particular potently inhibited MMP-2 and was found to inhibit tumor invasion and metastasis (*14*). Based on the fact that the sulfonamide group can be incorporated in metalloenzyme inhibitors to improve enzyme-inhibitor binding (*15*), arylsulfonyl pyrrolidine derivatives were designed by the current authors. In those derivatives, the 4-phenoxybenzenesulfonyl group and different aroyl groups are introduced into the trans-S-hydroxy-L-proline scaffold (Figure 1). This article describes the synthesis and evaluation of the biological activity of these MMP inhibitors.

2. Materials and Methods

2.1. Chemicals

Unless otherwise noted, all commercially available starting materials, reagents, and solvents were used without further purification. All reactions were monitored using TLC with 0.25 mm silica gel plates (60GF-254) and visualized with UV light, iodine stain, and ferric chloride. Silica gel or C18 silica gel was used for column chromatography purification. Flash chromatography was performed using the automated CombiFlash Rf system from Teledyne ISCO. ^{1}H NMR

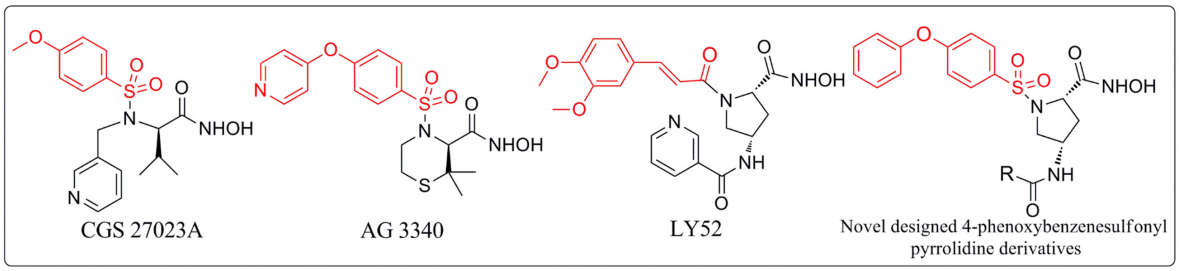


Figure 1. The structures of CGS27023A, AG3340, LY52, and novel designed 4-phenoxybenzenesulfonyl pyrrolidine derivatives.

and ^{13}C NMR spectra were recorded on a Bruker DRX spectrometer at 600 MHz; chemical shifts (d) were recorded in parts per million and the coupling constant (*J*) was measured in Hertz, and TMS served as an internal standard. ESI-MS was performed on an API 4000 spectrometer at the Weifang Medical University Analysis and Test Center in Weifang, Shandong, China.

2.2. *In vitro* MMP-2 and MMP-9 inhibition assay

Recombinant human MMP-2, MMP-9 and the fluorogenic substrate Mca-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH2 were all purchased from Enzo Life Sciences (Farmingdale, NY, USA). Compounds **4a-i** and the positive control LY52 were assayed for their inhibitory activities against MMP-2 and MMP-9 using the Fluorometric Drug Discovery Kit. Twenty μL of the enzyme solution and 20 μL of the tested compound with different gradient concentrations were added to the 96-well plate. The 100% group contained no inhibitor and the blank group contained no enzyme. After incubation for 45 min at 37°C, 10 μL of the fluorogenic substrate was added to start the reaction, and the resulting solution was measured at 328 nm/420 nm.

2.3. MTT assay

Cell proliferation was determined using an MTT (3-[4, 5-dimethyl-2-thiazolyl]-2, 5-diphenyl-2*H*-tertazolium bromide) assay. Cells were plated on 96-well plates (10000 cells/well) and cultured for 4 hours in RPMI1640 medium containing 10% FBS at 37°C in a 5% CO_2 humidified incubator, and then treated with various concentrations of the tested compounds (with LY52 as the positive control). After incubation for 48 h, 0.5% MTT was added to each well. Four h later, formazan formed by MTT was dissolved with DMSO for 15 min. The OD value was measured using an ELISA reader at 570 nm.

2.4. Colony formation

A549 cells were seeded on 6-well plates at a density of 500 cells/well and treated with the tested compounds at a concentration of 5 μM for 24 hours. After incubation for 2 wks, the cells in each well were washed twice with PBS, fixed with methanol for 15 min, and stained with 0.1% crystal violet for 10 min. Then colonies were photographed and counted under an inverted microscope.

2.5. Wound healing assay

A wound healing assay was performed by plating cells on 6-well culture dishes. After A549 cells were allowed to attach and reach 80% confluence, a scratch (1 mm) was made through the culture dish with a sterile plastic 200 μL micropipette tip to generate one homogeneous wound along each well. After wounding, non-adherent cells were removed and washed twice with PBS. Cells were further incubated without or with the indicated compounds for 48 h and the wound width was measured under a microscope using an ocular grid.

2.6. Transwell assay

Migration and invasion assays were performed on a 24-well plate using a transwell chamber (Darmstadt, Hesse, Germany) with a pore size of 8 microns. Then, 1×10^5 of A549 cells were added to the upper chamber pre-coated with Matrigel (BD Bioscience, Bedford, MA, USA). Each well of cells was seeded with the compound in RPMI 1640 medium containing 1% FBS in the upper chamber. The lower chamber contained 10% FBS as a chemoattractant. After incubation at 37°C for 24 h, the cells were carefully removed from the upper side of the filter with a cotton swab. The membrane was then fixed with methanol for 10 minutes and stained with 0.1% crystal violet, and cells were photographed and counted under an inverted microscope.

2.7. Tube formation assay

A 24-well chamber was coated with 100 μL Matrigel (BD Biosciences, Bedford, MA, USA) and allowed to gel for 30 min at 37°C. Then, 1×10^5 of human umbilical vein endothelial cells (HUVECs) per well were suspended in growth medium without or with increasing concentrations of compound **4e** or LY52. The formation of capillary tubes was identified after incubation for 8 h. Images were captured with an inverted microscope. Tube formation was determined

by counting branch points of the formed tubes, and the average numbers of branch points were calculated.

2.8. Rat thoracic aorta ring (TAR) assay

A 96-well chamber was coated with 100 μL Matrigel (BD Biosciences, Bedford, MA, USA) and allowed to gel for 30 min at 37°C. Thoracic aortas were removed from 6-wk-old male SD rats. After carefully removing the perivascular tissue, the thoracic aorta was roughly cut into a 1-mm-long ring, and each ring was embedded in an independent Matrigel-coated well. Aorta rings were treated every other d with tested compounds for 9 d and photographed on the 10^{th} d at 200× magnification.

2.9. *In vivo* H22 tumor transplant model

In this experiment, Kunming mice were subcutaneously inoculated with 1×10^7/mL murine H22 cells (*16*). Seven d after inoculation with H22 cells, the transplanted mice were randomly divided into four groups with 7 mice per group. The mice were treated with **4e** or LY52 at the specified dose for 5 d/wk for two wks (**4e** Low: 50 mg/kg/d; **4e** High: 100 mg/kg/d; LY52: 50 mg/kg/d). Mice in the blank group were treated with PBS. After 14 d of treatment, mice were sacrificed, and their lungs were fixed with Bouin solution and the number of lung nodules was counted.

2.10. Computational docking assay

A docking study was conducted as follows: the selected compound was constructed with a Sybyl/Sketch module and optimized using the Powell Energetic Gradient method with a Tripos force field with the convergence criterion set at 0.05 kcal/mol Å, and charges were assigned using the Gasteiger-Hückel method. The docking study of the selected compound **4e** with the active site of MMP-2 was performed using the Sybyl/FlexX module. The radius of the active site was 10.0 Å surrounding Zn^{2+} (PDB: 1HOV).

2.11. Statistical analysis

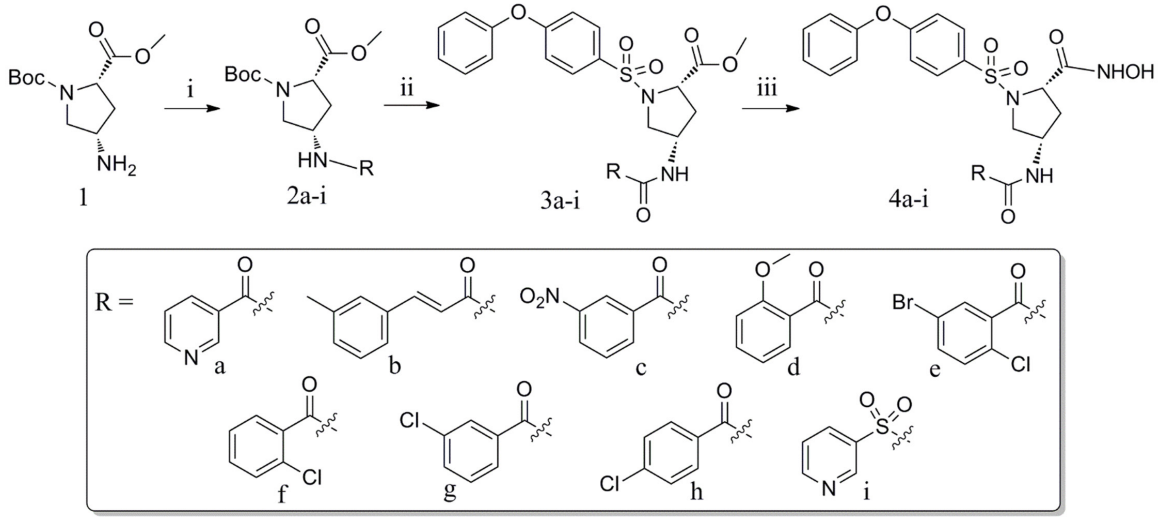
Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple range tests, and data are expressed as the mean ± SD. $p < 0.05$ was considered to indicate a statistically significant difference. Statistical analysis was performed using the software SPSS/Win 16.0 (SPSS, Chicago, USA).

3. Results

3.1. Chemistry

The target compounds were efficiently synthesized following the procedures shown in Scheme 1. The chemical structures of all target compounds are also listed and were analytically confirmed using ^{1}H-NMR and ^{13}C-NMR.

Starting from (2*S*,4*S*)-1-*tert*-butyl-2-methyl-4-aminopyrrolidine-1,2-dicarboxylate (**1**), intermediates (**2a-i**) were prepared *via* a condensation reaction with various aromatic acids or pyridine-3-sulfonic acid and isobutyl chloroformate (IBCF) as a condensation agent and triethylamine (TEA) as a base. After deprotection of the compounds **2a-i** in the presence of hydrogen chloride, the crude products without further purification were treated with 4-phenoxybenzene-1-sulfonyl chloride to yield compounds **3a-i**, which were treated with NH2OK in anhydrous methanol to yield the target compounds **4a-i**. For specific procedures for synthesis and spectroscopic data on all compounds, see Supplementary Data (*http://www.biosciencetrends.*



Scheme1. Reagents and conditions. (i) Isobutyl chloroformate, Et_3N, THF; (ii) HCl/EtOAC; 4-phenoxybenzene-1-sulfonyl chloride, DCM, TEA; (iii) NH_2OK, MeOH, 65%.

com/action/getSupplementalData.php?ID=65).

3.2. Suppression of the activity of MMP-2 and MMP-9 by synthesized compounds

The newly synthesized 4-phenoxybenzenesulfonyl pyrrolidine derivatives were assayed for their inhibitory activity against MMP-2 and MMP-9, and LY52 served as the positive control. The inhibition results (IC_{50}) are summarized in Figure 2. Compounds **4a-i** all displayed potent inhibitory activity against MMP-2 and MMP-9. Notably, **4a**, **4e**, and **4i** displayed more potent inhibitory activity against MMP-2 and MMP-9 than the other synthesized compounds and LY52; **4i** in particular displayed the most potent activity at the nanomole level (IC_{50} = 0.05 μM for MMP-2 and 0.08 μM for MMP-9).

3.3. Inhibition of cell growth by compounds **4a**, **4e**, and **4i**

Compounds **4a**, **4e** and **4i** were chosen to evaluate their antiproliferative activity against different tumor cell lines: A549 (lung cancer cells), ES-2 (ovarian clear cell carcinoma cells), Hela (human cervical carcinoma cells), K562 (chronic myelogenous leukemia cells), and MDA-MB-231 (human breast adenocarcinoma cells). LY52 served as a positive control. Results are shown in Figure 3A. Compound **4e** displayed the most potent activity against the proliferation of cancer cells among the tested compounds. To confirm the anti-growth activity of the tested compounds, a colony formation assay was performed to measure the effect of **4a**, **4e**, and **4i** at a concentration of 5 μM in A549 cells. As shown in Figures 3B and 3C, compound **4e** was superior to other compounds in suppressing clone formation by A549 cells.

3.4. Suppression of cell migration and invasion by compounds **4a**, **4e**, and **4i**

The activity of compounds **4a**, **4e**, and **4i** on the migration of A549 cells was assessed using a wound

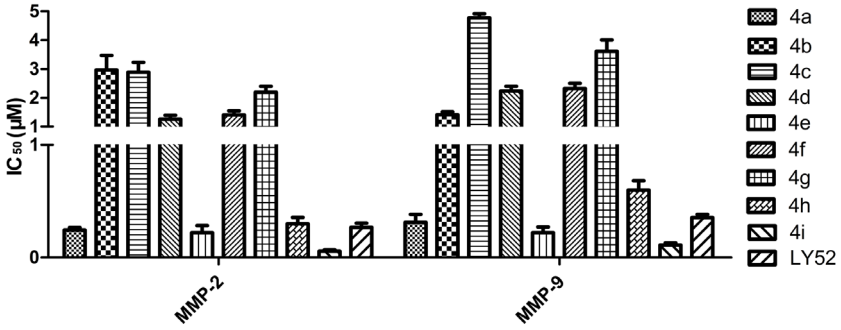


Figure 2. The inhibitory activities of the target compounds (4a-4i) against MMP-2 and MMP-9.

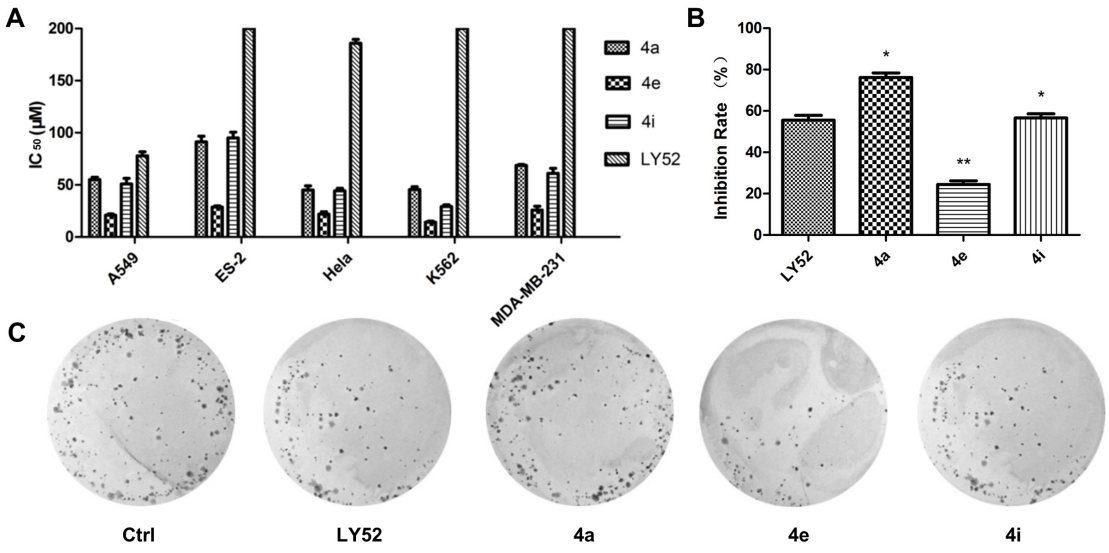


Figure 3. Inhibitory effects of 4a, 4e, and 4i on the growth of cancer cells. (A) IC_{50} values for **4a**, **4e**, and **4i** in A549, ES-2, Hela, K562, and MDA-MB-231 cells as determined by an MTT assay. **(B and C)** Inhibitory effect of **4a**, **4e**, and **4i** on clone formation of A549 cells. The rate of inhibition was estimated by counting the number of colonies containing > 50 cells, $^{*}p < 0.05$ *vs.* control; $^{**}p < 0.01$ *vs.* control.

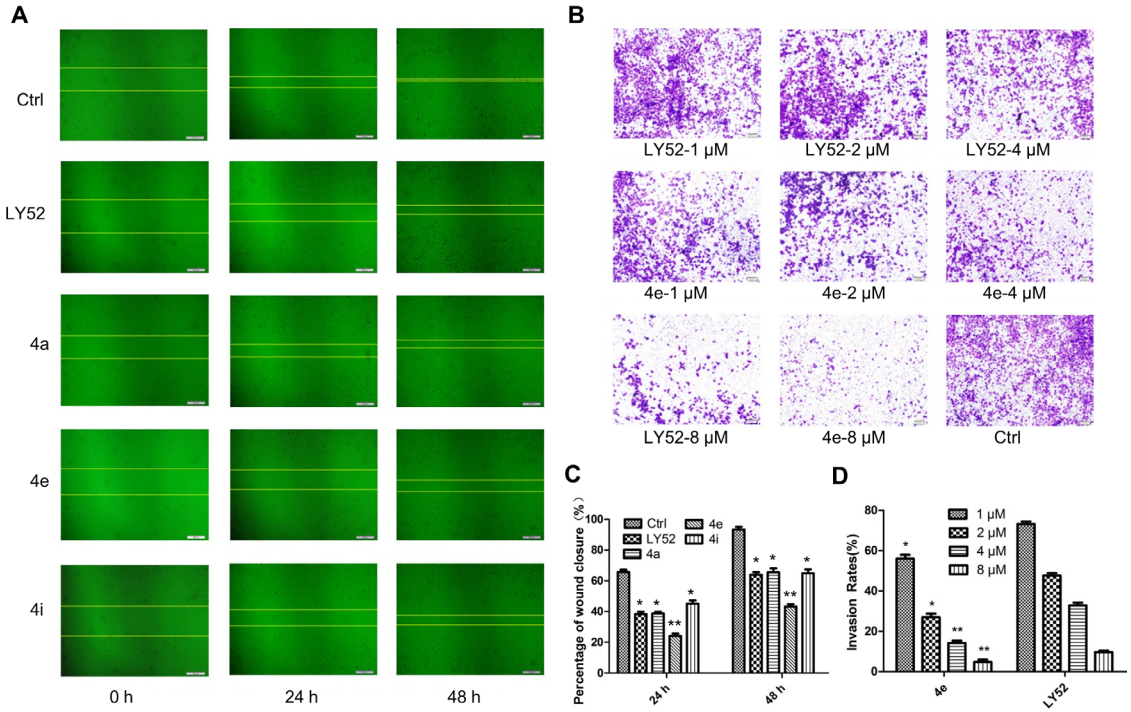


Figure 4. Inhibitory effects of 4a, 4e, and 4i on the migration and invasion of A549 cells as determined by a wound healing assay (A and C) and transwell assay (B and D). Cells were treated with **4a**, **4e**, or **4i** at a concentration of 5 μM for 48 h in a wound healing assay. Cells were treated with **4e** at incremental concentrations for 24 h. $^{*}p < 0.05$ *vs.* control; $^{**}p < 0.01$ *vs.* control.

healing assay. The concentration of all of the tested compounds was 5 μM. As shown in Figures 4A and 4C, compounds **4a**, **4e**, and **4i** markedly suppressed the migration of A549 cells as reflected by delayed wound closure. Compound **4e** was superior to other compounds in delaying wound closure. The anti-invasive activity of compound **4e** was evaluated using a transwell assay. Results indicated that it markedly reduced the number of invading cells in a dose-dependent manner (Figures 4B and 4D).

3.5. Suppression of angiogenesis by compound **4e** *in vitro*

To study its anti-angiogenesis action, compound **4e** was first evaluated for its cytotoxicity against HUVECs. Compound **4e** displayed relatively low cytotoxic activity with an IC_{50} of 22.42 μM. A HUVEC tube formation assay was then performed *in vitro* with **4e** and LY52 at concentrations of 1, 2, 4, and 8 μM. Results in Figure 5 indicate that **4e** reduced the number of branch points of HUVECs in a dose-dependent manner and almost completely inhibited tube formation at a concentration of 8 μM. Compared to HUVEC tube formation, the rat TAR model more closely resembles *in vivo* conditions during the process of angiogenesis. Compound **4e** was evaluated for microvessel formation at different concentrations (1, 2, 4, and 8 μM). Results indicated that the number of microvessels sprouting from aortic rings was significantly decreased by **4e** in a dose-dependent manner (Figure 6).

3.6. Suppression of cancer metastasis by compound **4e** *in vivo*

Treatment of A549 cells with compound **4e** at a concentration of 8 μM markedly decreased cancer cell migration and invasion *in vitro*. Encouraged by this finding, together with the potent anti-angiogenesis effects of **4e** *in vitro*, its anti-metastasis activity was examined in a H22 pulmonary metastasis model *in vivo*. As shown in Figures 7A and 7B, there were much fewer metastatic pulmonary nodes in mice treated with **4e** than in the LY52 group. Mice treated with **4e** did not experience any discernible adverse reactions or significant changes in body weight during the experiment.

3.7. Molecular docking results

Compound **4e** was chosen for a docking study to investigate its binding mode at the active site of MMP-2 (PDB: 1HOV). Results in Figure 8A suggest that the hydroxamic acid group can chelate with catalytic Zn^{2+} and that the 4-phenoxybenzenesulfonyl group can enter the S1' pocket, while the bromo- and chloro- substituted benzoyl group can occupy the S2' pocket. For a detailed understanding of the interactions of **4e** with the residues along the active site of the protein, a diagram of H-bond interactions was created (Figure 8B). The hydroxyl group and imido group of hydroxamic acid can form hydrogen bonds with Glu^{121} and the carbonyl group can form hydrogen bonds with His^{130}.

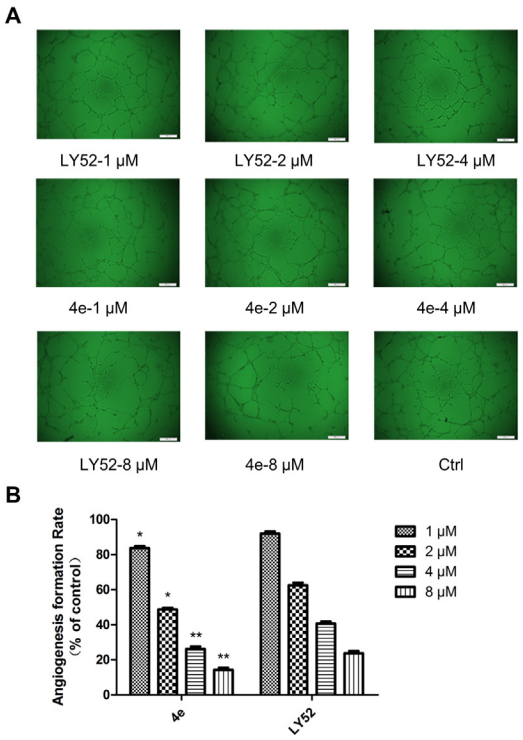


Figure 5. Compound 4e suppressed vascular endothelial cell tube formation *in vitro*. HUVECs were incubated with the indicated concentrations of **4e** or **LY52** for 8 h. The images were captured with an inverted microscope. Tube formation on Matrigel was determined by counting the branch points of formed tubes. $^{*}p < 0.05$ *vs.* control; $^{**}p < 0.01$ *vs.* control.

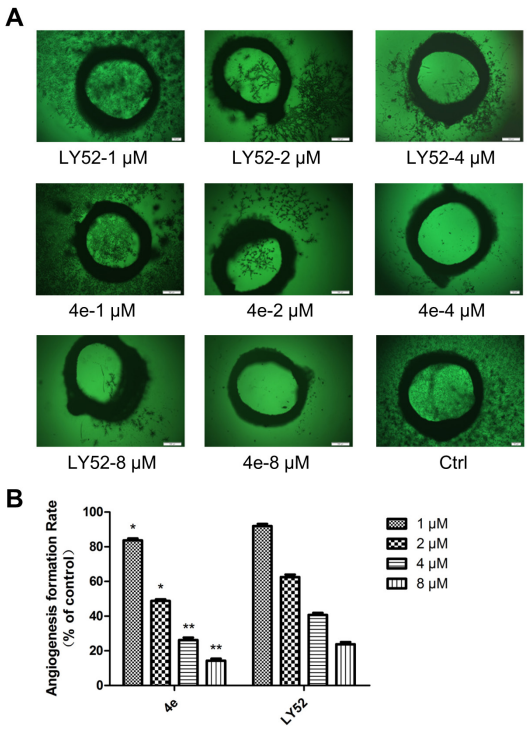


Figure 6. Compound 4e suppressed sprouting of microvessels from aortic rings. Thoracic aortas excised from 6-wk-old male SD rats were treated every other d with **4e** or **LY52** for 9 d and photographed on the 10th d at 200 × magnification. $^{*}p < 0.05$ *vs.* control; $^{**}p < 0.01$ *vs.* control.

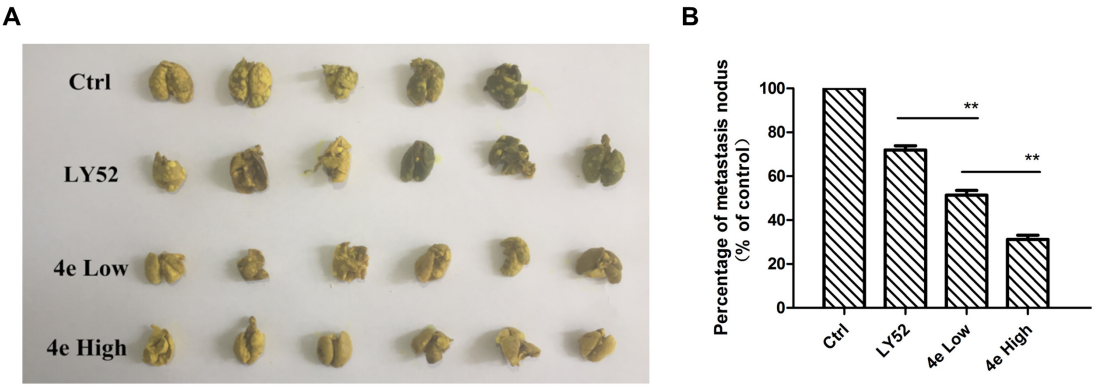


Figure 7. Compound 4e reduced metastatic pulmonary nodes in mice. Mice that were inoculated subcutaneously with H22 cells were treated with **4e** or **LY52** at the specified dose for 5 d/wk for two wks. At the end of experiment, the lungs were removed and fixed with Bouin solution and the number of lung nodules was counted. $^{*}p < 0.05$ *vs.* control; $^{**}p < 0.01$ *vs.* control.

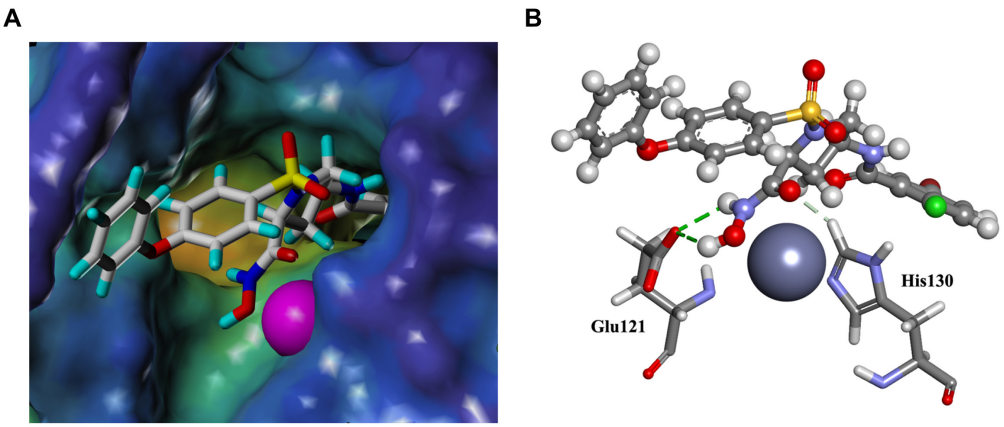


Figure 8. The FlexX docking (A) and binding mode (B) of compound 4e with MMP-2.

4. Discussion

Reported here is the synthesis and biological evaluation of 4-phenoxybenzenesulfonyl pyrrolidine derivatives as MMP-2/MMP-9 inhibitors. All of the synthesized compounds displayed potent inhibitory activity against MMP-2/MMP-9; **4a**, **4e**, and **4i** were more potent than the other compounds. These compounds' ability to suppress cell proliferation, migration, and invasion, angiogenesis *in vitro*, and cancer metastasis *in vivo* were also examined. Results suggested that the target compounds have a mild or moderate inhibitory effect on cancer cell proliferation. However, they significantly suppressed cell migration and capillary tube formation at low concentrations *in vitro*. Compound **4e** also significantly suppressed the metastasis of H22 cells in mice. These results suggest that the novel 4-phenoxybenzenesulfonyl pyrrolidine derivatives synthesized in this study have the potential to suppress cancer metastasis.

This study investigated the structure-activity relationship (SAR) of all target compounds. There is no obvious subtype selectivity between MMP-2 and MMP-9 for these 4-phenoxybenzenesulfonyl pyrrolidine derivatives. Among compounds **4c-h** containing a substituted benzoyl group, the bromo- and chloro-substituted compound **4e** had more potent inhibitory activity. Chloro- or methoxy- substitution at the *ortho* position of the benzoyl group results in no marked differences in inhibitory activity, but the *p*-chloro-substituted compound **4h** displayed more potent inhibitory activity. Among the synthesized compounds, compound **4i** containing a 3-pyridine-sulfonamide group displayed the most potent activity against MMP-2 and MMP-9. This result might be due to the contribution of electronegative nitrogen atoms, which can more easily form hydrogen bonds with the enzyme backbone, thus stabilizing the bond between the compound and the enzyme. However, results indicated that the anti-proliferative and anti-migration activity of **4i** was not superior to that of **4e**. Linking the enzyme-inhibiting activity of MMPs with their cellular potency is difficult because several other variables should be considered, such as cellular membrane permeability, metabolic stability, subcellular localization, and cellular xenobiotic export. There is also a possibility that compound **4e** has other targets involved in cell proliferation and migration, so further study is warranted.

In conclusion, the current study synthesized a series of 4-phenoxybenzenesulfonyl pyrrolidine derivatives as MMP-2/MMP-9 inhibitors. Representative compound **4e** inhibited the proliferation, migration, angiogenesis, and metastasis of cancer cells. These results indicate that **4e** is a promising candidate for further structural optimization and evaluation to develop MMP inhibitors in the future.

Acknowledgements

This work was supported by the Natural Science Foundation of Shandong Province (Grant No. ZR2019QH015), the National Natural Science Foundation of China (Grant No. 80813368), and the Higher Educational Science and Technology Program of Shandong Province (Grant No. J17KB098).

References

1. Jabłońska-Trypu A, Matejczyk M, Rosochacki SA. Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs. J Enzyme Inhib Med Chem. 2016; 31:177-183.
2. Scherer RL, McIntyre JO, Matrisian LM. Imaging matrix metalloproteinases in cancer. Cancer Metastasis Rev. 2008; 27:679-690.
3. Bäck M, Ketelhuth DF, Agewall S. Matrix metalloproteinases in atherothrombosis. Prog Cardiovasc Dis. 2010; 52:410-428.
4. Hu J, Van den Steen PE, Sang QX, Opdenakker G. Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases. Nat Rev Drug Discov. 2007; 6:480-498.
5. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer. 2002; 2:161-174.
6. Overall CM, Kleifeld O. Tumour microenvironment-opinion: Validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. Nat Rev Cancer. 2006; 6:227-239.
7. Rajeshwar PV, Corwin H. Matrix metalloproteinases (MMPs): Chemical-biological functions and (Q)SARs. Bioorg Med Chem. 2007; 15:2223-2268.
8. Tu GG, Xu WF, Huang HM, Li SH. Progress in the development of matrix metalloproteinase inhibitors. Curr Med Chem. 2008; 15:1388-1395.
9. Li YL, Xu WF. Design, synthesis, and activity of caffeoyl pyrrolidine derivatives as potential gelatinase inhibitors. Bioorg Med Chem. 2004; 12:5171-5180.
10. Cheng XC, Wang Q, Fang H, Tang W, Xu WF. Design, synthesis and evaluation of novel sulfonyl pyrrolidine derivatives as matrix metalloproteinase inhibitors. Bioorg Med Chem. 2008; 16:5398-5404.
11. Li YG, Zhang J, Xu WF, Zhu HW, Li X. Novel matrix metalloproteinase inhibitors derived from quinoxalinone scaffold (Part I). Bioorg Med Chem. 2010; 18:1516-1525.
12. Zhang J, Li XY, Jiang YQ, Feng JH, Li XG, Zhang YJ, Xu WF. Design, synthesis and preliminary evaluation of α-sulfonyl γ-(glycinyl-amino) proline peptidomimetics as matrix metalloproteinase inhibitors. Bioorg Med Chem. 2014; 22:3055-3064.
13. Zhang H, Li X, Wang XJ, Xu WF, Zhang J. Sulfonyl phosphonic 1,4-dithia-7-azaspiro[4,4]nonane derivatives as matrix metalloproteinase inhibitors: Synthesis, docking study and biological evaluation. Drug Discov Ther. 2017; 11:118-125.
14. Qu XJ, Yuan YX, Xu W.F, Chen MH, Cui SX, Meng H, Li YL, Makuuchi M, Nakata M, Tang W. Caffeoyl

pyrrolidine derivative LY52 inhibits tumor invasion and metastasis *via* suppression of matrix metalloproteinase activity. Anticancer Res. 2006; 26:3573-3578.
15. Cheng XC, Wang Q, Fang H, Xu WF. Roles of sulfonamide group in matris metalloproteinase inhibitors. Curr Med Chem. 2008; 15:368-373.
16. Jiang YQ, Li XY, Hou JN, Huang YX, Wang XJ, Jia YP, Wang QW, Xu WF, Zhang J, Zhang YJ. Synthesis and biological characterization of ubenimex-fluorouracil conjugates for anti-cancer therapy. Eur J Med Chem. 2018; 143:334-347.

Received February 20, 2020; Revised April 26, 2020; Accepted April 27, 2020.

**Address correspondence to:*
Jian Zhang and Jinbao Tang, Department of Medical Chemistry, School of Pharmacy, Weifang Medical University, 7166 West Baotong Road, Weifang, Shandong, China 261053.
E-mail: zhangjian_3323@163.com (JZ), tangjinbao2002@126.com (JT)

Released online in J-STAGE as advance publication May 9, 2020.

Brief Report

DOI: 10.5582/bst.2020.03024

A breathing movement sensor for chest radiography during inspiration in children aged less than 3 years: a prospective randomized controlled study

Mari Honda[1,§], Rei Haruyama[1,§], Yasuo Sugiura[1,2,*], Kaori Ohara[1], Kazuya Mochigi[3], Yuzuru Kono[1,3], Hiroyuki Shichino[4], Hideko Uryu[4], Tetsuya Mizoue[5], Terumi Marutani[6], Takashi Ebihara[6], Fumiya Uchiyama[7], Satoshi Makise[7], Hidechika Akashi[1]

[1] Bureau of International Health Cooperation, National Center for Global Health and Medicine, Tokyo, Japan;
[2] International Health Care Center, National Center for Global Health and Medicine, Tokyo, Japan;
[3] Department of Radiological Physics and Technology, National Center for Global Health and Medicine, Tokyo, Japan;
[4] Department of Pediatrics, National Center for Global Health and Medicine, Tokyo, Japan;
[5] Department of Epidemiology and Prevention, Center for Clinical Sciences, National Center for Global Health and Medicine, Tokyo, Japan;
[6] Department of Clinical Trial Management, Center for Clinical Sciences, National Center for Global Health and Medicine, Tokyo, Japan;
[7] Department of Radiology, National Center for Global Health and Medicine, Tokyo, Japan.

SUMMARY Chest radiographs should be obtained at the peak of inspiration so that radiological findings can be precisely interpreted. However, this is not easily achieved, particularly in young children who do not follow the instruction to hold their breath. We developed a sensor that detects the breathing movements and conducted a randomized controlled study to determine whether the sensor would increase the proportion of chest radiographs obtained in the inspiration phase. We recruited 124 infants and children aged less than 3 years, who visited the pediatric department of a general hospital in Tokyo, Japan, and allocated them into one of two groups: with-sensor and without-sensor groups. Overall, 81% of all images were obtained during inspiration. The proportion of chest radiographs taken during inspiration was not statistically different between the two groups (81% *vs.* 82%). In the with-sensor group, radiologic technologists were able to obtain chest radiographs of the same quality while not observing the chest movement, but the sensor. The use of the sensor did not increase the proportion of chest radiographs taken in the inspiration phase in this study. However, this null result may indicate the possibility of utilizing the sensor for automatizing chest radiography in the future.

Keywords chest radiographs, device, respiratory

1. Introduction

Since Wilhelm Conrad Röntgen discovered the X-rays in 1859, chest radiographs have been one of the most commonly used diagnostic tools in medicine, both in adults and children (*1*). A chest radiograph produces images of the body structures in that area, such as the airways, lungs, heart, blood vessels, diaphragm, and the bones of the spine and chest (*2*), and provides support for the diagnosis of various diseases, including pneumonia, lung and mediastinal tumors, and heart failure.

In order to interpret chest radiographs, standardized definitions of radiological findings are required as well as appropriate training to utilize the definitions (*3*). However, the quality of radiographic images is crucial to precisely interpret the radiological findings. The quality of chest radiographs is determined by several factors, such as deep inspiration, proper positioning, inclusion of all areas of interest, and absence of blurring or external objects overlying the lungs. "Performed at peak of inspiration, except for suspected foreign body aspiration" is one of the quality criteria for proper diagnosis in children (*4*). In adults, 10 posterior ribs should be visible above the diaphragm when chest radiographs are obtained at peak of inspiration, according to the World Health Organization manual of diagnostic imaging (*5*). The criteria for young children and infants are different because of their smaller body size and different body composition. The optimal inspiration depth is demonstrated by eight visible posterior ribs in children younger than 3 years and nine posterior ribs in children between 3 and 7 years old (*6*).

To take appropriate chest radiographs in the

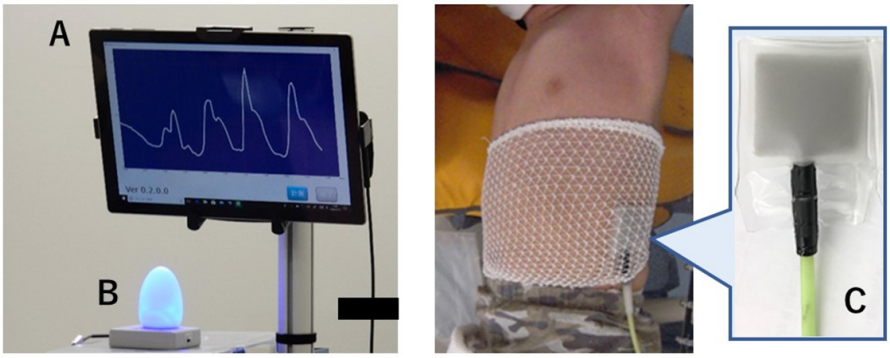


Figure 1. Images of the sensor device. (A) A monitor that shows the breathing waveforms; **(B)** An egg-shaped lamp indicating the inspiration phase; **(C)** An air pad detecting subtle body movement.

inspiration phase, patients are asked to stay still, take a deep breath, and then hold it for a few seconds. However, young children and infants are usually not able to hold their breath on command. Therefore, radiologic technologists judge the timing of the peak of inspiration by visually observing their thoracic movements. The high respiratory rates in children make it difficult to determine the appropriate timing. Furthermore, they usually do not cooperate and act up under unfamiliar circumstances. Few studies have analyzed whether chest radiographs were taken in the inspiration or the expiration phase. Tschauner *et al.* reported that only 49% of pediatric chest radiographs were obtained at the peak of inspiration (*6*).

Recent efforts to address this problem include training radiologic technologists and using assisting devices. Langen *et al.* developed a training program for radiologic technologists using video clips on how to obtain an infant's chest radiographs at the time of the deepest inspiration (*7*). After the simulation training with three infants in the video clips, radiologic technologists were more successful in performing chest radiography at the peak of inspiration, although the degree of improvement varied among individuals. Various types of body immobilization devices are also commonly used to reduce body movement and keep the individual in an appropriate position (*8*). However, to the best of our knowledge, there is no device that assists radiologic technologists to judge the timing of the peak of inspiration.

We developed and evaluated a sensor that detects the breathing movements in infants and young children aged less than 3 years. We hypothesized that the use of this sensor would increase the proportion of pediatric chest radiographs obtained during the inspiration phase.

2. Materials and Methods

2.1. Development of the breathing movement sensor

The original sensor was designed by THE YOSHIDA DENTAL MFG. CO., LTD. (Tokyo, Japan) based on the patent JP, 5607912, B, and was used to measure vital signs, such as heart rate and breathing rate. For the purpose of our study, the sensor was modified to focus on the breathing condition. It consists of an air pad (5.5 cm × 4 cm × 0.56 cm) that detects subtle body movement by the change in air pressure, a monitor that shows breathing waveforms (*i.e.*, ascending waves during inspiration and descending waves during expiration), and an egg-shaped lamp that lights in green and beeps at the peak of inspiration (Figure 1A, 1B). The air pad was not completely radiotransparent. Therefore, to avoid interference with the chest image, the air pad was placed on the left lateral abdomen, which moves to pull down the diaphragm for breathing, and covered by an elastic mesh bandage (Presnet® ALCARE Co., Ltd. Tokyo, Japan) (Figure 1C). We confirmed that the breathing waveforms produced by the air pad were consistent with those produced by CHESTAC-8900 (CHEST M.I., INC. Tokyo, Japan) in an adult test case, simultaneously using the air pad and the spirometer.

2.2. Randomized controlled study

We evaluated the effectiveness of this sensor by conducting a randomized controlled study to compare the proportion of chest radiographs obtained during the inspiration phase, with and without the sensor, among infants and young children aged less than 3 years. We conducted a pilot study of 10 patients to evaluate the feasibility before the main study.

2.2.1. Study population

We recruited children aged less than 3 years who visited the pediatric outpatient department during the regular working hours and were indicated to undergo chest radiography for clinical reasons by pediatricians at the Center Hospital of the National Center for Global Health and Medicine (NCGM), Tokyo, Japan, between

September 2018 and October 2019. Children with life-threatening conditions, severe chest wall malformation, and foreign body aspiration were excluded.

2.2.2. Sample size calculation

To identify the sample size for this study, we retrospectively assessed the pediatric chest radiographs taken at the hospital in May 2017. We found that 18 of the 59 radiographs (31%) were not taken during the inspiration phase (*i.e.* eight posterior ribs were visible above the diaphragm). We expected that by using the sensor this percentage would be reduced to 20%. With an 80% power, at the 5% significance level, the estimated sample size was calculated to be 124 (62 per group).

2.2.3. Randomization

We randomized participants by restricted randomization using random permuted blocks (*9*). The block size was two for 1:1 randomization to the intervention and control groups from numbers 1 to 124. We allocated 62 "intervention" cards and 62 "control" cards in 124 non-transparent envelopes according to the randomization results. The envelopes were sealed and numbered from 1 to 124, and each contained a card showing either "intervention" or "control". These envelopes were saved at the radiology department and were not opened until the participants arrived at the department. When a participant would come to the department for imaging ordered by a pediatrician, a radiologic technologist opened one envelope in sequential order. The technologist used the sensor when there was an "intervention" card in the envelope. There was no crossover between the pediatricians who ordered the chest radiographs, researchers who conducted the randomization assignment, and radiologic technologists who opened the envelopes.

2.2.4. Chest radiography

Chest radiography was performed by certified radiologic technologists at the radiology department of the NCGM. Since the department operates in shifts, every technologist in the department was involved in the study. Children were separated from their caregivers in the radiographing room. The technologist decided to obtain chest radiographs in the posterior-anterior or supine anterior-posterior position based on the child's age and condition. Immobilization devices were mostly used when radiographs were taken in the posterior-anterior position. After taking the radiograph, technologists recorded which modality they used to judge the imaging timing (*i.e.* breathing movement, waveform, light, sound, or a combination of these). One or two researchers attended almost all imaging procedures to observe the whole procedure.

2.2.5. Interpretation of chest radiographs

In this study, the inspiration phase in children younger than 3 years was defined by the presence of eight posterior ribs visible above the diaphragm. The two trained radiologists, with more than three years of experience, were assigned for interpreting the chest radiographs.

After 124 participants had undergone chest radiography, the two radiologists at the NCGM independently interpreted the radiographs and identified whether they were taken in the inspiration phase. Any discordance between the two readers was resolved by discussion to reach an agreement.

2.2.6. Statistical analysis

Data analysis was conducted using Stata 14 (Stata corporation, College Station, TX). The chi-squared test and Fisher's exact test were used to assess the correlation between sensor use and radiography in the inspiration phase. Stratified analyses were conducted by age and presence of cry.

2.3. Ethical approval

This study was approved by the Ethical Committee of the National Center for Global Health and Medicine (approval ID: NCGM-G-002310-00). Written informed consent was obtained from the caregivers of all participants.

3. Results and Discussion

A total of 125 children were recruited between September 20, 2018, and October 8, 2019. One child was excluded due to inadequate informed consent. Thus, 124 children were included in the analysis.

Table 1 shows the general characteristics of the study participants. The average age was 15.3 (0-32) months, with about half being 1 year old. Eighty-one percent of the participants were crying during the imaging. There were 61 participants in the without-sensor group and 63 in the with-sensor group.

Table 1. General characteristics of the study participants

	Without sensor	With sensor	Total
Age groups (y)			
0-1	21 (34%)	22 (35%)	43 (35%)
1-2	29 (48%)	31 (49%)	60 (48%)
2-3	11 (18%)	10 (16%)	21 (17%)
Crying			
Yes	51 (84%)	50 (79%)	101 (81%)
No	10 (16%)	13 (11%)	23 (19%)
	61	63	124

Table 2. Results of chest radiographs with and without sensor

	Without sensor	With sensor	Total	*p*
				0.9*
Expiration	11 (18%)	12 (19%)	23 (19%)	
Inspiration	50 (82%)	51 (81%)	101 (81%)	
	61	63	124	

*determined by chi-square test

Table 3. Result comparison between non-crying and crying participants

	Without sensor	With sensor	Total	*p*
No cry				1.0**
Expiration	4 (40%)	5 (38%)	9 (39%)	
Inspiration	6 (60%)	8 (62%)	14 (61%)	
Cry				1.0**
Expiration	7 (14%)	7 (14%)	14 (14%)	
Inspiration	44 (86%)	43 (86%)	87 (86%)	

**determined by Fisher's exact test

The proportion of radiographs taken in the inspiration phase was similar between the intervention (with-sensor) and control (without-sensor) groups (81% *vs.* 82%, $p = 0.9$; Table 2).

In the intervention group, the imaging timing was judged according to the waveform on the monitor in 31 cases (49%), the light signal of the egg-shaped lamp in 5 cases (8%), the chest movement of the study participants in 21 cases (33%), by the combination of light and waveform or sound and waveform in 3 cases (5%), and unknown in 3 cases (5%). After excluding the 21 radiographs taken by looking at the chest movement and 3 unknown cases, 87% (34/39) were obtained in the inspiration phase that examined the sole effect of the sensor.

When we stratified by age, we found that the proportion of images taken in the inspiration phase was much smaller among children 2-3 years old (7/21, 33%) than in those 0-1 (37/43, 86%) and 1-2 years old (57/60, 95%). However, there was no significant difference in the proportion of images taken in the inspiration phase between the intervention and control groups in all three age groups (Fisher's exact test: $p = 0.7$, $p = 1.0$, $p = 0.7$, respectively). When participants were not crying during imaging, only 61% (14/23) of the radiographs were obtained in the inspiration phase. However, the proportion of chest radiographs obtained in the inspiration phase was similar regardless of the sensor use (Table 3).

The quality of chest radiographs largely depends on the skills of technologists. This is particularly true for pediatric chest radiographs because the timing of the inspiration phase exclusively relies on the eyes of the technologist. To the best of our knowledge, this is the first attempt to develop and evaluate a sensor that detects the breathing movements of infants and young children aged less than 3 years to judge the imaging timing in the inspiration phase on chest radiography.

Contrary to our hypothesis, our study showed no statistical difference in the proportion of radiographs taken in the inspiration phase according to the sensor use. Moreover, the overall proportion of chest radiographs obtained in the inspiration phase in our study (81%) was higher than that in our pilot study (69%). This proportion is also higher than that reported in a previous study (49%) (*6*). One of the reasons might be observer bias (*10*). One or two researchers attended almost all imaging procedures during the study to observe the procedure and this might have affected the technologists' behavior. Additionally, in our pilot study, we analyzed all chest radiographs, including those obtained in the emergency department during non-working hours, which may have had lower quality than those taken at the radiology department during regular working hours. At the emergency department in our hospital, there is less staff and no immobilization device for pediatric chest radiography. Without the device, radiologic technologists usually have to hold infants and young children to maintain their good position during the procedure, which may distract them from optimizing the imaging timing.

Although the proportion of chest radiographs obtained in the inspiration phase in the overall results was high, the participants who did not cry and those in the 2-3 years age group was relatively low. Radiologic technologists often had difficulty in detecting chest movement in non-crying children. Moreover, elder children might be difficult to keep in the required position during the procedure. The immobilization device in our hospital could be used for children with body weight up to 15 kg, but some children in our study did not allow us to use it. Unfortunately, the sample size of non-crying participants and also those in the 2-3 years age group were both not sufficient to conduct sub-analyses in this study. In a future study, it might be intriguing to target non-crying children or recruit adults having difficulties communicating and/or following directions.

Among the 39 chest radiographs obtained by observing the waveform, light, or according to the beep sound of the sensor, 34 (87%) were taken in the inspiration phase. This result indicates that this type of sensor could be utilized for automatizing chest radiography. Ideally, artificial intelligence could be used to analyze the patterns of a respiratory waveform to automatically shoot chest radiographs at the peak of inspiration. This new device may reduce the workload and occupational radiographic exposure of radiologic technologists without influencing the quality of images. Respiratory gating in computed tomography (CT), positron emission tomography/CT, or magnetic resonance imaging, in particular for radiation therapy,

have been already put into practical use (*11*). Our sensor is simpler than those systems and could, therefore, lead to a better quality of chest radiographs in a cost effective way. Moreover, in response to economic growth and the changes in social development, there has been an increasing demand for diagnostic imaging in low- and middle-income countries. Although there is often a shortage of qualified radiologic technologists, this device could be of assistance in those countries.

The study has several limitations. First, double-blinding was not feasible. The two radiologists who interpreted the chest radiographs were blinded to the participants' allocation; however, the radiologic technologists and participants recognized it because of the use of the sensor in the intervention group and non-use in the control group. We could have placed a nonfunctional air pad in the control group; however, the radiologic technologists would still not have been blinded. Second, the subgroup analysis by category or severity of the diseases was not conducted since the focus of our study was on the effect of the sensor regardless of disease or condition within our criteria. Third, our estimation of the proportion of chest radiographs obtained in the inspiration phase in the control group was lower than the actual result. Therefore, our sample size was too small to have sufficient statistical power. Finally, we could not make children stop crying during the procedure even though the crying affects the chance to obtain radiographs in the inspiration phase (*12*). In general, children cry during the radiographic examination because they feel stress, fear, or anxiety (*13*). For future research, it might be interesting to include adult participants who were not able to move and communicate with others.

In conclusion, in this study, the use of the body movement sensor did not increase the proportion of chest radiographs taken during the inspiration phase among infants and young children aged less than 3 years. However, this null result may indicate the possibility of utilizing the sensor for automatizing chest radiography in the future.

Acknowledgements

This research was supported by a grant from the National Center for Global Health and Medicine, Japan (19A2010). The sensor applied in the current research was provided by THE YOSHIDA DENTAL MFG. CO. LTD. (Tokyo, Japan) during the study period based on the research contract between the company and the National Center for Global Health and Medicine, Japan.

We thank all members of the Center Hospital of the National Center for Global Health and Medicine for their contribution to this study, as follows: Ayumi Mizukami, Junko Yamanaka, Keiji Goishi, Mizue Tanaka, Masao Kaneshige, Minsoo Lee-Yoshimoto, Mami Shimada, Satoshi Takasago, Tomohisa Akamatsu, Yuri Suzuki, Yukari Atsumi, Tomomi Ota, Haruka Aihara, Mayu Koto, Chihiro Ajimi, Yuko Tsuchida, Haruka Yamanaka, Hideki Funazaki, Yoshinobu Yajima, Futoshi Matsunaga, Azusa Minagawa, Ken Matsumoto, Satomi Yamamoto, Takumi Iwase, Daisuke Yamazaki, Daisuke Ishibashi, Ayumi Suetaka, Yoshikazu Takahashi, Tomoko Oikawa, Nao Fujiki, Shun Otsuka, Keiichi Kurihara, Kenta Hashimoto, Kento Mitani, Kotaro Kijima, Megumi Fujii, Daisuke Muramatsu, Kazuki Shinagawa, Masashi Hayano, Tsuyoshi Sakai, Hiroyuki Takano, Tsubasa Sugihara, Misato Terashima, Masaki Nagai, and Toshihiko Hashizume.

Financial Disclosures: The authors declare no conflicts of interest associated with this manuscript.

Trial Registration: UMIN-Clinical Trial Registry; UMIN000031052; January 29, 2018.

References

1. Greene R. Radiologic history exhibit. Francis H. Williams, MD: Father of chest radiology in North America. Radio Graphics. 1991; 11:325-332.
2. Puddy E, Hill C. Interpretation of the chest radiograph. Continuing Education in Anaesthesia, Critical Care, & Pain. 2007; 7:71-75.
3. Cherian T, Mulholland EK, Carlin JB, Ostensen H, Amin R, de Campo M, Greenberg D, Lagos R, Lucero M, Madhi SA, O'Brien KL, Obaro S, Steinhoff MC. Standardized interpretation of paediatric chest radiographs for the diagnosis of pneumonia in epidemiological studies. Bull World Health Organ. 2005; 83:353-359.
4. European Commission. European guidelines on quality criteria for diagnostic radiographic images in paediatrics. Luxembourg, 1996; pp. 27. *https://www.sprmn.pt/pdf/EuropeanGuidelinesEur16261.pdf* (accessed March 23, 2020).
5. Ostensen H, Pettersson H, eds. The WHO manual of diagnostic imaging. World Health Organization, 2003; chapter3, pp. 6.
6. Tschauner S, Marterer R, Gubitz M, Kalmar PI, Talakic E, Weissensteiner S, Sorantin E. European guidelines for AP/PA chest X-rays: routinely satisfiable in a paediatric radiology division? Eur Radiol. 2016; 26:495-505.
7. Langen HJ, Kohlhauser-Vollmuth C, Sengenberger C, Bielmeier J, Jocher R, Eschmann M. Performing chest x-rays at inspiration in uncooperative children: the effect of exercises with a training program for radiology technicians. Radiol Res Pract. 2014;2014:312846.
8. Kohda E, Tsutsumi Y, Nagamoto M, Gomi T, Terada H, Kawawa Y, Masaki H, Shiraga N. Revisit image control for pediatric chest radiography. Radiat Med. 2007; 25:60-64.
9. Efird J. Blocked randomization with randomly selected block sizes. Int J Environ Res Public Health. 2011; 8:15-20.
10. Mahtani K, Spencer EA, Brassey J, Heneghan C. Catalogue of bias: observer bias. BMJ Evid Based Medicine. 2018; 23:23-24.
11. Frood R, McDermott G, Scarsbrook A. Respiratory-gated PET/CT for pulmonary lesion characterisation-promises and problems. Br J Radiol. 2018; 91:20170640.
12. Frank ED, Long BW, Smith BJ. Merrill's atlas of

radiographic positioning and procedures - E-Book. Elsevier Health Sciences, 2013; pp. 118.
13. Björkman B, Golsäter M, Enskär K. Children's anxiety, pain, and distress related to the perception of care while undergoing an acute radiographic examination. J Radiol Nurs. 2014; 33:69-78.

Received March 23, 2020; Revised April 7, 2020; Accepted May 11, 2020.

[§]These authors contributed equally to this work.
**Address correspondence to:*
Yasuo Sugiura, Bureau of International Health Cooperation, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan.
E-mail: yasugiura@it.ncgm.go.jp

Released online in J-STAGE as advance publication May 17, 2020.

Communication

DOI: 10.5582/bst.2020.03069

Asymptomatic carriers of COVID-19 as a concern for disease prevention and control: more testing, more follow-up

Jiao Zhang[1], Shoucai Wu[2], Lingzhong Xu[1,3,*]

[1] School of Public Health, Cheeloo College of Medicine, Shandong University, Ji'nan, Shandong, China;
[2] Department of Geriatrics, Qilu Hospital, Cheeloo College of Medicine, Shandong Univeristy, Ji'nan, Shandong, China;
[3] NHC Key Laboratory of Health Economics and Policy Research, Cheeloo College of Medicine, Shandong University, Ji'nan, Shandong, China.

SUMMARY Following a containment phase of two months, China has transitioned to the mitigation phase. However, China still faces the risk of COVID-19 spreading due to not only to sporadic new cases and imported cases but also asymptomatic carriers. According to daily reports from the National Health Commission of the People's Republic of China from March 31, 2020 to April 7, 2020, the number of new asymptomatic cases reported daily greatly exceeded that of new imported cases. As of 24:00 on April 7, there were a total of 1,095 asymptomatic cases with COVID-19 under medical observation on the Chinese mainland, including 358 imported cases. A growing number of studies have indicated that asymptomatic carriers are infectious to an extent and can potentially transmit COVID-19. At present, China's measures for managing asymptomatic carriers are 14 days of centralized quarantine and observation; in principle, people with two consecutive negative nucleic acid tests (at an interval of at least 24 hours) can be released from quarantine. However, asymptomatic carriers will not be included in confirmed cases unless they develop clinical manifestations while in quarantine. As "silent spreaders", asymptomatic carriers warrant attention as part of disease prevention and control. The testing and follow-up of asymptomatic carriers should be expanded to include people in close contact with patients with confirmed COVID-19 and asymptomatic cases, clusters of outbreaks, and key areas and populations with a high risk of infection.

Keywords coronavirus disease 2019 (COVID-19), asymptomatic infections, China

According to a World Health Organization report, China has transitioned to the mitigation stage following a two-month containment phase (*1*). Currently, the sporadic cases of COVID-19 have appeared in China for several days, but the epidemic is still rapidly worsening in Europe and the United States. The number of confirmed imported cases of COVID-19 has exceeded that of autochthonous cases, placing pressure on patient treatment, disease control and investigation, nucleic acid detection, and quarantine facilities of cities of entry. According to daily reports from the National Health Commission of the People's Republic of China, this situation has arisen as the number of new imported cases has exceeded that of new domestic cases since March 13, 2020 (Figure 1). Although the number of imported cases is still low, there may be imported cases for a considerable period of time. China still faces the dual risk of a COVID-19 spread due to sporadic new cases and imported cases. At present, most regions in China are focusing on the prevention and control of imported cases from overseas, and all overseas arrivals must be quarantined in designated places for 14 days. However, asymptomatic carriers are "silent spreaders" and also warrant attention in terms of disease prevention to contain the epidemic.

On February 5, 2020, the "Diagnosis and Treatment Protocol for COVID-19 (Fifth ed., Trial)" was released by the National Health Commission of the People's Republic of China, and asymptomatic infection was first included as a source of infection (*2*). Recently, asymptomatic carriers have been detected in Liaoning, Zhejiang, Shandong, and other provinces of China, and suspected asymptomatic carriers have even appeared in some areas. The existence of asymptomatic infections is one feature that distinguishes the COVID-19 epidemic from the SARS epidemic in 2003. Moreover, a study by Chu *et al*. (*3*) found that SARSCoV-2 replicated more efficiently but induced significantly less of a host interferon and proinflammatory response than SARS-CoV, which suggests that SARS-CoV-2 is transmitted person-to-person in a highly efficient manner and that it frequently causes asymptomatic infections. Some of the first detailed

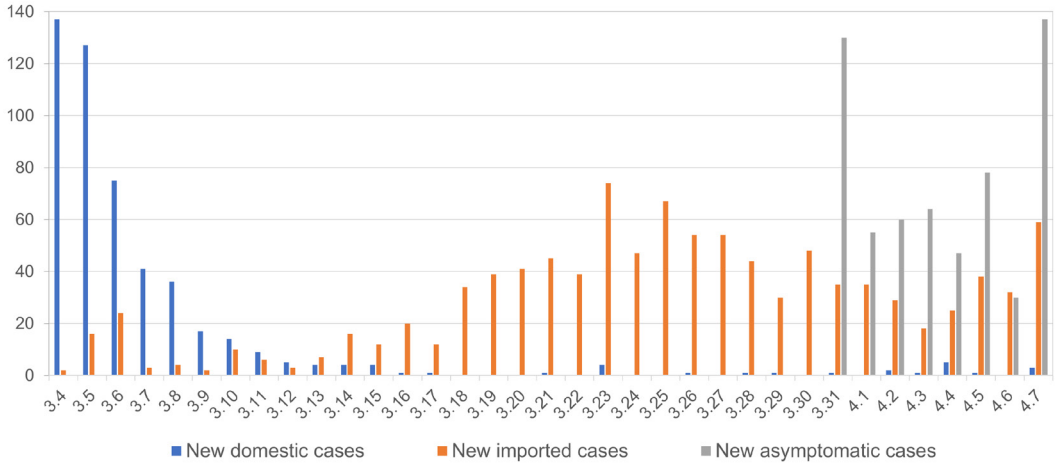


Figure 1. New COVID-19 cases in China from March 4, 2020 to April 7, 2020. Data source: National Health Commissionof the People's Republic of China , *http://www.nhc.gov.cn/xcs/yqtb/list_gzbd.shtml*

estimates suggested that covert cases of COVID-19 could represent some 60% of all infections (*4*).

Furthermore, asymptomatic carriers may be highly infective during the incubation period. A recent study found that the viral load detected in asymptomatic carriers was similar to that in symptomatic patients, which suggests the transmissible potential of asymptomatic or minimally symptomatic patients (*5*). Scientists with the Ningbo Center for Disease Control and Prevention in East China's Zhejiang Province recently found that 6.3% of the close contacts of patients with confirmed COVID-19 were ultimately infected with the virus; 4.4% of the close contacts of asymptomatic carriers were ultimately infected (*6*). Another recent study involving 24 cases found that asymptomatic carriers have a certain level of infectivity and that the period of viral infection can be up to 29 days (*7*). The researchers in that study pointed out the importance of identifying and isolating asymptomatic carriers and patients with mild symptoms in order to contain the epidemic (*7*).

The National Health Commission of the People's Republic of China has included asymptomatic cases in its daily report since April 1 (*8*). From March 31 to April 7, the number of new asymptomatic cases reported daily greatly exceeded that of new imported cases (Figure 1). As of 24:00 on April 7, 2020, there were 1,095 asymptomatic carriers under medical observation on the Chinese mainland, including 358 imported cases (*9*). At present, China's measures for managing asymptomatic carries are 14 days of centralized quarantine and observation; in principle, people with two consecutive negative nucleic acid tests (at an interval of at least 24 hours) can be released from quarantine. However, asymptomatic carriers will not be included in confirmed cases unless they develop clinical manifestations while in quarantine (*10*).

As "silent spreaders", asymptomatic carriers warrant attention as part of disease prevention and control; more efforts should be made to monitor, track, quarantine, and treat asymptomatic carriers. The testing and follow-up of asymptomatic carriers should be expanded to include people in close contact with patients with confirmed COVID-19 and asymptomatic cases, clusters of outbreaks, and key areas and populations with a high risk of infection. Once asymptomatic carriers are identified, an epidemiological investigation should be promptly conducted, the sources of infection for those cases should be determined as early as possible, and relevant information should be published publicly and openly.

References

1. WHO. Coronavirus disease 2019 (COVID-19) Situation Report – 76 2020. *https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200405-sitrep-76-covid-19.pdf?sfvrsn=6ecf0977_2* (accessed April 6, 2020).
2. National Health Commission of the People's Republic of China . Notice on the issuance and dissemination of the Diagnosis and Treatment Protocol for COVID-19 (Fifth ed., draft) 2020. *http://www.nhc.gov.cn/yzygj/s7653p/202002/3b09b894ac9b4204a79db5b8912d4440.shtml* (accessed April 8, 2020). (in Chinese)
3. Chu H, Chan JF-W, Wang Y, *et al*. Comparative replication and immune activation profiles of SARS-CoV-2 and SARS-CoV in human lungs: An ex vivo study with implications for the pathogenesis of COVID-19. Clin Infect Dis. 2020; doi: 10.1093/cid/ciaa410.
4. Qiu J. Covert coronavirus infections could be seeding new outbreaks. Nature 2020; doi: 10.1038/d41586-020-00822-x.
5. Zou L, Ruan F, Huang M, *et al*. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med. 2020; 382:1177-1179.
6. Chen Y, Wang A, Yi b, Ding KQ, Wang HB, Wang JM, Shi HB, Wang SJ, Xu GZ. The epidemiological characteristics of infection in close contacts of COVID-19 in Ningbo City. Chinese Journal of Epidemiology. 2020; 41:668-672. (in Chinese)
7. Hu Z, Song C, Xu C, Jin G, Chen Y, Xu X, Ma H, Chen W,

Lin Y, Zheng Y, Wang J, Hu Z, Yi Y, Shen H. Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China. Sci China Life Sci. 2020; doi: 10.1007/s11427-020-1661-4.
8. State Council of China. April 1, 2020. Press Conference of the State Council's Organization for COVID-19 Prevention and Control. *http://www.gov.cn/xinwen/gwylflkjz77/index.htm* (accessed April 4, 2020). (in Chinese)
9. National Health Commissionof the People's Republic of China. The latest updates on COVID-19 outbreaks as of 24:00 on April 7. *http://www.nhc.gov.cn/xcs/yqtb/202004/5e2b6f0bd47d48559582242e3878447d.shtml* (accessed April 18, 2020). (in Chinese)
10. People's Governmentof the People's Republic of China. The State Council's Organization for COVID-19 Prevention and Control: Notice on issuance and dissemination of Practices for Management of Asymptomatic Carriers with COVID-19. *http://www.gov.cn/zhengce/content/2020-04/08/content_5500371.htm* (accessed April 8, 2020). (in Chinese)

Received April 9, 2020; Revised April 14, 2020; Accepted April 20, 2020.

Address correspondence to:
Lingzhong Xu, Department of Social Medicine and Maternal & Child Health, School of Public Health, Shandong University, No. 44 Wenhua Xi Road, Ji'nan 250012, China.
E-mail: lzxu@sdu.edu.cn

Released online in J-STAGE as advance publication April 22, 2020.

Communication

DOI: 10.5582/bst.2020.03154

Does immune privilege result in recovered patients testing positive for COVID-19 again?

Jufeng Xia (Shunichi Arai)[1,2,*]

[1] Graduate School of Frontier Science, The University of Tokyo, Tokyo, Japan;
[2] Department of Immunology, Arai Japan Medical Institute, Tokyo, Japan.

SUMMARY Recently, an increasing number of reports have indicated that a few patients who were believed to have recovered from COVID-19 initially tested negative but later tested positive. Several hospitals in different countries have detected SARS-CoV-2 RNA in the semen and cerebrospinal fluid of patients with severe COVID-19. Given the fact that the testes and central nervous system are both immune privilege sites and the fact that Ebola virus and Zika virus can avoid immune clearance and continue proliferating and spreading by hiding in those sites, the question of whether SARS-CoV-2 is present in immune privilege sites, it attacks those sites, and it spreads again after proliferating in those sites needs to be investigated.

Keywords SARS-CoV-2, COVID-19, immune privilege, re-detectable positive, semen, CNS

Since December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused nearly 4 million cases of coronavirus disease 2019 (COVID-19) worldwide, including over 274,000 deaths, as of 11 May 2020 (*1*). Generally, COVID-19 is less severe and less fatal than SARS, but some patients, and especially those who are elderly with co-morbidities, are prone to develop more severe symptoms and require emergent medical interventions (*2*). An increasing number of patients with COVID-19 have been discharged and undergone regular follow-up and observation. Studies have reported that some recovered patients have test positive again for SARS-CoV-2 RNA (*3*-*5*). There may be two reasons why patients test positive again for SARS-CoV-2 RNA: first, the patients were re-infected by another carrier of the virus; second, the virus was not completely cleared and instead reappeared in recovered patients. Since there is little evidence that recovered patients were re-infected, Vineet Menachery, a virologist at the University of Texas Medical Branch, said testing positive after recovery could simply mean that the test result was a false negative and that the patient was still infected (*6*). The leads to the question of whether the virus is still in the bodies of patients and, if so, where it is hiding. After considering a number of possibilities, the current authors hit upon immune privilege.

Certain sites in the human body have immune privilege, meaning that they are able to tolerate the introduction of antigens without eliciting an inflammatory immune response. Immunologically privileged sites include: the eyes, the placenta and fetus, the testicles, the central nervous system (CNS), and the anagen hair follicles (*7*) (Figure 1). Immune privilege is thought to be an evolutionary adaptation to protect vital structures from the potentially damaging effects of an inflammatory immune response. Inflammation in the brain or eye can lead to loss of organ function, while immune responses directed against a fetus can lead to a miscarriage. Thus, these are niches where viruses may be protected from the host immune response. Immunoprivileged sites gained attention as places where viruses can persist after disease recovery during the 2013-16 West African Ebola virus outbreak (*8*). In addition, Zika virus can also be detected in the semen of recovered patients (*9*). There are reports of patients who had these viruses in their semen months after they were originally infected even though the viruses had been cleared from elsewhere in their bodies. Ebola virus was still detectable in the semen of some survivors for more than three years, and Ebola virus transmission through sexual intercourse can occur months after the patient has recovered. In one of the most extreme cases, Zika virus was detected in the semen of a man in Italy at least 134 days after symptoms of the disease (mainly a fever) first emerged, even though blood and saliva samples revealed no trace of the virus. A semen test on day 188 still indicated the presence of virus (his blood and saliva were not tested). The testes seem to be a particularly understudied area among immune-privileged sites, which also include the eye and the

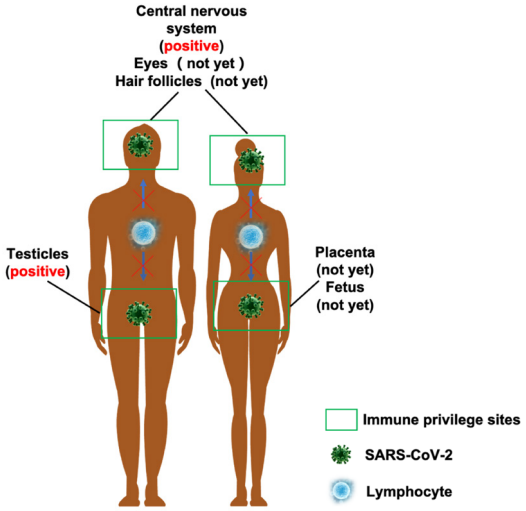


Figure 1. SARS-CoV-2 could hide in immune privilege sites to avoid immune clearance. To date, SARS-CoV-2 has been detected in the central nervous system and semen but not in the eyes, hair follicles, placenta, or fetus.

brain (*10*). Coincidentally, SARS-CoV-2 shares some characteristic with those viruses.

A study of 38 patients undergoing treatment for severe COVID-19 at a Chinese People's Liberation Army General Hospital looked for SARS-CoV-2 in their semen (*11*). Fifteen of the patients provided a semen sample during the acute phase of their illness and 23 shortly after recovering. SARS-CoV-2 RNA was found in semen samples from 4 of the 15 patients with acute disease and 2 of the 23 recovering patients. These new findings differ from the results of an earlier study involving 12 COVID-19 patients and a case report which suggested that semen of infected patients tested negative (*12,13*). However, the earlier studies focused on patients with mild disease after they had recovered, whereas the more recent study focused on hospitalized patients with severe disease, and all samples in this latest study were collected while symptoms were evident or very shortly after recovery. In fact, all of the semen samples from recovering patients that were found to have viral RNA were collected 2 or 3 days after recovery. Thus, different findings from the earlier studies and the more recent one are probably the result of differences in disease severity and the timing of sample collection. Other studies found that infected patients had neurologic manifestations (*e.g.* cerebrovascular disease and impaired consciousness) and skeletal muscle injury, and SARS-CoV-2 RNA was even detected in the cerebrospinal fluid of an infected patient (*13,14*).

However, the presence of viral RNA in immunologically privileged sites in patients does not necessarily mean that an infectious virus is present. Thus, what must be determined is whether infectious viruses can also be isolated from immunologically privileged sites in COVID-19 patients and survivors. Isolation of SARS-CoV-2 in those sites would lead to 3 questions: first, will the virus attack eyes, CNS, testes, or a fetus; second, will SARS-CoV-2 spread through sexual transmission or pregnancy; and third, can detection of SARS-CoV-2 in immunologically privileged sites serve as a prognostic marker or is it a necessary indicator for recovered patients, and especially those who with severe disease. In the future, more studies need to investigate whether immunologically privileged sites play an important role in SARS-CoV-2infection and the recurrence of COVID-19. In the meantime, the sensible move would be for patients recovering from COVID-19 to remain in self-isolation until further research determines how long the virus remains in immunologically privileged sites.

References

1. Coronavirus disease (COVID-19) outbreak situation. *https://www.who.int/emergencies/diseases/novel-coronavirus-2019* (accessed May 11, 2020).
2. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, *et al*. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020; 382:1708-1720.
3. Lan L, Xu D, Ye G, Xia C, Wang S, Li Y, Xu H. Positive RT-PCR test results in patients recovered from COVID-19. JAMA. 2020; doi: 10.1001/jama.2020.2783.
4. Ling Y, Xu SB, Lin YX, Tian D, Zhu ZQ, Dai FH, Wu F, Song ZG, Huang W, Chen J, Hu BJ, Wang S, Mao EQ, Zhu L, Zhang WH, *et al*. Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients. Chin Med J (Engl). 2020; 133:1039-1043.
5. Zhang B, Liu S, Dong Y, Zhang L, Zhong Q, Zou Y, Zhang S. Positive rectal swabs in young patients recovered from coronavirus disease 2019 (COVID-19). J Infect. 2020; pii: S0163-4453(20)30233-4.
6. Can You be Re-infected after Recovering from Coronavirus? Here's what we know about COVID-19 immunity. *https://time.com/5810454/coronavirus-immunity-reinfection/* (accessed April 3, 2020).
7. Rajabi F, Drake LA, Senna MM, Rezaei N. Alopecia areata: a review of disease pathogenesis. Br J Dermatol. 2018; 179:1033-1048.
8. Uyeki TM, Erickson BR, Brown S, McElroy AK, Cannon D, Gibbons A, Sealy T, Kainulainen MH, Schuh AJ, Kraft CS, Mehta AK, Lyon GM 3rd, Varkey JB, Ribner BS, Ellison RT, *et al*. Ebola virus persistence in semen of male survivors. Clin Infect Dis. 2016; 62:1552-1555.
9. Purpura LJ, Choi MJ, Rollin PE. Zika virus in semen: Lessons from Ebola. Lancet Infect Dis. 2016; 16:1107-1108.
10. Nicastri E, Castilletti C, Liuzzi G, Iannetta M, Capobianchi MR, Ippolito G. Persistent detection of Zika virus RNA in semen for six months after symptom onset in a traveller returning from Haiti to Italy, February 2016. Euro Surveill. 2016; 21.
11. Li D, Jin M, Bao P, Zhao W, Zhang S. Clinical characteristics and results of semen tests among men with coronavirus disease 2019. JAMA Netw Open. 2020; 3:e208292.
12. Song C, Wang Y, Li W, Hu B, Chen G, Xia P, Wang W, Li C, Diao F, Hu Z, Yang X, Yao B, Liu Y. Absence of

2019 novel coronavirus in semen and testes of COVID-19 patients. Biol Reprod. 2020; pii: ioaa050.
13. Paoli D, Pallotti F, Colangelo S, Basilico F, Mazzuti L, Turriziani O, Antonelli G, Lenzi A, Lombardo F. Study of SARS-CoV-2 in semen and urine samples of a volunteer with positive naso-pharyngeal swab. J Endocrinol Invest. 2020; doi: 10.1007/s40618-020-01261-1.
14. Xiang P, Xu XM, Gao LL, Wang HZ, Xiong HF, Li RH. First case of 2019 novel coronavirus disease with encephalitis. ChinaXiv. 2020: T202003, 00015.

Received May 9, 2020; Revised May 14, 2020; Accepted May 15, 2020.

*Address correspondence to:
Jufeng Xia, Graduate School of Frontier Science, The University of Tokyo, Tokyo, Japan.
E-mail: xia-tky@umin.ac.jp

Released online in J-STAGE as advance publication May 16, 2020.

Communication

DOI: 10.5582/bst.2020.01110

Mobile field hospitals, an effective way of dealing with COVID-19 in China: sharing our experience

Zhi Chen[1,§], Shan He[2,§], Fan Li[3], Junxiang Yin[4], Xiaoliang Chen[4,*]

[1] Critical Care Medicine, Jiangxi Provincial People's Hospital Affiliated to Nanchang University, Nanchang, China;
[2] Pediatrics, The First People's Hospital of Yunnan Province, Kunming, China;
[3] Pulmonary and Critical Care Medicine, Jiangxi Provincial People's Hospital Affiliated to Nanchang University, Nanchang, China;
[4] Hepatobiliary Surgery, Jiangxi Provincial People's Hospital Affiliated to Nanchang University, Nanchang, China.

SUMMARY During the COVID-19 outbreak, China made great progress in controlling the epidemic, and the number of confirmed and suspected cases continues to decrease thanks to the various efforts employed. Mobile field hospitals have played a huge role in the centralized management of patients and they have effectively reduced transmission. This article describes some of our experiences operating mobile field hospitals in order to provide a reference and to better inform countries that are dealing with this crisis.

Keywords coronavirus, epidemic, COVID-19, mobile field hospital, patient management

1. Introduction

A novel coronavirus has caused severe pneumonia in Wuhan, China since December 2019. In order to better describe the global spread of this severe respiratory infection (SARI), the condition was designated COVID-19 (*1*) by the World Health Organization on February 12, 2020. Thus far, COVID-19 has infected more than 120,000 people and led to more than 3,500 deaths globally (*2,3*), and those numbers are still increasing.

China was the first place affected by this disease, and Chinese medical personnel and the local government were initially caught off guard and overwhelmed by this sudden event (*4*). In Wuhan, a labor shortage and a lack of medical facilities presented major obstacles. In response, numerous medical personnel from the rest of China were dispatched; mobile field hospitals also arrived to build temporary hospitals, 2 for patients in critical condition and 15 for patients with mild symptoms. During the outbreak, mobile field hospitals played a great role in epidemic control. This article describes some of our experience operating mobile field hospitals in the City of Wuhan, China.

2. The configuration of a mobile field hospital

2.1. The general characteristics of a mobile hospital

Mobile field hospitals were located at sites with good ventilation, abundant space, and convenient patient transport. The medical staff consisted of a medical team from a regional hospital, two emergency medical teams from the national government, one local medical assistance team, and one biosafety level 3 (BLS-3 or P3) mobile laboratory (Figure 1A). There were approximately 1,000 medical personnel and 1,000-1,500 beds in each mobile field hospital (*5*).

In terms of their specialties, personnel comprised four teams that were respectively in charge of managing information, providing medical care, controlling nosocomial infections, and managing logistics.

The information management group is responsible for formulating plans, deciding work flows, dividing and coordinating work, gathering, processing, announcing, and reporting information, coordinating patient transfer and admission, managing personnel schedules, and handling problems.

The medical team is responsible for deciding treatment plans, devising and implementing core regulations, allocating physicians and nurses, summarizing medical information, and arranging the schedules of medical personnel.

The nosocomial infection control team is responsible for devising and implementing nosocomial infection control regulations, providing protective equipment and training, and inspecting and supervising nosocomial infection control.

The logistics team is responsible for distributing resources, providing daily essentials, maintaining

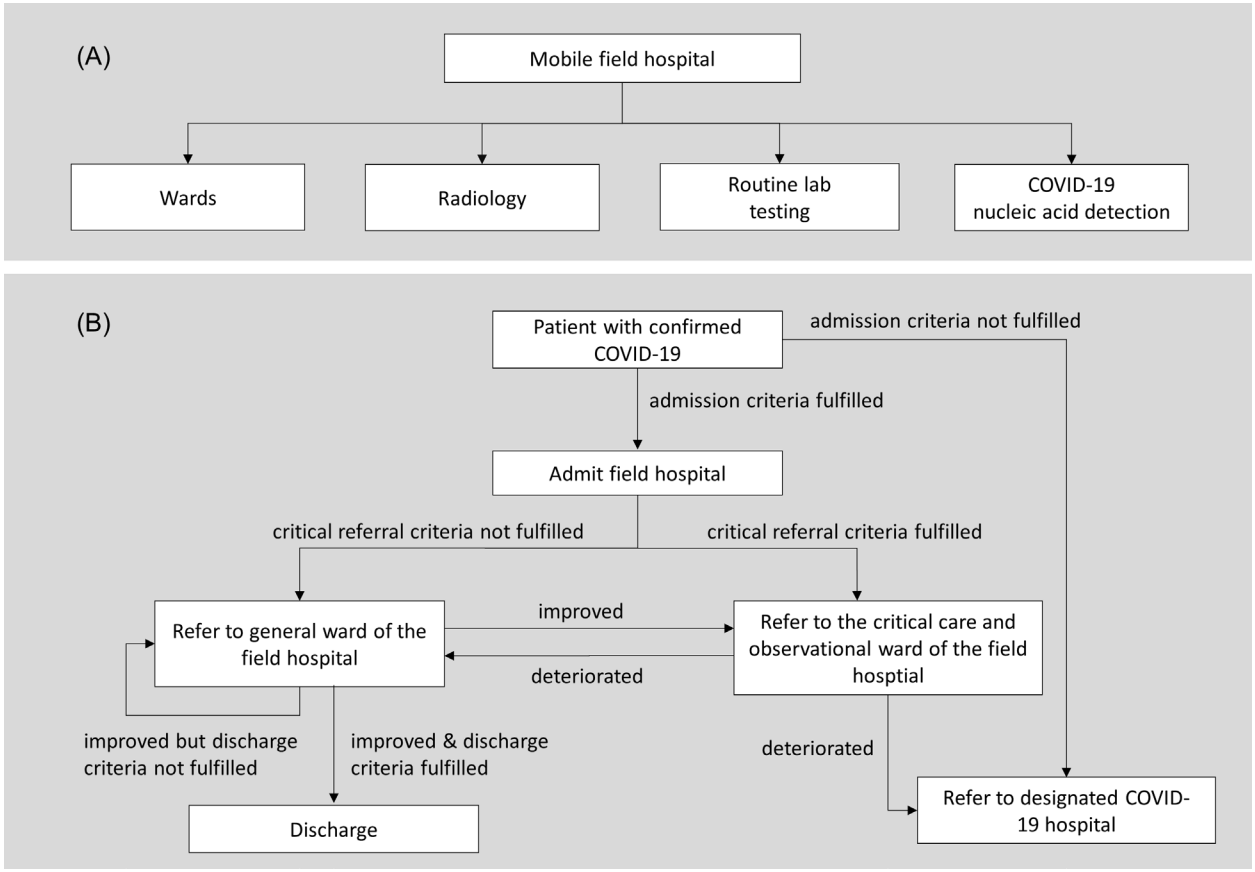


Figure 1. (A) Organization of a mobile field hospital. (B) Flow chart for patient management in a mobile field hospital.

facilities, preparing medication, environmental sanitation, medical waste management, and sewage.

The mobile field hospital was divided into four sections based on function: wards, Radiology, routine lab testing, and SARS-CoV-2 nucleic acid detection. Wards were designed for patient care and observation and were equipped with one critical care and observation unit and three infectious isolation units. Radiology consists of several mobile imaging vehicles performing radiography, computed tomography, and ultrasonography. Routine lab testing consists of several mobile laboratories providing routine lab testing such as blood tests and blood biochemical panels. Nucleic acid detection consists of a BLS-3 mobile lab that is responsible for SARS-CoV-2 nucleic acid detection and confirming the diagnosis of COVID-19.

2.2. Principles for management of a mobile field hospital

The general rules: Specialized admission, centralized quarantine, modular management, standardized treatment, and two-way referral of patients. A flow chart for patient management is shown in Figure 1B.

Admission criteria: Based on *Chinese Guidelines for the Diagnosis and Management of COVID-19*, version 5.0 (*6*), patients with confirmed COVID-19 must fulfill all criteria in Table 1A.

Discharge criteria: The patient must fulfill at least 3 criteria in Table 1B.

Criteria for admission to the critical care and observation unit. Any of the criteria in Table 1C must be fulfilled.

Criteria for referral to a hospital specializing in critical care. Any of the criteria in Table 1D must be fulfilled.

3. Conclusion

The biggest advantage of a mobile field hospital is its convenient centralized management of patients with mild symptoms. Those patients are able to walk and mingle with healthy people; without centralized management, they could spread the infection rapidly in the community and impede control of the disease. After mobile field hospitals were set up, regular hospitals were spared and patients with confirmed COVID-19 were isolated and treated in a centralized location, greatly helping to control the epidemic. More than 12,000 patients with COVID-19 have been treated by 15 mobile field hospitals over the past 30 days, and the rate of referral to a designated hospital has been around 35%.The mobile field hospitals were literally "a field of life". This article has described our experiences operating mobile field hospitals to deal with COVID-19 in China. Topics described here include the location of the hospital, regulations for patient management, and principles for patient admission and referral. The hope is that this information can serve as

Table 1. Rules for patient management at a mobile field hospital

(A): Admission criteria
1. Mildly symptomatic patients.
2. Nucleic acid testing is positive and/or a pulmonary CT scan is indicated.
3. Capable of independent living, older than 18 and younger than 65.
4. No severe underlying cardiopulmonary, neurological, or renal condition.
5. No history of a mental disorder.
6. SaO_2 greater than 93% and a respiratory rate less than 24 breaths per minute at rest.
7. Able to walk without assistance.

(B): Discharge criteria
1. Afebrile for at least 3 days.
2. Respiratory symptoms have improved significantly.
3. Pulmonary inflammation has decreased markedly according to Radiology.
4. Nucleic acid testing is negative two consecutive times (with at least 1 day between tests).

(C): Admission to the critical care and observation unit.
1. Tachypnea or a respiratory rate greater than 30 breaths per minute at rest.
2. SaO_2 less than 93% at rest.
3. $PaO_2/FiO_2 < 300$.
4. Other potentially fatal underlying risk factors.

(D): Criteria for referral to a hospital specializing in critical care.
1. Patients in the critical care and observation unit whose condition fails to improve or deteriorates after initial interventions.
2. Persistent hyperthermia for two days with a body temperature higher than 38.5 ºC after proper treatment.
3. Younger than 18 or older than 65.
4. Has a severe underlying cardiopulmonary, neurological, or renal condition, including the need to undergo dialysis.
5. Living with others.
6. Has a mental disorder like hypomania.
7. Other specific reasons (to be noted).

a reference and better inform countries that are dealing with COVID-19.

Acknowledgement

This research was supported by a grant from the Jiangxi Provincial Commission of Health and Family Planning, China (project number 20181004).

References

1. Sohrabi C, Alsafi Z, O'Neill N, Khan M, Kerwan A, Al-Jabir A, Iosifidis C, Agha R. World Health Organization declares global emergency: A review of the 2019 novel coronavirus (COVID-19). Int J Surg. 2020; 76:71-76.
2. Wu YC, Chen CS, Chan YJ. The outbreak of COVID-19: An overview. J Chin Med Assoc. 2020; 83:217-220.
3. Cascella M, Rajnik M, Cuomo A, Dulebohn SC, Di Napoli R. Features, Evaluation and Treatment Coronavirus (COVID-19). In: StatPearls (Treasure Island (FL), 2020.
4. Guan WJ, Ni ZY, Hu Y, *et al*. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020; doi: 10.1056/NEJMoa2002032.
5. The Novel Coronavirus-related Pneumonia Emergency Response Team for Protection and Disinfection of Key Locations, Chinese Center for Disease Control and Prevention. Health protection guidelines of mobile field hospitals during the outbreak of novel coronavirus -related pneumonia. Zhonghua Yu Fang Yi Xue Za Zhi. 2020; 54:E006. (in Chinese)
6. Lin L, Li TS. [Interpretation of the "Guidelines for the Diagnosis and Treatment of Novel Coronavirus (2019-nCoV) Infection from the National Health Commission (Draft Version 5)"]. Zhonghua Yi Xue Za Zhi. 2020; 100:E001. (in Chinese)

Received March 15, 2020; Revised March 26, 2020; Accepted March 27, 2020.

[§]These authors contributed equally to this work.
**Address correspondence to:*
Xiaoliang Chen, Hepatobiliary Surgery, Jiangxi Provincial People's Hospital Affiliated to Nanchang University, Nanchang, China.
E-mail: chenxiaoliang99@163.com

Released online in J-STAGE as advance publication April 1, 2020.

Communication

DOI: 10.5582/bst.2020.03094

Introduction on collective quarantine of close contacts of patients with COVID-19 for medical observation in China: from the perspective of frontline staff

Chenjie Dong[1,§], Yuan Tian[1,§], Wenzhong Xu[2], Jiangjiang He[3,*], Duo Chen[3], Jie Zhu[4], Ping Lu[4]

[1] Jiading Health Affair Management Center, Shanghai, China;
[2] Jiading District Health Commission, Shanghai, China;
[3] Department of Health Policy Research, Shanghai Health Development Research Center (Shanghai Medical Information Center), Shanghai, China;
[4] Malu Community Health Service Center, Jiading District, Shanghai, China.

SUMMARY The World Health Organization (WHO) has deemed coronavirus disease 2019 (COVID-19) to be a pandemic. The strict prevention and control measures taken by China have proven to be effective, creating a window of opportunity for other countries. The tracking and management of contacts of patients with COVID-19 are important components of prevention and control measures. This article briefly describes the placement of close contacts of patients with COVID-19 under collective quarantine for medical observation in China from the perspective of frontline staff. This article focuses on a community in the Jiading District of Shanghai to provide a reference for placement of close contacts of patients with COVID-19 under collective quarantine for medical observation in other countries and regions.

Keywords COVID-19, close contact, community, collective quarantine for medical observation

1. Introduction

The World Health Organization (WHO) deemed coronavirus disease 2019 (COVID-19) to be a pandemic (*1*); as of April 10, 2020, there were 1,521,252 confirmed cases and 92,798 deaths globally (*2*). Eight fundamental measures to respond to COVID-19 were cited by the WHO (*3*); identifying, tracking, and isolating contacts, a crucial task, was mentioned several times (*3-5*). On January 20, 2020, the Chinese Government classified COVID-19 as a category B infectious disease under the Law on the Prevention and Treatment of Infectious Diseases but it is regulating the disease as a category A infectious disease. COVID-19 was categorized as a quarantinable infectious disease pursuant to the Frontier Health and Quarantine Law (*6*). China implemented measures such as "Four Early Actions" (early detection, reporting, treatment, and isolation) and "Four Specifications" (the infected will be treated in dedicated facilities by senior medical professionals from around the country and with all necessary resources), and these strong prevention and control measures created a window of opportunity for other countries (*7*). The measures taken by China have proven to be effective (*8-11*). The tracking and management of contacts of patients with COVID-19 were both cited in the prevention and control suggestions offered by WHO (*12,13*) and in Chinese guidelines on prevention and control measures (*14*).

Here, a town in Jiading District, Shanghai is cited as an example. This article briefly describes the placement of close contacts of patients with COVID-19 under collective quarantine for medical observation in China from the perspective of frontline staff.

2. Overview of collective quarantine for medical observation

Since the outbreak of COVID-19, the WHO and Chinese Government have issued several control and treatment protocols and implemented responses to prevent the transmission of the virus. Individual provinces and cities in China have initiated first-level public health emergency responses (*15*). The tracking and management of contacts of patients with COVID-19 is an important component of prevention and control measures. The WHO issued a guidance document on collective quarantine for medical observation (*16*), the Chinese Government published a series of related documents (*14,20,21*), and Shanghai issued prevention and control documents based on the aforementioned documents that were particular to the region (*17-19,22*).

The documents provide guidance on the tracking and management of close contacts of patients, the selection of a site for collective quarantine, staff assignment and responsibilities, disinfection and protection, *etc*. Details on those documents are shown in Table 1.

2.1. Related departments, personnel, and their responsibilities

Individuals in China that need to be quarantined for medical observation and isolation efforts follow WHO guidelines. Tasks are mainly classified into management and coordination, medical and health care, and logistical support. Details on departments, personnel, and their responsibilities are shown in Table 2.

2.2. Tasks

Tasks at the quarantine sites are performed according to the required 14-day quarantine. Specific timepoints include the preparatory stage, preliminary observation (Day 1), intermediate observation (Day 2-Day 13), late observation (the afternoon of Day 13), and the day on which the individual is discharged from medical observation (Day 14). Staff should use self-protection and conduct regular disinfection according to related documents every time they come in contact with individuals under observation and they should enter and leave the quarantine area through specific entrances and exits. Detailed tasks are shown in Table 3.

2.3. Procedures for referral and first aid

When individuals under observation are in discomfort, they will be diagnosed and treated by general practitioners at the collective quarantine sites. If the individual needs to be referred, a Proof of Treatment Form from a Collective Quarantine Site for Medical Observation is needed. At that point, the community/subdistrict is responsible for designating people and a vehicle to transport the individual. If the individual is suspected of having COVID-19 or under other special circumstances, community health center administrative staff at the quarantine site will contact a regional emergency center or fever clinic at a nearby medical facility, and designate people and a vehicle to transport the individual to the fever clinic along with regional first-aid personnel. Accompanying personnel should be in level-one protection and the transporting vehicle should be disinfected. In line with the protocol, if the individual is excluded as having COVID-19, the community/subdistrict will arrange a vehicle and personnel to pick the individual up. If the individual is confirmed as being infected, then the hospital that received the individual will refer the individual to the Shanghai Public Health Clinical Center for further treatment. The referral flowchart is shown in Figure 1.

3. Factors that influence the effectiveness of medical observation

From the perspective of frontline staff, there are 6 factors that may influence the effectiveness of medical observation of close contacts of patients with COVID-19:

i) The ability to lead and mobilize: The Chinese political system enables the Chinese Government to implement a series of powerful measures for epidemic prevention and control. Government at all levels and the entire society were mobilized, which was highly efficient (*23*). Hotels that met requirements were selected to serve as quarantine sites for medical observation, and the logistical management teams led by the government distributed and allocated resources in a standardized manner (*24,25*).

ii) Inter-agency collaboration: Epidemic prevention and control is a task for society as a whole. It is not a job for the health department alone. A government at all levels approach is required (*26*). Staff at

Table 1. Documents related to placement of close contacts of patients with COVID-19 under collective quarantine for medical observation in China

Date Issued	Issuing Institutions	File Name (*Ref.*)
2020.01.28	Shanghai Municipal Health Commission	Notice on comprehensive health screening of people arriving in Shanghai and isolation and observation (quarantine) of key groups for prevention and control of COVID-19 in Shanghai (*17*).
2020.02.07	Shanghai Municipal People's Congress	Decision to implement extensive efforts to prevent and control COVID-19 (*18*).
2020.02.11	Shanghai Government	Notice on further implementing strict individual prevention and control measures (*19*).
2020.02.29	National Health Commission	Notice on the further standardization and enhancement of disinfection efforts amid the COVID-19 epidemic (*20*).
2020.03.04	National Health Commission	Notice on the issuance of the Diagnosis and Treatment Protocol for COVID-19 (7^{th} ed., draft) (*21*).
2020.03.07	National Health Commission	Notice on the issuance of the Protocol for Prevention and Control of COVID-19 (6^{th} ed.) (*14*).
2020.03.13	Shanghai Municipal Health Commission	Notice on the issuance of the Shanghai Protocol for Prevention and Control of COVID-19 (5^{th} ed.) (*22*).

Table 2. Departments, personnel, and corresponding responsibilities at the collective quarantine sites for medical observation of close contacts of patients with COVID-19

Category of tasks	Related Department	Personnel (code)	Responsibilities
Management and coordination	Government departments	Community/subdistrict administrative staff (A)	Organize, manage and coordinate all persons sent by each department and assign tasks; better allocate materials; deal with all emergencies.
	Health Administration department	Health administrative staff (B)	Assist A to manage and coordinate personnel within the health system and to better allocate health resources.
		Health supervision and enforcement personnel (C)	Supervise health and enforce the law, evaluate areas of isolation; evaluate pollutants and sewage treatment.
Medical and health care	Medical and health care facilities	Community health center administrative staff (D)	Manage and coordinate the quarantine site, arrange food and materials, assign tasks to site staff; convey information on individuals under observation and arrange material reserves and supplies.
		General practitioner (E)	Receive and instruct individuals under observation; perform medical evaluation and routine diagnosis and treatment of individuals under observation; train the staff and individuals under observation in the prevention and control of the epidemic; disinfect contaminated areas.
		Psychologists (F)	Provide psychological assessment, counseling, and treatment to individuals under observation.
		Public health physicians (G)	Perform an infection risk assessment and epidemiological investigation of individuals under observation; disinfect contaminated areas.
		Nurses (H)	Provide care to individuals under observation, train staff and the individuals under observation in the prevention and control of the epidemic; prepare disinfectants and instruct disinfection workers to thoroughly disinfect public areas and partially contaminated areas; dispose of medical waste and provide related instructions.
		Pharmacists (I)	Instruct individuals under observation in taking medication.
		CDC professionals (J)	Collect, analyze, and transmit information on individuals under observation; guide quarantine sites to prevent and control the epidemic.
Logistical support	Fire department	Firemen (K)	Evaluate and supervise fire safety and respond to fires.
	Public security and civil affairs authorities	Policemen (L)	Evaluate and ensure safety.
	Security department	Security staff (M)	Provide safety; keep order in quarantine sites; deliver materials like daily essentials and food.
	Foreign affairs department	Interpreters (N)	Interpret, communicate, and coordinate; convey information to foreigners.
	Sanitation and cleaning	Cleaner (O)	Clean surroundings and rooms, dispose of waste collectively.
		Disinfection worker (P)	Disinfect public areas, partially contaminated areas, and vehicles.
		Sanitation worker (Q)	Collect and transport waste and medical waste.
	Social organizations	Volunteers (R)	Register information on and communicate with individuals under observation; register and report on the needs of individuals under observation and give feedback.
	Hotels	Hotel staff (S)	Operate hotels and ensure the supply of materials.

quarantine sites consisted of agency officials, health administrators, medical personnel, firemen, policemen, security personnel, foreign affair personnel, sanitation workers, volunteers, and hotel staff. This inter-agency collaboration contributed to the coherence of epidemic prevention and control.

iii) The establishment of health emergency teams and funding and material support: An experienced and well-trained team can calmly deal with a public health emergency and play a major role. The development and continuous training of health emergency teams can improve their capabilities. Abundant funding and materials can ensure the consistency of collective quarantine under medical observation and help medical personnel to perform tasks in an orderly manner.

iv) Efficient transmission of information: Convenient and efficient transmission and integration of information facilitates inter-agency collaboration, promotes efficiency, and facilitates the effective and reasonable allocation of human resources and materials to ensure highly efficient epidemic prevention and control. Valid news can prevent the spread of rumors and reduce risks caused by misinformation (*26*).

v) The psychological status of individuals under observation: Individuals under observation can be affected by their physical health or by psychological

Table 3. Efforts at sites for medical observation of close contacts of patients with COVID-19

Time	Efforts	Details	Executor
Preparatory stage	Location selection	Select hotels that meet requirements as collective quarantine sites for medical observation.	A, B
	Evaluation	Evaluate pollutants, sewage treatment, and quarantine areas.	C
		Fire safety assessment.	K
		Security assessment.	L
	Training	Provide preliminary training to all staff.	D, E, F, H
Day 1	Collection of information	Collect and confirm information on individuals under observation.	D
	Accommodations	Arrange rooms and order meals for individuals under observation.	D
	Task assignment	Assign the task of receiving individuals under observation.	D
	Reception of individuals for observation	Contact medical personnel who will transfer individuals and confirm information.	E
		Inform close contacts of patients about issues related to collective quarantine for medical observation.	E
		Have individuals sign a Consent to Medical Observation Form for close contacts of patients with COVID-19 in Shanghai and the Shanghai Jiading Form for Placement in Collective Quarantine.	E
		Inform people under medical observation at quarantine sites about self-protection and disinfection and provide friendly reminders about the psychological support hotline.	E
	Evaluation	Preliminary evaluation of infection risks: perform an epidemiological investigation.	E
		Disease evaluation: inquire about clinical symptoms and medical history.	E
		Evaluate the mental health of individuals under observation.	F
	Check-in guidance	Instruct individuals under observation to enter rooms via a specific entryway and describe things, equipment, and devices in the room and how to use them.	E
		Inform individuals of how to take their temperature and train them in self-protection and disinfection.	E
	Documentation of information	Record information on individuals under observation and complete the health status information registration form.	E
	Reassessment of infection risk	Communicate by phone, confirm and supplement the individual's epidemiological history.	G
	Routine communication	Communicate by phone and ascertain the individual's living conditions and report their needs to D.	R
	Taking an individual's temperature	Take the individual's temperature twice a day.	E
	Waste disposal	Household waste of asymptomatic individuals under observation can be disposed of as general household waste after disinfection; that of individuals with symptoms should be disposed of as infective medical waste.	O, Q, G, H
	Environment disinfection	Train disinfection workers to disinfect surroundings.	P
Day 2-Day 13	Routine communication	Communicate by phone and ascertain the individual's living conditions and report their needs to D.	R
	Evaluation and observation	Evaluate infection risk and prior disease risk and deal with those risks, inspect rooms, and take the individual's temperature.	E
	Disease diagnosis and treatment	Diagnose and treat individuals with a risk of infection and disease, and refer them if treatment is not feasible (see the Referral flowchart for close contacts under medical observation)	E
	Psychological counseling	Provide psychological counseling to depressed individuals.	F
Day 13	Preliminary evaluation before discharging	Inquire about medical history and take the individual's temperature.	E
	Providing information	Deliver a Notification of Discharge from Isolation.	E
	Documentation of information	Complete the Notification of Discharge from Medical Observation for Close Contacts of Patients with COVID-19 in Shanghai.	E
Day 14	Re-evaluation before discharge	Ask individuals about their medical history and take their temperature.	E
	Discharge from medical observation	Give out Discharging Notification to Close Contacts of Patients with COVID-19 in Shanghai and one mask to those who are healthy.	E
	Guidance upon departure	Instruct individuals to leave via specific exits.	E
	Final disinfection	Perform a final disinfection of the room.	E
	Information documentation	Record related information on Registration Log of Medical Observation Discharging.	E

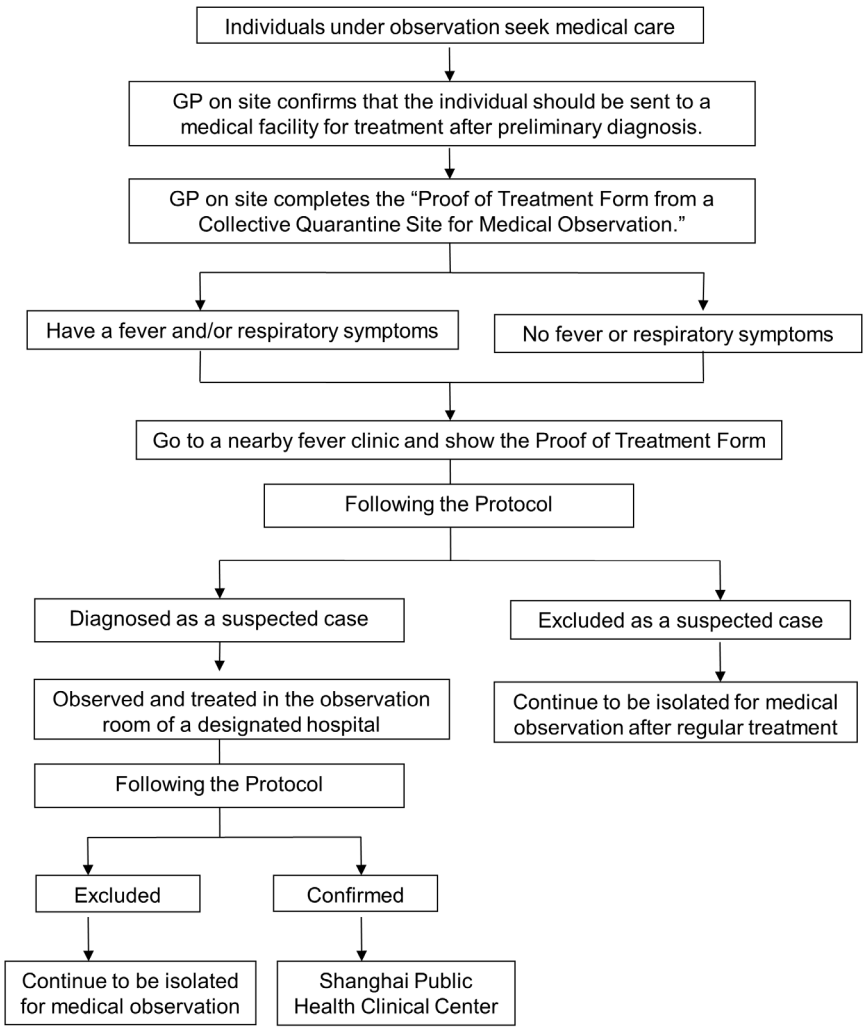


Figure 1. Referral flowchart for close contacts of patients with COVID-19 under medical observation in Shanghai.

factors. The diversity of needs and whether those needs are met have an impact on individuals, including the risk of disease during observation and mental health. During the preliminary stage of observation, a psychological assessment should be performed on individuals under observation as early as possible, and effective interventions should be implemented to help facilitate medical observation.

vi) Attention to key groups: During medical observation, more attention should be paid to key groups (such as children, the elderly, pregnant women, patients with chronic diseases, and patients with particular diseases). They have lower immunity and need special care. An early assessment of the risk of infection and disease should be performed for these groups, and effective interventions should be promptly implemented to help facilitate medical observation.

According to information on the distribution of cases of COVID-19 from the Chinese Center for Disease Control and Prevention (*27*), the number of cases tended to decline in mid-February in most regions of China. As of March 31, 2020, there were 81,554 confirmed cases, 76,238 cases cured, and 3,312 deaths (*28*). Currently, important results have been achieved by China's phased epidemic prevention and control. Local transmission of COVID-19 has basically been blocked on the Chinese mainland. The overall status of the epidemic is improving (*29*). However, there is a possibility of recurrence, and attention should be paid to cured patients who later test positive for the virus, patients with false-negative results, asymptomatic carriers, and new patients (*30*). Overseas, the pandemic is rapidly spreading, bring greater pressure on China to regulate imported cases of COVID-19. More strict management of travelers arriving in China is needed (*31*). When individuals who have already arrived in China are placed under medical observation, attention must be paid to different cultures and different values in order to avoid unnecessary conflicts and to sustain the effectiveness of medical observation. In the long run, attention should be paid to post-traumatic stress disorder (PTSD) among individuals under observation. A psychological support hotline was instituted and guidelines on psychological support, counseling, and crisis interventions were issued in China to alleviate the psychological harms caused by the epidemic and to

actively prevent, alleviate, and control the social effects of the epidemic on the mental state of those affected (*32-36*). These efforts aim to identify high-risk groups and avoid the incidence of extreme events.

Acknowledgements

This paper was supported by grants from the Shanghai Jiading District Health Commission for a "Study of the community strategies for prevention and control of major infectious diseases in Jiading District" (Project number: 2020JDHP12) (to Chenjie Dong, Yuan Tian and Ping Lu) and the China Medical Board Collaborating Project "Establishing Health Policy Transformation Network of China (Project number: CMB-CP 14-190)" (to Jiangjiang He and Duo Chen).

References

1. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. *https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020* (accessed March 11, 2020).
2. World Health Organization. WHO. Coronavirus disease (COVID-2019) situation reports. *https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/* (accessed March 29, 2020).
3. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19 - 9 March 2020. *https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---9-march-2020* (accessed March 9, 2020).
4. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19 - 25 March 2020.https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---25-march-2020 (accessed March 25, 2020).
5. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19 - 27 March 2020. *https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---27-march-2020* (accessed March 27, 2020).
6. National Health Commission. Announcement by the National Health Commission of the People's Republic of China. No.1 2020. *http://www.nhc.gov.cn/xcs/zhengcwj/202001/44a3b8245e8049d2837a4f27529cd386.shtml* (accessed January 20, 2020). (in Chinese)
7. World Health Organization. WHO-AUDIO Executive Board EB146 Coronavirus Briefing. *https://www.who.int/docs/default-source/coronaviruse/transcripts/who-audio-executive-board-eb146-coronavirus-briefing-script-04feb2020-final.pdf?sfvrsn=70a66dfc_2* (accessed February 4, 2020).
8. Bureau of Disease Prevention and Control. Report of the WHO-China Joint Mission on Novel Coronavirus Disease 2019 (COVID-19) is issued. *http://www.nhc.gov.cn/jkj/s3578/202002/87fd92510d094e4b9bad597608f5cc2c.shtml?wYNOrhhjiR4y=1582949699287* (accessed February 29, 2020). (in Chinese)
9. Department of International Cooperation. Transcript of a Press Conference of the WHO-China Joint Mission on COVID-19. *http://www.nhc.gov.cn/gjhzs/s3578/202002/1fa99f55972740f681d47cde0d1b2522.shtml* (accessed February 24, 2020). (in Chinese)
10. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19 - 28 February. 2020.*https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---28-february-2020* (accessed February 28, 2020).
11. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19 - 20 March 2020. *https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---20-march-2020* (accessed March 20, 2020).
12. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19 - 6 March 2020. *https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---6-march-2020* (accessed March 6, 2020).
13. World Health Organization. WHO Director-General's opening remarks at the mission briefing on COVID-19 - 26 February. 2020.*https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-mission-briefing-on-covid-19---26-february-2020* (accessed February 26, 2020).
14. National Health Commission. Notice from the National Health Commission on the issuance of the Protocol for Prevention and Control of COVID-19 (6th ed.). *http://www.nhc.gov.cn/xcs/zhengcwj/202003/4856d5b0458141fa9f376853224d41d7.shtml* (accessed March 7, 2020). (in Chinese)
15. Huanqiu. Latest news! 22 provinces and cities initiate a primary public health emergency response. *https://china.huanqiu.com/article/9CaKrnKp3Vd* (accessed January 25, 2020). (in Chinese)
16. World Health Organization. Considerations for quarantine of individuals in the context of containment for coronavirus disease (COVID-19). *https://www.who.int/publications-detail/considerations-for-quarantine-of-individuals-in-the-context-of-containment-for-coronavirus-disease-(covid-19)* (accessed March19, 2020).
17. Notice on comprehensive health screening of people arriving in Shanghai and isolation and observation (quarantine) of key groups for prevention and control of COVID-19 in Shanghai. *https://hpq.sh.gov.cn/zw/009002/009002016/009002016004/009002016004004/20200225/7b0d41ae-9ba1-465b-9892-e08c320d5a23.html* (accessed January 28, 2020). (in Chinese)
18. Shanghai Government website. Decision of the Shanghai Municipal People's Congress to implement extensive efforts to prevent and control COVID-19. *http://www.shanghai.gov.cn/nw2/nw2314/nw32419/nw48516/nw48545/nw48635/u26aw63497.html* (accessed February 7, 2020). (in Chinese)
19. Shanghai Government website. Notice from the Shanghai Government on further implementing strict individual prevention and control measures. *http://www.shanghai.gov.cn/nw2/nw2314/nw32419/nw48516/nw48545/nw48635/u26aw63661.html* (accessed February 11, 2020). (in Chinese)

20. National Health Commission. Notice on the further standardization and enhancement of disinfection efforts amid the COVID-19 epidemic. *http://www.nhc.gov.cn/xcs/zhengcwj/202002/d1ae51c7899d4faaa763a2e67ebbd2e5.shtml* (accessed February 29, 2020). (in Chinese)
21. General Office of the National Health Commission. Notice on issuance of the Diagnosis and Treatment Protocol for COVID-19 (7th ed., draft). *http://www.nhc.gov.cn/xcs/zhengcwj/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml* (accessed March 3, 2020). (in Chinese)
22. Shanghai Municipal Health Commission. Notice on the issuance of the Shanghai Protocol for Prevention and Control of COVID-19 (5th ed.). *http://wsjkw.sh.gov.cn/jbfk2/20200313/19d2f5ed7e174e0db5b895da0a1e0b20.html* (accessed February 24, 2020). (in Chinese)
23. People's Daily Online. The unrivalled advantage of the system of socialism with Chinese characteristics. *http://world.people.com.cn/n1/2020/0315/c1002-31632120.html* (accessed March 15, 2020). (in Chinese)
24. General Office of the National Health Commission. Notice of the General Office of the National Health Commission on enhancing efforts by primary care facilities to prevent and control COVID-19. *http://www.gov.cn/zhengce/zhengceku/2020-01/27/content_5472401.htm* (accessed January 26, 2020). (in Chinese)
25. Chinese Thoracic Society, Chinese Society of General Practice, Chinese Association of Chest Physicians, *et al.* Expert recommendations for the prevention and control of SARS-CoV-2 virus infection in primary care (First Edition), Chinese Journal of General Practitioners. 2020; 19 :175-192. (in Chinese)
26. World Health Organization. Munich Security Conference. *https://www.who.int/dg/speeches/detail/munich-security-conference* (accessed February 15, 2020).
27. Chinese Center for Disease Control and Prevention. Prevalence and distribution of COVID-19. *http://2019ncov.chinacdc.cn/2019-nCoV/* (accessed January 26, 2020). (in Chinese)
28. National Health Commission. The prevalence of COVID-19 as of 12 AM on March 31, 2020. *https://www.xuexi.cn/lgpage/detail/index.html?id=1939773170044809532&item_id=1939773170044809532* (accessed April 1, 2020). (in Chinese)
29. CCTV. National Health Commission: China's epidemic prevention and control achieved important results in stages. *http://news.cctv.com/2020/03/13/ARTIYYzn3IrVYHkGsZRBRtwa200313.shtml* (accessed March 13, 2020). (in Chinese)
30. The Paper. Alerts: Nanshan Zhong suggests maintaining the current prevention and control measures and Wenhong Zhang emphasizes the necessity of strict prevention and control measures. *https://www.thepaper.cn/newsDetail_forward_6732253* (accessed March 28, 2020). (in Chinese)
31. CNR. Premier Li Keqiang held a meeting of the leaders of China's COVID-19 response, stressing measures to prevent the resurgence of the epidemic domestically and calling for further efforts to prevent imported cases arriving by land and water. *http://china.cnr.cn/news/20200327/t20200327_525031811.shtml* (accessed March 27, 2020). (in Chinese)
32. Bureau of Disease Prevention and Control, National Health Commission. Notice on the issuance of guidelines for emergency psychological interventions related to the COVID-19 epidemic. *http://www.nhc.gov.cn/xcs/zhengcwj/202001/6adc08b966594253b2b791be5c3b9467.shtml* (accessed January 26, 2020). (in Chinese)
33. Bureau of Disease Prevention and Control, National Health Commission. Notice on the institution of a hotline to provide psychological support in response to the epidemic. *http://www.nhc.gov.cn/xcs/zhengcwj/202002/8f832e99f446461a87fbdceece1fdb02.shtml* (accessed February 2, 2020). (in Chinese)
34. Bureau of Disease Prevention and Control, National Health Commission. Notice on the issuance of guidelines for a psychological support hotline during the COVID-19 epidemic. *http://www.nhc.gov.cn/xcs/zhengcwj/202002/f389f20cc1174b21b981ea2919beb8b0.shtml* (accessed February 7, 2020). (in Chinese)
35. General Office of the National Health Commission. Notice on enhancing psychological support and social services in relation to prevention and control of COVID-19. *http://www.nhc.gov.cn/xcs/zhengcwj/202003/a9b0bcb3bb7445298c480c5003c51d6d.shtml* (accessed March 5, 2020). (in Chinese)
36. Bureau of Disease Prevention and Control, National Health Commission. Notice on the issuance of a plan for psychological counseling of people affected by COVID-19. *http://www.nhc.gov.cn/xcs/zhengcwj/202003/0beb22634f8a4a48aecf405c289fc25e.shtml* (accessed March 18, 2020). (in Chinese)

Received April 16, 2020; Revised April 29, 2020; Accepted May 7, 2020.

[§]These authors contributed equally to this work.
**Address correspondence to:*
Jiangjiang He, Shanghai Health Development Research Center (Shanghai Medical Information Center), No.1477 Beijing (W) Road, Jing'an District, Shanghai, China 200040.
E-mail: hejiangjiang@shdrc.org

Released online in J-STAGE as advance publication May 9, 2020.

Communication

DOI: 10.5582/bst.2020.03066

A practice of anesthesia scenario design for emergency cesarean section in patients with COVID-19 infection based on the role of standard patient

Yin Kang[1], Longjiao Deng[1], Dengwen Zhang[1], Yuehong Wang[1], Gang Wang[1], Li Mei[1], Guobin Zhou[1], Haihua Shu[1,2,*]

[1] Department of Anesthesiology, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China;
[2] The Second School of Clinical Medicine, Southern Medical University, Guangzhou, Guangdong, China.

SUMMARY The new coronavirus (COVID-19) has been characterized as a world pandemic by WHO since March 11, 2020. Although it is likely that COVID-19 transmission is primarily via droplets and close contact, airborne transmission and fecal-oral route remains a possibility. The medical staff working in the operating room, such as anesthesiologists, surgeons and nurses, are at high risk of exposure to virus due to closely contacting patients. The perioperative management is under great challenge while performing surgeries for patients suffering COVID-19, including emergency cesarean section, which is one of the most common surgeries under such circumstances. How to prevent medical staff from cross-infection is an issue of great concern. In this article, we give a practice of anesthesia scenario design for emergency cesarean section in a supposed standard patient suffering COVID-19, aimed to optimize the work flow and implement the protective details through simulation of a real operation scenario, which may be useful for training and clinical practice of anesthesia management for patients suffering COVID-19 or other fulminating infectious diseases.

Keywords COVID-19, SARS-CoV-2, anesthesia management, cesarean section, scenario design

1. Introduction

The new coronavirus (COVID-19) outbroke in many countries of the world and has been characterized as a world pandemic by the World Health Organization on March 11, 2020 (*1*). It is now generally considered that the transmission happens mainly through respiratory droplets and close contact (*2,3*), and in some exceptional cases airborne or aerosols may also be involved (*4*). The medical staff working in the operating room (OR) are at high risk of exposure to virus due to close contact with patients, procedures of surgeries and anesthesia being possible to produce airborne or aerosols in a comparitivly confined space. However, urgent and emergency surgeries for confirmed or highly suspected COVID-19 patients, especially emergency cesarean sections are inevitable. Thus, it is important to provide systemic preventive precautions and training to improve the personal protective ability during perioperative anesthesia management.

In this article, we provide our practice and experience for planning a training program for an anesthesia scenario based on the role of a standard patient to optimize work flow and implement protective details.

2. Methods and Results

2.1. Establishment of emergency response team

In order not to disturb regular operation and anesthesia work, we recruited doctors and nurses based on voluntary registration and established emergency response teams (ERTs) including 6 formal ERTs and 3 backup ERTs. Each ERT included two senior anesthesiologists, a resident doctor and an anesthesia nurse with department head. The ERT has been regularly arranged and scheduled and each ERT takes charge of one week of anesthesia work for surgical patients with confirmed or highly-suspected COVID-19 in turns. The staff included in ERT must be strictly trained and practiced wearing and undressing personal protective equipment (PPE), including adequate precautions, good hand hygiene, properly fitted N-95 masks and other essential PPE.

2.2. Establishment of surgical and anesthesia rule and workflow for cesarean section based on the role of a standard patient

It is necessary to establish an understandable rule and workflow for surgeons, anesthesiologists and nurses, which must be strictly obeyed and performed by all departments to obtain interdisciplinary cooperation (Figure 1). It is important to classify the normal, suspicious and confirmed patients by screening the patient from COVID-19 before being admitted to the hospital or being sent to OR.

2.3. Anesthesia scenario setting

A suspected COVID-19 pregnant woman had been sent to the appointed fever clinic in our hospital. The on-duty physician immediately performed the detection of nucleic acid nose or throat swab after consulting the infection expert and reporting to the Hospital Infection Administration Division. In accordance with the contingency plan, all departments were on their way: the Prevention and Health Section was responsible for the infectious disease report; the Hospital Infection Administration Division directed nosocomial infection control; and the director of the Infectious Disease Department carried out medical treatment and called for obstetric consultation. 4 hours later the nucleic acid test was shown to be positive for coronavirus. Obstetricians decided that this confirmed COVID-19 patient needed emergency cesarean because the woman had a mild pneumonia with complete placenta previa and 38 weeks gestation and the fetal heart rate was getting faster in the last half hour. Dedicated OR equipped with a negative pressure system was standby and surgery related staff were in place immediately.

2.4. Anesthesia scenario process

The process of anesthesia scenario setting for this emergency cesarean section is summarized in Figure 2.

Report: The notice of emergency cesarean with a confirmed COVID-19 pregnant woman was immediately reported to the department head and on call ERT.

Preoperative preparation: ERT members moved quickly based on their own responsibility (Table 1). Equipment, consumables, drugs and so on should follow the strict principle of "only in" policy, which means they can't be taken out of the dedicated OR. Only the necessary items need to be brought in. Possible use or backup items are placed in a nearby clean OR, including anesthetics for general anesthesia, intubation supplies with visual laryngoscope, crystal and colloid, pressurized bag, arterial pressure transducer, central venous catheter, emergency cart and difficult airway cart.

Personnel protection and staff arrangement: All the in-room staff were in place and carried out level 3 PPE under the guidance of infection specialist (Figure 3A and 3B). Related instruments, equipment and drugs needed for the surgery were checked again. A senior anesthesiologist and resident doctor were in the room, another senior anesthesiologist and the anesthesia nurse

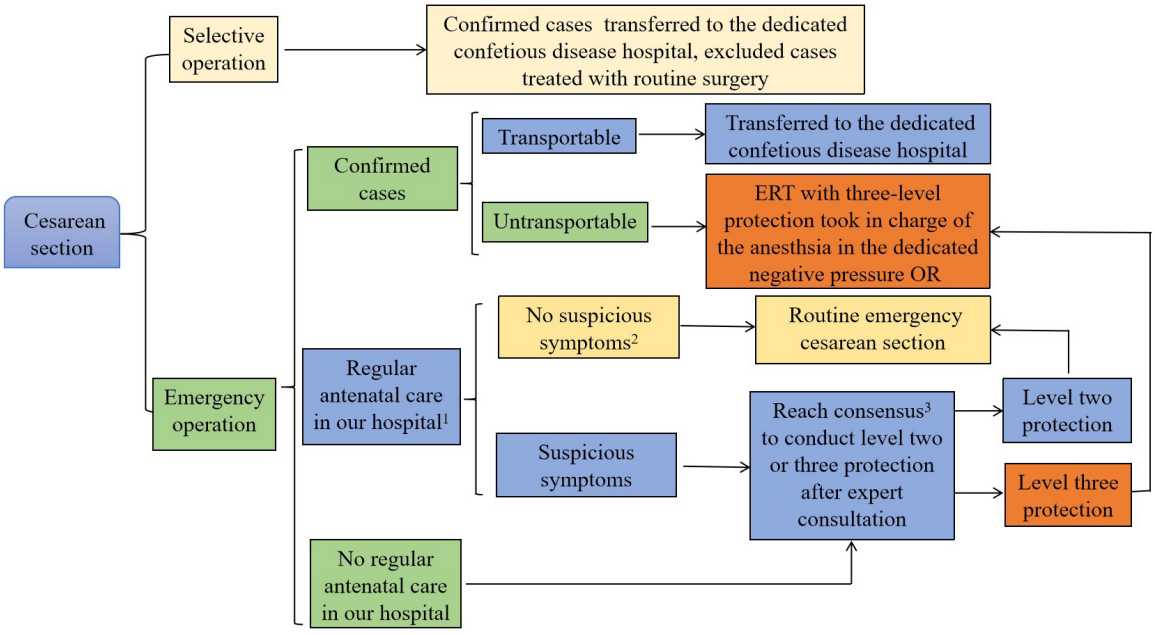


Figure 1. The surgical and anesthesia rule and workflow for cesarean section. Annotate:1) Regular antenatal care in our hospital (including whole blood cell count and nucleic acid test for COVID-19, expert consultation and chest CT screening if necessary). 2) Suspicious symptoms including contact history; fever, cough and other respiratory symptoms; chest CT screening not excluded. 3) Consensus among the anesthesiology director, obstetrics director and operating room director.

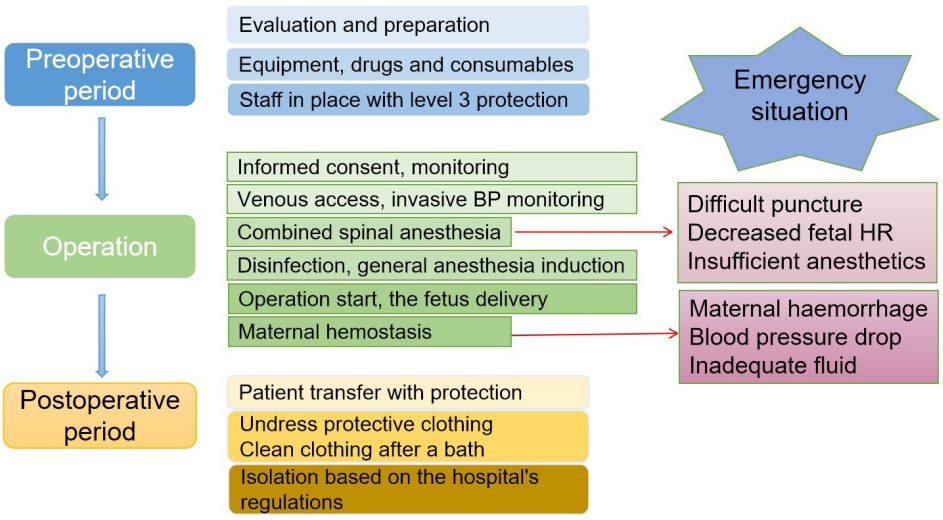


Figure 2. Flow chart of the anesthesia scenario setting-emergency cesarean section with a confirmed COVID-19 pregnancy woman.

were on standby to provide guidance or support in a clean OR nearby. Department head (team leader) took the responsibility of communication and cooperation with other departments.

Informed consent and monitoring: Patient was sent to the negative pressure OR strictly following planned route in advance with a clean area, semi-contaminative (buffer area) and contaminative area, as shown in Figure 3C. The patient signed the informed consent paper after entering the OR. The patient wore a protective mask all the time, and supplementary oxygen was given over the mask through a nasal catheter with low oxygen flow 2-3 L/min. Two venous accesses and invasive BP monitoring were established.

Prevention and treatment of emergency or unexpected situations during operation: Emergencies including hypotension, nausea and vomiting, and arrhythmia and massive bleeding may occur. 15 minutes after the fetus' delivery, massive bleeding occurred and blood pressure dropped quickly to 60/35 mmHg within 5 minutes. Routine treatment proceeded with fluid infusion and vasopressor drugs. The backup ERT members were ready to replenish the colloid solution, vasoactive drugs and apply for blood transfusion. A backup anesthesiologist was ready to participate in the rescue whenever necessary. The newborn was handled by a neonatologist and sent to the neonatal unit for isolated medical observation. The group remembered to change gloves and disinfect hands, avoiding contamination of other areas of the OR.

Patient Transit: The patient was tranfered to the Infection Disease Department through the dedicated patient delivery route.

Postoperative Clean and Sterilize: After handover of the patient, the relevant medical staff undressed from the PPE under the guidance of an infectious specialist, and put on clean clothing after a bath and had rest based on the hospital's regulations. The OR and anesthesia machine will be sterilized following the guidance of the institute. The nurse will deal with the disinfection and treatment of postoperative medical items and waste.

3. Discussion

Emergency surgery for confirmed or suspected COVID-19 patients may take a longer time to prepare than general emergency operations because of the special requirements for preoperative wear of PPE and patient transfer. Therefore, it should need rapid action with close cooperation and orderly preparation to shorten the waiting time. Repeat practice and training of perioperative management is necessary for this kind of patient, and is important. Spinal anesthesia is still recommended as the primary choice of anesthesia for cesarean delivery in a confirmed COVID-19 pregnant woman (*5*). However, general anesthesia can be used in patients with contraindications of spinal anesthesia or serve as a backup plan in case of spinal anesthesia failure or intraoperative conversion to general anesthesia.

The ERT in our department are scheduled on a weekly basis. This schedule will minimally disturb regular anesthesia work and allow staff to obtain enough rest after operations. Each member of the ERT has his own responsibility (Table 1).

The dedicated operating room for COVID-19 surgeries should be equipped with a negative pressure system. Patients with general anesthesia should be fully preoxygenated under autonomous respiration to avoid high flow oxygenation as it may increase the production of viral droplets and aerosols (*6*). Two layers of wet gauze can be used to cover the patient's mouth and nose. The use of a breathing circuit filter is recommended during surgery (Figure 3D) because its use can effectively prevent the anesthesia machine from contamination by bacteria and viruses (*7*). To avoid potential exposure risk

Table 1. Anesthesia Precaution Checklist for COVID-19 infected patients

Itmes	Confirmation (√)
1. Preoperative preparation: **a. ERT anesthesiologists:** ● Communication with relevant departments ● Preoperative evaluation ● Informed consent paper for anesthesia **b. ERT nurse:** ● Preparation of drugs, equipment and consumables in dedicated OR ● Preparation of drugs, equipment and consumables in a backup clean OR nearby ● Anti-mist treatment for goggles and glasses with iodophor or bath lotion half an hour in advance	
2. Personal protective equipment: ● Level 3 personal protective equipment under the guidance of infection specialist: hand hygiene, operating scrubs, disposable hair cover, n95 mask, surgery mask, goggles/face shield, protective clothing overall, medical latex gloves, boot covers, isolation gown	
3. Patient transfer and reconfirmation: ● Dedicated access to transport patients. The patient must wear a surgical mask or N95 mask all the time ● Reconsideration of drugs, equipment and consumables by the in-room anesthesiologists ● Other ERT members and nurse on standby in the clean OR nearby ● Spare drugs, consumables, emergency cart, difficult airway cart in place ● Effective and timely communication between ERT members through network video or walkie-talkie	
4. Patient entry to the dedicated OR: ● Signature of informed consent for anesthesia ● Monitoring, establishment of venous access	
5. Anesthesia: **a. Spinal anesthesia** ● Patient with a surgical mask or N95 mask ● Supplementary oxygen given over the mask ● A backup plan of general anesthesia **b. General anesthesia** ● Visual laryngoscope with disposable laryngeal lenses as first choice ● Breath filters installed between the proximal end of the endotracheal tube and the distal end of the circuit ● Fully preoxygenation with appropriate oxygen flow ● Two layers of wet gauze to cover the patient's mouth and nose ● Modified rapid sequence induction with sufficient muscle paralysis ● Avoid coughing and/or bucking ● Oral or tracheal suction performed with a closed suction system after intubation	
6. Unexpected difficult airway: ● Place Laryngeal mask if it meets the needs of surgery ● Notify pending support if endotracheal intubation required	
7. Treatment of intubation utensils: ● Take off the outer layer gloves and put on new latex gloves after hand hygiene ● Alcohol to sterilize the laryngoscope handle three times before putting in the prepared specimen bag separately for further treatment postoperation	
8. Supplement of drugs and consumables during operation: ● Sent in the OR from the backup OR nearby through the semi-contaminated area ● Blood transfusion: all the prepared work done by the backup ERT member before transfusion	
9. patient transit: ● Be clear about the dedicated patient delivery routes ● Inform the infectious department and get ready for this patient ● Clean files and patient contacted files separately stored ● Attention to staff protection in transit	
10. Isolation: ● Undress PPE and put on clean clothing after a bath ● Isolation based on the hospital's regulations	

of aerosols or droplets contamination, modified rapid sequence induction with sufficient muscle paralysis is recommended. Opioid analgesics should be given after loss of consciousness and muscle relaxant to reduce stiffness of the chest wall. It is also very important to take measures to avoid coughing and/or bucking during anesthesia induction, intubation, extubation and the recovery period (*5,8*). Video laryngoscopes are a good choice for normal trachea intubation. For spinal anesthesia, the infected patient must wear a surgical mask or N95 mask all the time.

Maintain smooth communication among departments. The team leader and the backup ERT stay in a clean OR nearby in case of emergency or need of rescue. The transfer route, protection requirements of patient and transported staff should be clearly aware before the surgery and exactly implemented after the operation.

Anesthesia drills for different types of operations are required at regular intervals to get continuous feedback and improvements. On this basis, we have developed the "Anesthesia Precaution Checklist for COVID-19 infected patients" (Table 1) and require that each item of the checklist be ascertained or implemented before entering the infectious surgical room.

In conclusion, the ERT members should be fully

Figure 3 Some methods to protect medical staff from infection in operation room during the practice of anesthesia scenario. (**A**) The procedure of wearing PPE. Operating scrubs→shoe covers→hand hygiene→n95 mask→surgical mask→hair cover→gloves→protective clothing→boot covers→gloves→goggles/face shield→isolation gown→gloves; (**B**) The procedure of undressing PPE. (Contaminative area) Isolation gown→outer gloves→hand hygiene→new gloves→goggles/face shield→hand hygiene→(first buffer area) unclasp the boot covers→outer gloves→hand hygiene→new gloves→protective clothing→hand hygiene→(second buffer area) hand hygiene→hair cover→gloves→new gloves→surgical mask→n95 mask→hand hygiene→shoe covers and shoes→gloves→(clean area) clean clothing after a bath; (**C**) The area division around negative pressure OR and the patient in-and -out route. Annotate:1) Dedicated elevator for patient transmit, 2) Buffer area, 3) Dedicated OR, 4) Clean OR. Areas in grey mean semi-contaminative area, areas in white are clean while area in yellow is the contaminative area. The red lines represent the in-and-out route for covid-19 patients. The green line is for the evacuation of in-room medical staff; (**D**) The placement of the breath filters.

trained in advance based on safe medical practices and nosocomial infection prevention protocols. Everyone must make sure they can wear and take off the PPE correctly through training and appraisals. Anesthesia drills for different types of operations are required at regular intervals to get continuous feedback and improvements.

Acknowledgements

This project was supported by Medical Affairs Department and Scientific Research department of Guangdong Provincial People's Hospital and the Scientific Research Initial Funding of Guangdong Provincial People's Hospital awarded to Dr. Shu HH.

References

1. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. *https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020* (accessed March 11, 2020).
2. Wang D, Hu B, Hu C, *et al.* Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020; e201585. doi:10.1001/jama.2020.1585.
3. National Health Commission & State Administration of Traditional Chinese Medicine. Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version7). *https://www.who.int/docs/default-source/wpro---documents/countries/china/covid-19-briefing-nhc/1-clinical-protocols-for-the-diagnosis-and-treatment-of-covid-19-v7.pdf?sfvrsn=c6cbfba4_2* (accessed March 3, 2020).
4. van Doremalen N, Bushmaker T, Morris DH, *et al.* Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. N Engl J Med. 2020; doi:10.1056/NEJMc2004973.
5. Chen X, Liu Y, Gong Y, *et al.* Perioperative Management of Patients Infected with the Novel Coronavirus: Recommendation from the Joint Task Force of the Chinese Society of Anesthesiology and the Chinese Association of Anesthesiologists. Anesthesiology. 2020; doi:10.1097/ALN.0000000000003301.
6. Respiratory care committee of Chinese Thoracic Society. Zhonghua Jie He He Hu Xi Za Zhi. 2020; 17:E020. (in Chinese)
7. Wilkes AR, Benbough JE, Speight SE, Harmer M. The bacterial and viral filtration performance of breathing system filters. Anaesthesia. 2000; 55:458-465.
8. Wax RS, Christian MD. Practical recommendations for critical care and anesthesiology teams caring for novel coronavirus (2019-nCoV) patients. Can J Anaesth. 2020; doi: 10.1007/s12630-020-01591-x.

Received April 7, 2020; Revised April 16, 2020; Accepted April 19, 2020.

**Address correspondence to:*
Haihua Shu, Department of Anesthesiology, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, 106# Zhongshan Second Road, Guangzhou, Guangdong, 510080, China.
E-mail: shuhaihua@hotmail.com or 2763346275@qq.com

Released online in J-STAGE as advance publication April 22, 2020.

Communication

DOI: 10.5582/bst.2020.03098

Suggestions on surgical treatment during coronavirus disease 2019 (COVID-19) pandemic

Zhiming Zhao[1], Mengyang Li[2], Rong Liu[1,*]

[1] The Second Department of Hepatopancreatobiliary Surgery, The First Medical Center, Chinese People's Liberation Army General Hospital, Beijing, China;
[2] Department of Hepatopancreatobiliary Surgery, The Fourth Medical Center, Chinese Peoples Liberation Army General Hospital, Beijing, China.

SUMMARY Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2 virus, is now generating a global epidemic, leading to a severe public health emergency. Until April 12, 2020 around 1,700,954 confirmed cases and 105,633 deaths have been reported all over the world. The World Health Organization (WHO) has declared COVID-19 as a Public Health Emergency of International Concern. Under this circumstance, surgical activities should be carefully evaluated to avoid excessive occupation of limited medical resources, and to reduce the possibility of hospital infection. China has achieved an inspiring achievement on epidemic control. Here, we reviewed available studies on surgical activities during the outbreak, in combination with our current experience, with the aim of providing feasible suggestions on surgical issues during the COVID-19 pandemic.

Keywords COVID-19, surgery, public emergency

1. Introduction

With the pandemic of coronavirus disease 2019 (COVID-19), most medical resources have been allocated to its treatment. Diagnosis and treatment of other diseases have been seriously restricted. As far as surgical activities, it is suggested that except for the dedicated COVID-19 center, other hospitals should only provide urgent or time-sensitive elective surgical services after deliberated evaluation (*1*). The exhaustion of medical resources and the risk of infection during operations should be a concern. COVID-19 spreads mainly through respiratory droplets produced by infected patients. Operations, including endoscopy, laparoscopy, intubation and extubation, tend to transmit droplets and aerosols. Surgeons, anesthetists and operating nurses within the operating room are at high risk of exposure and infection. Personal protective equipment (PPE), including N95 mask, disposable medical protective clothing, surgical cap, sterile gloves, goggles and face shield, should be provided to all staff if surgery is recommended (*1*).

2. Emergency surgery

Screening of symptoms and exposure risk should be performed for all patients at time of their arrival. If patient's vital signs are unstable, emergency surgery should be performed, with the highest level of protection measures conducted. If the patient's vital signs are stable, suspected patients should be streamed into dedicated fever clinic, with all non-urgent surgery postponed. For the non-suspected, if operation is needed, further nasopharyngeal swab (NPS) or chest CT still should be examined. After all screening is finished and patients triaged, requested surgical activity is subsequently triggered (*2*).

For suspected or infected patients, a separate path for transport should be pre-designed. A dedicated negative-pressure operating room should be prepared. Experienced surgeons outside the learning curve and standardized surgical procedures are recommended to decrease the operation time, in order to minimize the possibility of infection. Well-trained operating room nurses and anesthesia should be in position for pre-operative preparation. Personal protection equipment is recommended for all participating medical staff (*3*).

Special attention should be paid to pregnant women whose general condition is proved to be worse than others (*4*). Thus, caesarean section for infected or suspected pregnant women is the most challenging emergency surgery. Compared with vaginal delivery, caesarean has the advantage of minimized in-patient stay and reduced physical exertion. Evaluation of

maternal disease condition, fetal intrauterine status and gestational age determines the timing of caesarean (*5*).

Application of laparoscopic technique and energy devices for bleeding control should be carefully evaluated. Zheng *et al*. reported that aerosolization of blood through energy devices has a risk of spreading virus (*6*). This leads to concern about the safety of urgent minimal-invasive surgery, such as appendicectomy and cholecystectomy. Thus, prevention of aerosol dispersal, lowering pneumoperitoneum pressure, lowering electrocautery power setting and using bipolar cautery should be implemented in laparoscopic surgery (*6*).

In addition, some traditional urgent surgeries can be replaced by alternative treatment. Patients with uncomplicated appendicitis can receive interval appendectomy after nonoperative management, which has proved to have a similar outcome compared with upfront appendectomy (*7*). Likewise, percutaneous transhepatic-gallbladder drainage can be performed in acute cholecystitis followed by cholecystectomy in 3 months (*8*). These strategies enable surgery teams to be effectively allocated to other urgent surgeries, and can reduce the utilization frequency of operating rooms.

3. Elective surgery

Comprehensive consideration of limited medical resources and severity of the epidemic are needed to assess the benefit of elective surgery. England's NHS hospitals have suspended non-urgent elective surgery at least 3 months to reserve resources in the face of the COIVD-19 outbreak (*9*). A recent study suggested that the median safe postponement period is 3 weeks (*10*).

For benign diseases, we suggest that follow-up and assessment should be performed though telephone or on-line inquiry, and surgery should be postponed until the end of outbreak.

For oncological diseases, Liang *et al*. reported that cancer patients are more susceptible to coronavirus infection, probably due to compromised immune system and effects of chemotherapy (*11*). The Society of Surgical Oncology (SSO) provided recommendations for the management of different cancers during the COVID-19 outbreak, including breast cancer, colorectal cancer, endocrine tumor, gastric and esophageal cancer, hepato-pancreato-biliary cancer, melanoma, peritoneal surface malignancy and sarcoma (*12*). All these recommendations are based on evidence and reported experiences. However, treatment decisions for each individual patient should be made on a case-by case discussion, considering the cancer biology, alternative strategy, COVID-19 epidemic situation and time schedule of surgery team. We suggest that decisions should be made based on triage. In general, patients can be divided into three categories: *i*). Surgical procedures should be performed without delay; *ii*). Surgery can be postponed without severe impact on prognosis; *iii*). Surgery can be replaced by other treatment (*13*).

The first category refers to patients with severe complications during tumor progression. For example, obstruction or perforation as a complication of colorectal cancer is recommended to receive surgical treatment (*12*).

For the second category, postponed surgery may reduce the possibility of infection with no significant impact on prognosis (*14*). A recent study from Annals of Surgery proved that surgery can be postponed for at least 4 weeks in 48% of cancer types. For patients receiving neoadjuvant chemotherapy, the median safe postponement period is 8 weeks (*10*).

For those surgeries that can be replaced by other treatment, we should note that chemotherapy is reported to raise susceptibility to COVID-19 (*11*). In addition, checkpoint inhibitors, which can induce hyperinflammation, may raise a cytokine storm and interstitial mononuclear inflammatory infiltration in a pneumonia condition theoretically. Lung immune-related adverse events caused by immune-check point inhibitors can overlap with COVID-19 related pneumonia (*15*). Thus, decisions should be made deliberately based on evidence and MDT discussion. Accordingly, intensive personal protection should be provided to these patients to decrease the risk of infection.

Robot assisted surgery has the advantage of reducing hospital stay and minimizing the number of directly exposed medical staff. Additionally, with support of robotic arms, pneumoperitoneum pressure can be reduced to 8 mmHg. It can decrease the infection risk for both patients and surgeons. Thus, it is suggested to be implemented for undeferred complex oncological surgery (*16*).

4. Anesthesia

Because of the transmission pathway through respiratory droplets, anesthesia, involving intubation and extubation, is at an extremely high risk. Awakened intubation should be prohibited. During the intubation and extubation operation, non-essential staff should leave the operating room. All items should obey the "Only-In" principle. The anesthetist and other remaining staff should wear FFP3 filter and personal protective equipment properly, and a negative pressure operating room is necessary (*3*). In the pandemic area, anesthesiologists and ventilators limit general anesthesia. Even for urgent surgery, epidural anesthesia and local anesthesia should be considered under some circumstances (*17*).

5. Patient transport

Transport of diagnosed or suspected infected patients

Table 1. Key points of suggestions

Emergency surgery
1). Screening of symptoms and exposure risk should be performed followed by NPS.
2). For infected or suspected patients, highest level of PPE should be implemented.
3). Separate path should be pre-designed.
4). Dedicated negative-pressure operating room and experienced surgeon are recommended.
5). Application of laparoscopy and energy devices should be carefully evaluated.
6). For infected pregnant women, caesarean is recommended compared with vaginal delivery.
7). Traditional emergency surgery can be replaced by alternative treatment.

Elective surgery
1). For infected patients, elective surgery should be postponed
2). For uninfected patients, non-time-sensitive elective surgery should be postponed
3). For benign disease, telemedicine is suggested for follow-up and assessment
4). For malignancies, surgery can be performed when a severe complication occurs. Otherwise, median safe postponement period is 3 weeks.
5). Robot assisted surgery has advantage of minimizing the number of exposed surgeons in complex oncological surgery

Anesthesia
1). Non-essential staff should not enter the operating room during Intubation and extubation.
2). Epidural anesthesia and local anesthesia is suggested under some circumstances.

Patient transport
1). For non-intubated patient with no oxygen requirement, surgical mask is suggested.
2). For non-intubated patient with oxygen requirement, face mask over surgical mask is recommended.
3). For intubated patient, dedicated ventilator rather than bag-valve mask is suggested.
4). Disinfection measures must be taken immediately if contamination occurs during transport.

before or after surgery needs proper protection measures. A non-intubated patient is recommended to wear a surgical mask. Oxygen can be delivered through face mask over surgical mask. Intubated patient should be transported with ventilator rather than bag-valve mask. Staff participating in transport should wear proper personal protection equipment (*18*). A separate path must be pre-designed to segregate these patients from the non-infected. Make sure that there is no extra stay in anesthetic bay and recovery room before arriving at the dedicated COVID-19 ward (*3*). If contamination occurs in transport, disinfection measures must be taken by dedicated cleaning team.

6. Blood supply

The sudden outbreak of COVID-19 has seriously influenced blood supply. Decreased donation limits the reservation of blood derivatives, which in turn, restricts surgical activity. In addition, blood safety also raises attention. Although SARS-CoV-2 virus mainly affects respiratory tract, viral RNA also can be detected in plasma and serum, resulting in the theoretical possibility of transmission through blood products. It has been reported that Wuhan Blood Center has already started to test for SARS-CoV-2 RNA from blood donations since February 10. Meanwhile, appropriate measures are taken to exclude suspected infected donors, including: *i*). Taking body temperature before donation; *ii*). Detailed inquiry for related symptoms, and travel history to epidemic areas within 28 days of donors and relatives; *iii*). Telephone follow-up to all blood donors for subsequent physical condition after donation; *iv*). Callback blood products from infected donors (*19*).

7. Conclusion

Except for all the recommendation above (Table1), we suggest that surgical schedule should be made based on careful consideration of the availability of dedicated operating room, the exhaustion of PPE, the demand for ICU beds, the need for blood supply, etc. Standardized treatment procedures for diagnosed or suspected COVID-19 patients must be provided to all relative staff. Don and doff of PPE must be strictly trained for all medical staff. Face-to-face interaction is recommended only in emergencies. Telemedicine should be considered whenever possible to reduce the risk of exposure. We should note that present control interventions are based on symptoms. Primary screening is performed for patients with relative symptoms, such as fever and cough. Asymptomatic carriers, may be a blind spot of COVID-19 screening, leading to an unanticipated resurgence (*20*). Exposure risk evaluation and contact history inquiry can help to identify some asymptomatic patients, but the rest may be a threat to medical security. Although most of the asymptomatic patients are in the incubation period, and will develop relative symptoms later, we are still concerned about the presence of chronic asymptomatic carriers, just like Typhoid Mary.

Abating spread has not been observed all over the world, and limitations of medical resources may not be improved in a short time period. We still need a long time to overcome this difficulty. The present suggestions are based on published studies and our experience in clinical practice during the COVID-19 outbreak in China. We wish that these recommendations are useful in the battle against COVID-19 infection around the world.

References

1. Huang Z, Zhao S, Li Z, Chen W, Zhao L, Deng L, Song B. The battle against Coronavirus Disease 2019 (COVID-19): Emergency management and infection control in a radiology department. J Am Coll Radiol. 2020; pii: S1546-1440(20)30285-4. doi: 10.1016/j.jacr.2020.03.011.
2. Ti LK, Ang LS, Foong TW, Ng BSW. What we do when a COVID-19 patient needs an operation: operating room preparation and guidance. Can J Anaesth. 2020; doi: 10.1007/s12630-020-01617-4.
3. Coccolini F, Perrone G, Chiarugi M, *et al*. Surgery in COVID-19 patients: operational directives. World J Emerg Surg. 2020; 15:25.
4. Parazzini F, Bortolus R, Mauri PA, Favilli A, Gerli S, Ferrazzi E. Delivery in pregnant women infected with SARS-CoV-2: A fast review. Int J Gynaecol Obstet. 2020; doi: 10.1002/ijgo.13166.
5. Qi H, Luo X, Zheng Y, Zhang H, Li J, Zou L, Feng L, Chen D, Shi Y, Tong C, Baker PN. Safe delivery for COVID-19 infected pregnancies. BJOG. 2020; doi: 10.1111/1471-0528.16231.
6. Zheng MH, Boni L, Fingerhut A. Minimally invasive surgery and the novel coronavirus outbreak: lessons learned in China and Italy. Ann Surg. 2020; doi: 10.1097/SLA.0000000000003924.
7. Polites SF, Azarow KS. Perspectives on Pediatric Appendicitis and Appendectomy During the Severe Acute Respiratory Syndrome Coronavirus 2 Pandemic. J Laparoendosc Adv Surg Tech A. 2020.
8. Tan HY, Jiang DD, Li J, He K, Yang K. Percutaneous Transhepatic Gallbladder Drainage Combined with Laparoscopic Cholecystectomy: A Meta-Analysis of Randomized Controlled Trials. J Laparoendosc Adv Surg Tech A. 2018; 28:248-255.
9. Iacobucci G. Covid-19: all non-urgent elective surgery is suspended for at least three months in England. BMJ. 2020; 368:m1106.
10. Turaga K, Girotra S. Are we harming cancer patients by delaying their cancer surgery during the COVID-19 pandemic? Ann Surg. 2020; *https://journals.lww.com/annalsofsurgery/Documents/Are%20we%20harming%20cancer%20patients.pdf* (accessed April 22, 2020).
11. Liang W, Guan W, Chen R, Wang W, Li J, Xu K, Li C, Ai Q, Lu W, Liang H, Li S, He J. Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. Lancet Oncol. 2020; 21:335-337.
12. Bartlett DL, Howe JR, Chang G, *et al*. Management of cancer surgery cases during the COVID-19 pandemic: considerations. Ann Surg Oncol. 2020; doi: 10.1245/s10434-020-08461-2.
13. Ficarra V, Novara G, Abrate A, *et al*. Urology practice during COVID-19 pandemic. Minerva Urol Nefrol. 2020; doi: 10.23736/S0393-2249.20.03846-1.
14. Lambertini M, Toss A, Passaro A, Criscitiello C, Cremolini C, Cardone C, Loupakis F, Viscardi G, Meattini I, Dieci MV, Ferrara R, Giusti R, Maio MD. Cancer care during the spread of coronavirus disease 2019 (COVID-19) in Italy: young oncologists' perspective. ESMO Open. 2020; 5. pii: e000759.
15. Bersanelli M. Controversies about COVID-19 and anticancer treatment with immune checkpoint inhibitors. Immunotherapy. Immunotherapy. 2020; doi: 10.2217/imt-2020-0067.
16. Kimmig R, Verheijen RHM, Rudnicki M, Council S. Robot assisted surgery during the COVID-19 pandemic, especially for gynecological cancer: a statement of the Society of European Robotic Gynaecological Surgery (SERGS). J Gynecol Oncol. 2020;31:e59. doi: 10.3802/jgo.2020.31.e59.
17. Wax RS, Christian MD. Practical recommendations for critical care and anesthesiology teams caring for novel coronavirus (2019-nCoV) patients. Can J Anaesth. 2020; 67:568-576.
18. Givi B, Schiff BA, Chinn SB, Clayburgh D, Iyer NG, Jalisi S, Moore MG, Nathan CA, Orloff LA, O'Neill JP, Parker N, Zender C, Morris LGT, Davies L. Safety Recommendations for Evaluation and Surgery of the Head and Neck During the COVID-19 Pandemic. JAMA Otolaryngol Head Neck Surg. 2020; doi: 10.1001/jamaoto.2020.0780.
19. Chang L, Yan Y, Wang L. Coronavirus Disease 2019: Coronaviruses and Blood Safety. Transfus Med Rev. 2020; pii: S0887-7963(20)30014-6. doi: 10.1016/j.tmrv.2020.02.00320.
    Ye F, Xu S, Rong Z, Xu R, Liu X, Deng P, Liu H, Xu X. Delivery of infection from asymptomatic carriers of COVID-19 in a familial cluster. Int J Infect Dis. 2020; pii: S1201-9712(20)30174-0. doi: 10.1016/j.ijid.2020.03.042

Received April 12, 2020; Revised April 24, 2020; Accepted April 25, 2020.

**Address correspondence to:*
Rong Liu, The Second Department of Hepatopancreatobiliary Surgery, The First Medical Center, Chinese People's Liberation Army General Hospital, Beijing 100853, China.
E-mail: liurong301@126.com

Released online in J-STAGE as advance publication April 27, 2020.

BioScience Trends. 2020; 14(3):E2.

DOI: 10.5582/bst.2020.E2

RETRACTED: Polyphosphate-induced matrix metalloproteinase-3-mediated proliferation in rat dental pulp fibroblast-like cells is mediated by a Wnt5 signaling cascade.

This article entitled “Polyphosphate-induced matrix metalloproteinase-3-mediated proliferation in rat dental pulp fibroblast-like cells is mediated by a Wnt5 signaling cascade” (*1*) has been retracted at the request of the authors due to research misconduct.

Reference

1. Ozeki N, Yamaguchi H, Hase N, Hiyama T, Kawai R, Kondo A, Nakata K, Mogi M. Polyphosphate-induced matrix metalloproteinase-3-mediated proliferation in rat dental pulp fibroblast-like cells is mediated by a Wnt5 signaling cascade. *Biosci Trends. 2015; 9(3):160-168. DOI: 10.5582/ bst.2015.01041.*

BioScience Trends. 2020; 14(3):E3.

DOI: 10.5582/bst.2020.E3

RETRACTED: Products of dentin matrix protein-1 degradation by interleukin-1β-induced matrix metalloproteinase-3 promote proliferation of odontoblastic cells.

This article entitled “Products of dentin matrix protein-1 degradation by interleukin-1β-induced matrix metalloproteinase-3 promote proliferation of odontoblastic cells” (*1*) has been retracted at the request of the authors due to research misconduct.

Reference

1. Hase N, Ozeki N, Hiyama T, Yamaguchi H, Kawai R, Kondo A, Nakata K, Mogi M. Products of dentin matrix protein-1 degradation by interleukin-1β-induced matrix metalloproteinase-3 promote proliferation of odontoblastic cells. *Biosci Trends. 2015; 9(4):228-236. DOI: 10.5582/bst.2015.01092.*

BST
BioScience Trends

Guide for Authors

1. Scope of Articles

BioScience Trends (Print ISSN 1881-7815, Online ISSN 1881-7823) is an international peer-reviewed journal. *BioScience Trends* devotes to publishing the latest and most exciting advances in scientific research. Articles cover fields of life science such as biochemistry, molecular biology, clinical research, public health, medical care system, and social science in order to encourage cooperation and exchange among scientists and clinical researchers.

2. Submission Types

Original Articles should be well-documented, novel, and significant to the field as a whole. An Original Article should be arranged into the following sections: Title page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments, and References. Original articles should not exceed 5,000 words in length (excluding references) and should be limited to a maximum of 50 references. Articles may contain a maximum of 10 figures and/or tables. Supplementary Data are permitted but should be limited to information that is not essential to the general understanding of the research presented in the main text, such as unaltered blots and source data as well as other file types.

Brief Reports definitively documenting either experimental results or informative clinical observations will be considered for publication in this category. Brief Reports are not intended for publication of incomplete or preliminary findings. Brief Reports should not exceed 3,000 words in length (excluding references) and should be limited to a maximum of 4 figures and/or tables and 30 references. A Brief Report contains the same sections as an Original Article, but the Results and Discussion sections should be combined.

Reviews should present a full and up-to-date account of recent developments within an area of research. Normally, reviews should not exceed 8,000 words in length (excluding references) and should be limited to a maximum of 10 figures and/or tables and 100 references. Mini reviews are also accepted, which should not exceed 4,000 words in length (excluding references) and should be limited to a maximum of 5 figures and/or tables and 50 references.

Policy Forum articles discuss research and policy issues in areas related to life science such as public health, the medical care system, and social science and may address governmental issues at district, national, and international levels of discourse. Policy Forum articles should not exceed 3,000 words in length (excluding references) and should be limited to a maximum of 5 figures and/or tables and 30 references.

Communications are short, timely pieces that spotlight new research findings or policy issues of interest to the field of global health and medical practice that are of immediate importance. Depending on their content, Communications will be published as "Comments" or "Correspondence". Communications should not exceed 1,500 words in length (excluding references) and should be limited to a maximum of 2 figures and/or tables and 20 references.

Editorials are short, invited opinion pieces that discuss an issue of immediate importance to the fields of global health, medical practice, and basic science oriented for clinical application. Editorials should not exceed 1,000 words in length (excluding references) and should be limited to a maximum of 10 references. Editorials may contain one figure or table.

News articles should report the latest events in health sciences and medical research from around the world. News should not exceed 500 words in length.

Letters should present considered opinions in response to articles published in *BioScience Trends* in the last 6 months or issues of general interest. Letters should not exceed 800 words in length and may contain a maximum of 10 references. Letters may contain one figure or table.

3. Editorial Policies

For publishing and ethical standards, BioScience Trends follows the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (*http://www.icmje.org/recommendations*) issued by the International Committee of Medical Journal Editors (ICMJE), and the Principles of Transparency and Best Practice in Scholarly Publishing (*https://doaj.org/bestpractice*) jointly issued by the Committee on Publication Ethics (COPE), the Directory of Open Access Journals (DOAJ), the Open Access Scholarly Publishers Association (OASPA), and the World Association of Medical Editors (WAME).

BioScience Trends will perform an especially prompt review to encourage innovative work. All original research will be subjected to a rigorous standard of peer review and will be edited by experienced copy editors to the highest standards.

Ethics: *BioScience Trends* requires that authors of reports of investigations in humans or animals indicate that those studies were formally approved by a relevant ethics committee or review board. For research involving human experiments, a statement that the participants gave informed consent before taking part (or a statement that it was not required and why) should be indicated. Authors should also state that the study conformed to the provisions of the Declaration of Helsinki (as revised in 2013). When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.

Conflict of Interest: All authors are required to disclose any actual or potential conflict of interest including financial interests or relationships with other people or organizations that might raise questions of bias in the work reported. If no conflict of interest exists for each author, please state "There is no conflict of interest to disclose".

Submission Declaration: When a manuscript is considered for submission to *BioScience Trends*, the authors should confirm that 1) no part of this manuscript is currently under consideration for publication elsewhere; 2) this manuscript does not contain the same information in whole or in part as manuscripts that have been published, accepted, or are under review elsewhere, except in the form of an abstract, a letter to

the editor, or part of a published lecture or academic thesis; 3) authorization for publication has been obtained from the authors' employer or institution; and 4) all contributing authors have agreed to submit this manuscript.

Cover Letter: The manuscript must be accompanied by a cover letter prepared by the corresponding author on behalf of all authors. The letter should indicate the basic findings of the work and their significance. The letter should also include a statement affirming that all authors concur with the submission and that the material submitted for publication has not been published previously or is not under consideration for publication elsewhere. The cover letter should be submitted in PDF format. For example of Cover Letter, please visit: Download Centre (*https://ircabssagroup.com/downcentre*).

Copyright: When a manuscript is accepted for publication in *BioScience Trends*, the transfer of copyright is necessary. A JOURNAL PUBLISHING AGREEMENT (JPA) form will be e-mailed to the authors by the Editorial Office and must be returned by the authors as a scan. Only forms with a hand-written signature are accepted. This copyright will ensure the widest possible dissemination of information. Please note that your manuscript will not proceed to the next step in publication until the JPA Form is received. In addition, if excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article.

Peer Review: *BioScience Trends* uses single-blind peer review, which means that reviewers know the names of the authors, but the authors do not know who reviewed their manuscript. The external peer review is performed for research articles by at least two reviewers, and sometimes the opinions of more reviewers are sought. Peer reviewers are selected based on their expertise and ability to provide high quality, constructive, and fair reviews. For research manuscripts, the editors may, in addition, seek the opinion of a statistical reviewer. Consideration for publication is based on the article's originality, novelty, and scientific soundness, and the appropriateness of its analysis.

Suggested Reviewers: A list of up to 3 reviewers who are qualified to assess the scientific merit of the study is welcomed. Reviewer information including names, affiliations, addresses, and e-mail should be provided at the same time the manuscript is submitted online. Please do not suggest reviewers with known conflicts of interest, including participants or anyone with a stake in the proposed research; anyone from the same institution; former students, advisors, or research collaborators (within the last three years); or close personal contacts. Please note that the Editor-in-Chief may accept one or more of the proposed reviewers or may request a review by other qualified persons.

Language Editing: Manuscripts prepared by authors whose native language is not English should have their work proofread by a native English speaker before submission. If not, this might delay the publication of your manuscript in *BioScience Trends*.

The Editing Support Organization can provide English proofreading, Japanese-English translation, and Chinese-English translation services to authors who want to publish in *BioScience Trends* and need assistance before submitting a manuscript. Authors can visit this organization directly at *http://www.iacmhr.com/iac-eso/support.php?lang=en*. IAC-ESO was established to facilitate manuscript preparation by researchers whose native language is not English and to help edit works intended for international academic journals.

4. Manuscript Preparation

Manuscripts are suggested to be prepared in accordance with the "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals", as presented at *http://www.ICMJE.org*.

Manuscripts should be written in clear, grammatically correct English and submitted as a Microsoft Word file in a single-column format. Manuscripts must be paginated and typed in 12-point Times New Roman font with 24-point line spacing. Please do not embed figures in the text. Abbreviations should be used as little as possible and should be explained at first mention unless the term is a well-known abbreviation (*e.g.* DNA). Single words should not be abbreviated.

Title page: The title page must include 1) the title of the paper (Please note the title should be short, informative, and contain the major key words); 2) full name(s) and affiliation(s) of the author(s), 3) abbreviated names of the author(s), 4) full name, mailing address, telephone/fax numbers, and e-mail address of the corresponding author; and 5) conflicts of interest (if you have an actual or potential conflict of interest to disclose, it must be included as a footnote on the title page of the manuscript; if no conflict of interest exists for each author, please state "There is no conflict of interest to disclose"). Please visit Download Centre and refer to the title page of the manuscript sample.

Abstract: The abstract should briefly state the purpose of the study, methods, main findings, and conclusions. For articles that are Original Articles, Brief Reports, Reviews, or Policy Forum articles, a one-paragraph abstract consisting of no more than 250 words must be included in the manuscript. For Communications, Editorials, News, or Letters, a brief summary of main content in 150 words or fewer should be included in the manuscript. Abbreviations must be kept to a minimum and non-standard abbreviations explained in brackets at first mention. References should be avoided in the abstract. Three to six key words or phrases that do not occur in the title should be included in the Abstract page.

Introduction: The introduction should be a concise statement of the basis for the study and its scientific context.

Materials and Methods: The description should be brief but with sufficient detail to enable others to reproduce the experiments. Procedures that have been published previously should not be described in detail but appropriate references should simply be cited. Only new and significant modifications of previously published procedures require complete description. Names of products and manufacturers with their locations (city and state/country) should be given and sources of animals and cell lines should always be indicated. All clinical investigations must have been conducted in accordance with Declaration of Helsinki principles. All human and animal studies must have been approved by the appropriate institutional review board(s) and a specific declaration of approval must be made within this section.

Results: The description of the experimental results should be succinct but in sufficient detail to allow the experiments to be analyzed and interpreted by an independent reader. If necessary, subheadings may be used for an orderly presentation. All figures and tables must be referred to in the text.

Discussion: The data should be interpreted concisely without repeating material already presented in the Results section. Speculation is permissible, but it must be well-founded, and discussion of the wider implications of the findings is encouraged. Conclusions derived from the study should be included in this section.

Acknowledgments: All funding sources should be credited in the Acknowledgments section. In addition, people who contributed to the work but who do not meet the criteria for authors should be listed along with their contributions.

References: References should be numbered in the order in which they appear in the text. Citing of unpublished results, personal communications, conference abstracts, and theses in the reference list is not recommended but these sources may be mentioned in the text. In the reference list, cite the names of all authors when there are fifteen or fewer authors; if there are sixteen or more authors, list the first three followed by *et al*. Names of journals should be abbreviated in the style used in PubMed. Authors are responsible for the accuracy of the references. The EndNote Style of *BioScience Trends* could be downloaded at **EndNote** (*https://ircabssagroup.com/examples/BioScience_Trends.ens*).

Examples are given below:

Example 1 (Sample journal reference):

Inagaki Y, Tang W, Zhang L, Du GH, Xu WF, Kokudo N. Novel aminopeptidase N (APN/CD13) inhibitor 24F can suppress invasion of hepatocellular carcinoma cells as well as angiogenesis. Biosci Trends. 2010; 4:56-60.

Example 2 (Sample journal reference with more than 15 authors):

Darby S, Hill D, Auvinen A, *et al*. Radon in homes and risk of lung cancer: Collaborative analysis of individual data from 13 European case-control studies. BMJ. 2005; 330:223.

Example 3 (Sample book reference):

Shalev AY. Post-traumatic stress disorder: Diagnosis, history and life course. In: Post-traumatic Stress Disorder, Diagnosis, Management and Treatment (Nutt DJ, Davidson JR, Zohar J, eds.). Martin Dunitz, London, UK, 2000; pp. 1-15.

Example 4 (Sample web page reference):

World Health Organization. The World Health Report 2008 – primary health care: Now more than ever. *http://www.who.int/whr/2008/whr08_en.pdf* (accessed September 23, 2010).

Tables: All tables should be prepared in Microsoft Word or Excel and should be arranged at the end of the manuscript after the References section. Please note that tables should not in image format. All tables should have a concise title and should be numbered consecutively with Arabic numerals. If necessary, additional information should be given below the table.

Figure Legend: The figure legend should be typed on a separate page of the main manuscript and should include a short title and explanation. The legend should be concise but comprehensive and should be understood without referring to the text. Symbols used in figures must be explained. Any individually labeled figure parts or panels (A, B, *etc*.) should be specifically described by part name within the legend.

Figure Preparation: All figures should be clear and cited in numerical order in the text. Figures must fit a one- or two-column format on the journal page: 8.3 cm (3.3 in.) wide for a single column, 17.3 cm (6.8 in.) wide for a double column; maximum height: 24.0 cm (9.5 in.). Please make sure that the symbols and numbers appeared in the figures should be clear. Please make sure that artwork files are in an acceptable format (TIFF or JPEG) at minimum resolution (600 dpi for illustrations, graphs, and annotated artwork, and 300 dpi for micrographs and photographs). Please provide all figures as separate files. Please note that low-resolution images are one of the leading causes of article resubmission and schedule delays.

Units and Symbols: Units and symbols conforming to the International System of Units (SI) should be used for physicochemical quantities. Solidus notation (*e.g*. mg/kg, mg/mL, mol/mm^2/min) should be used. Please refer to the SI Guide *www.bipm.org/en/si/* for standard units.

Supplemental data: Supplemental data might be useful for supporting and enhancing your scientific research and *BioScience Trends* accepts the submission of these materials which will be only published online alongside the electronic version of your article. Supplemental files (figures, tables, and other text materials) should be prepared according to the above guidelines, numbered in Arabic numerals (*e.g.*, Figure S1, Figure S2, and Table S1, Table S2) and referred to in the text. All figures and tables should have titles and legends. All figure legends, tables and supplemental text materials should be placed at the end of the paper. Please note all of these supplemental data should be provided at the time of initial submission and note that the editors reserve the right to limit the size and length of Supplemental Data.

5. Submission Checklist

The Submission Checklist will be useful during the final checking of a manuscript prior to sending it to *BioScience Trends* for review. Please visit Download Centre and download the Submission Checklist file.

6. Online Submission

Manuscripts should be submitted to *BioScience Trends* online at *http://www.biosciencetrends.com*. The manuscript file should be smaller than 5 MB in size. If for any reason you are unable to submit a file online, please contact the Editorial Office by e-mail at *office@biosciencetrends.com*

7. Accepted Manuscripts

Proofs: Galley proofs in PDF format will be sent to the corresponding author *via* e-mail. Corrections must be returned

to the editor (*proof-editing@biosciencetrends.com*) within 3 working days.

Offprints: Authors will be provided with electronic offprints of their article. Paper offprints can be ordered at prices quoted on the order form that accompanies the proofs.

Page Charge: Page charges will be levied on all manuscripts accepted for publication in *BioScience Trends* ($140 per page for black white pages; $340 per page for color pages). Under exceptional circumstances, the author(s) may apply to the editorial office for a waiver of the publication charges at the time of submission.

Misconduct: *BioScience Trends* takes seriously all allegations of potential misconduct and adhere to the ICMJE Guideline (*http://www.icmje.org/recommendations*) and COPE Guideline (*http://publicationethics.org/files/Code_of_conduct_for_journal_editors.pdf*). In cases of suspected research or publication misconduct, it may be necessary for the Editor or Publisher to contact and share submission details with third parties including authors' institutions and ethics committees. The corrections, retractions, or editorial expressions of concern will be performed in line with above guidelines.

(As of June 2020)

BioScience Trends
Editorial and Head Office
Pearl City Koishikawa 603,
2-4-5 Kasuga, Bunkyo-ku,
Tokyo 112-0003, Japan.
E-mail: office@biosciencetrends.com