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Editorial

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The strategy behind Japan's response to COVID-19 from 2020-2021 and future challenges posed by the uncertainty of the Omicron variant in 2022

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SUMMARY Japan has experienced five waves of the COVID-19 pandemic so far. Four states of emergency were declared, and the Tokyo 2020 Olympic (July 23-August 8, 2021) and Paralympic Games (August 24-September 5, 2021) were held during the fifth wave of the pandemic. Although a record 5,773 new cases were reported in Tokyo on August 13, the number abruptly decreased afterwards, and only 9 new cases were confirmed in Tokyo on November 1, 2021. The high vaccination rates (79.2% of the total population has received the first dose and 77.8% has received the second dose as of December 24, 2021) and behavioral changes (such as mask wearing rate in public places remains close to 100%) are considered to be important factors in curbing the spread of the virus. However, the new Omicron variant poses future challenges due to its uncertainty. A cumulative total of 231 cases of the Omicron variant were reported in Japan between November 30 and December 25, 2021. Preliminary data indicated that the Omicron variant could be more contagious but less deadly than the Delta variant. Since mankind may be forced to coexist with COVID-19, efforts such as vaccination campaigns will need to continue and behavioral changes will become increasingly important as the "new normal" to reduce population density and contact with people. This is evinced at least in Japan's successful practices in fighting the past five waves of the pandemic.

Keywords COVID-19, SARS-CoV-2, Delta variant, Omicron variant, vaccination, behavioral changes, Japan

COVID-19 has spread around the world since the first case was identified in December 2019, and has become a public health emergency of international concern (*1-3*). In Japan, the first domestic case of COVID-19 transmission was reported on January 16, 2020 (*4*), and the pandemic is about to enter its third year. COVID-19 was designated as a designated infectious disease as of February 1, 2020 and then classified under pandemic influenza as of February 13, 2021 (*5*). Japan's basic policy for COVID-19 is to curb the outbreak of infection, maintain the medical care provision system, and focus on dealing with the severely ill.

Japan has experienced five waves of the COVID-19 pandemic so far (Figure 1). As one of the most important response strategies, four states of emergency have been declared since April 2020, three of which were declared in 2021. During this period, Japan hosted the Tokyo 2020 Olympic (July 23-August 8, 2021) and Paralympic Games (August 24-September 5, 2021) and began a massive vaccination campaign.

Instead of a complete lockdown since the outbreak of COVID-19, Japan has been trying to control the infection through self-restraint request policy. Thankfully, personal protective measures were thoroughly implemented, such as wearing masks, handwashing, and avoiding confined spaces, crowded places, and close-contact settings. More importantly, the behavioral changes adopted to contain COVID-19 during the four declared states of emergency reduced population density and contact with people, including teleworking and staggered office hours, curbing the flow of people during vacation week (*6,7*); facilities, shops, restaurants and bars that were considered to be at higher risk for COVID-19 transmission (*e.g.*, those associated with nighttime activities) were requested to close or reduce their business hours.

During the Tokyo Summer Olympics, Japan experienced its fifth wave of the pandemic, with a high number of new infections each day; a record 5,773 new cases were reported in Tokyo on August 13, and 25,975 new infections were reported nationwide on August 20, 2021 (*8*). But with the lifting of the fourth national state of emergency on September 30, 2021, the nationwide

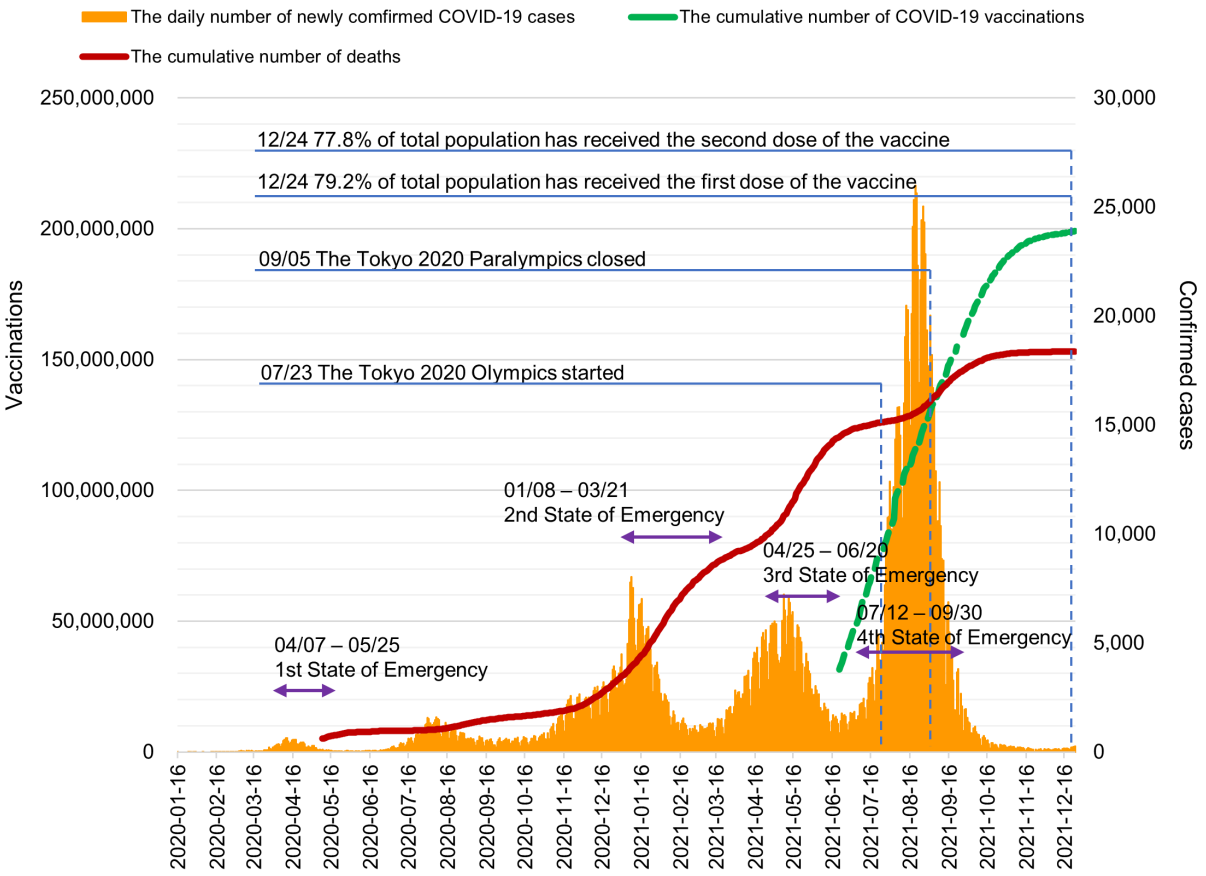

Figure 1. Number of cases reported with COVID-19 and the national response to the pandemic in Japan. Data source: *https://www.mhlw.go.jp/stf/covid-19/kokunainohasseijoukyou.html*

pandemic was effectively contained and the number of new confirmed cases abruptly decreased. According to the Ministry of Health, Labour, and Welfare (*8*), the number of confirmed COVID-19 cases in Japan fell below 1,000 per day starting on October 7, and 227 cases were confirmed across Japan on October 31, 2021. In Tokyo, only 9 new cases were confirmed on November 1, 2021.

Many experts have agreed that the reasons for the decrease in the number of infections were "effective decrease in human flow", "thorough infection control measures", "the effectiveness of vaccines", and "weather conditions" (*9*). High vaccination rates and the universal wearing of masks in particular are considered to be important factors in curbing the spread of the virus.

Although the vaccination campaign in Japan started late, the pace of progress has been impressive. According to data from Prime Minister's Office of Japan (*10*), the total number of vaccine doses administered as of December 24, 2021 has reached 199,120,144. Nationwide, 79.2% of the total population has received the first dose of the vaccine and 77.8% has received the second dose; 92.0% of the population age 65 or older received the first dose of vaccine and 91.7% of that population received the second dose. Vaccination campaigns are actively encouraging a third dose, and 385,209 people have received the third dose.

Another factor is the universal wearing of masks. In fact, "being courteous when coughing", "complying with social distancing", and "wearing masks" seem to ingrained habits that are followed during flu season in Japan. Since the outbreak of COVID-19, the government has been urging the general public through websites, television, and other media to thoroughly implement personal protective measures, including wearing masks. This was requested by the government and not mandated for the general public to obey (*e.g.*, fines were not imposed), but thankfully these patterns of behavior have been fully adopted and the current rate of mask wearing among the public is still close to 100%. Such a pattern of behavior, consciously adopted by the general public in Japan, differs from some countries that have dropped requirements for face coverings indoors and in other settings.

Although the past five waves of the pandemic have been effectively contained nationwide, the new Omicron variant poses future challenges because of its uncertainty. Since the first case of infection with the Omicron strain was reported by South Africa to the WHO on November 24, 2021, the Omicron variant had been identified in 110 countries across all six WHO Regions as of December 22, 2021 (*11*). In Japan, the first case of infection with the Omicron strain was confirmed on November 30, 2021 (*12*); who entered the country from Namibia in

Southern Africa. As of December 25, 2021, Japan had reported a cumulative total of 231 cases of the Omicron variant (*13*), with the majority of those detected through airport and quarantine testing. Concerns are building again in Japan as more infections involving the Omicron variant continue to emerge.

Data on experimental evaluation of and epidemiological information on the Omicron strain is mounting but nonetheless limited. Preliminary data indicated that the transmission of the Omicron strain is much higher than that of the Delta strain in countries with documented community transmission, with a doubling time of 2-3 days (*11*). Although the severity of the disease in individuals infected with the Omicron strain needs to be studied further over a sufficient period of observation while determining factors such as age, history of SARS-CoV-2 infection, and vaccination history, initial research suggests that it is less deadly than the Delta variant. This means Omicron may be less risky for each of us (lower severity), but riskier for all of us (higher transmissibility). Given the persistent mutation of the virus, if the SARS-CoV-2 virus becomes more transmissible and it continues to coexist with human beings over the long term, then presumably the disease's severity will decrease and it will become akin to "another type of influenza".

In Japan, the uncertainty of the Omicron variant and a potential sixth wave of the pandemic represent challenges for the future. National measures will continue, including vaccination campaigns, border quarantine, domestic surveillance of mutant strains *via* PCR testing, and genomic surveillance. In addition, the Japanese Government and experts in Japan continue to highly recommend basic infection control measures by individuals.

Since mankind may be forced to coexist with COVID-19, efforts such as vaccination campaigns will need to continue and behavioral changes will become increasingly important as the "new normal" to reduce population density and contact with people. This is evinced at least in Japan's successful practices in fighting the past five waves of the pandemic.

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Review

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Effect of dehydroepiandrosterone on atherosclerosis in postmenopausal women

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SUMMARY In China, cardiovascular disease (CVD) has surpassed malignant tumours to become the disease with the highest mortality rate, and atherosclerosis (AS) is an important pathological cause of CVD. Dehydroepiandrosterone (DHEA) is the most abundant steroid hormone in circulating human blood and is a precursor of estrogen and androgen. DHEA is converted into a series of sex hormones in local peripheral tissues where its acts physiologically. DHEA also acts therapeutically, thereby avoiding the adverse systemic reactions to sex hormones. DHEA inhibits AS, thus inhibiting the development of CVD, and it improves the prognosis for CVD. The incidence of CVD in postmenopausal women is substantially higher than that in premenopausal women, and that incidence is believed to be related to a decrease in ovarian function. The current review analyzes the mechanisms of postmenopausal women's susceptibility to AS. They tend to have dyslipidemia, and their vascular smooth muscle cells (VSMCs) proliferate and migrate more. In addition, oxidative stress and the inflammatory response of endothelial cells (ECs) are more serious in postmenopausal women. This review also discusses how DHEA combats AS by countering these mechanisms, which include regulating the blood lipid status, protecting ECs (including coping with oxidative stress and inflammatory reactions of the vascular endothelium, inhibiting apoptosis of ECs, and inducing NO production) and inhibiting the proliferation and migration of VSMCs. As a result, DHEA has great value in preventing AS and inhibiting its progression in postmenopausal women.

Keywords dehydroepiandrosterone, atherosclerosis, postmenopause, vascular smooth muscle cells, endothelial cells, blood lipid

1. Introduction

Cardiovascular disease (CVD) is a common disease that jeopardizes the health of postmenopausal women (*1*) and atherosclerosis (AS) is the most critical pathological cause of CVD (*2*). Premenopausal women rarely suffer from CVD. However, the incidence of CVD in postmenopausal women is 2-6 times higher than that in premenopausal women of the same age group (*3*), due to its close relationship to a postmenopausal estrogen deficiency (*4*) (Figure 1). A study (*5*) involving 879 women suggested that menopause was significantly associated with the risk of developing carotid plaques. Females with an earlier onset of menopause (< 45 years) had a significantly higher atherosclerotic plaque volume than those with an intermediate (45-52 years) or later onset of menopause (> 52 years), irrespective of other cardiovascular risk factors (*6*). The mean carotid intima-media thickness (CIMT) of the common carotid artery in postmenopausal women was significantly thicker than that in premenopausal women, with a mean difference of 0.068 mm (*7*). A recent prospective cohort study (*8*) also found that an elevated or persistently high level of Aβ1- 40, an aging peptide, is related to the rate of progression of subclinical AS in postmenopausal women and negatively correlated with levels of DHEA-S. An increasing number of women are prescribed hormone replacement therapy (HRT) after menopause or ovarian resection to prevent and treat CVD, osteoporosis, Alzheimer's disease, and other related long-term postmenopausal complications (*9-12*).

Dehydroepiandrosterone (DHEA) is the precursor of estrogen and androgen and is thought to prevent the development of AS (*13*). Dehydroepiandrosterone

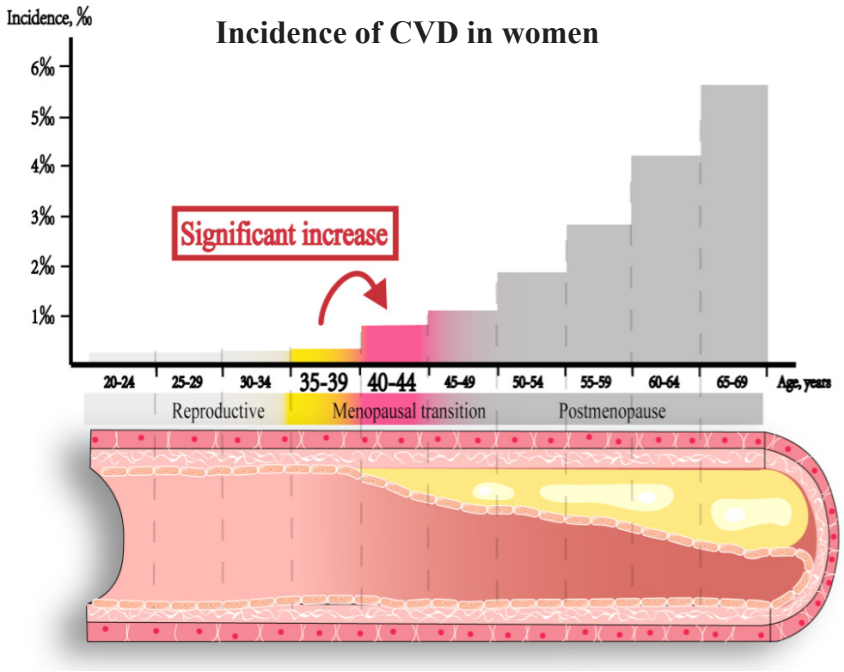

Figure 1. Incidence of CVD and vascular status in women. The incidence of CVD in women was stable at around 0.25‰, and it increased significantly after the age of 40. The intima-media thickness of blood vessels in women also increased, with more lipid deposition.

sulfate (DHEA-S) is the metabolite of DHEA, and the level of DHEA-S is significantly inversely correlated with the incidence of CVD (*14-17*). For postmenopausal women with coronary risk factors, a lower DHEA-S level means a higher mortality due to CVD (*18*). The new immunosenescence paradigm proposed in recent years offers an explanation. Senescence leads to the loss of DHEA, which causes semi-activated macrophages to be immunosuppressed and unable to differentiate, while releasing pro-inflammatory cytokines in an unregulated manner. These dysfunctional cells accumulate in vascular tissue and lead to the development of AS (*19*). That said, DHEA also has positive effects on the brain, bones, emotions, and sexual function of postmenopausal women, so its clinical use warrants consideration.

2. Metabolism and pathway of DHEA

As early as 1934, DHEA was successfully separated from urine. In 1944, Munson discovered the sulfated form of DHEA (*20*). DHEA, also known as 3β-hydroxyandrost-5-en-17-one, is the most abundant steroid circulating in human blood and is synthesized from cholesterol.

2.1. Generation of DHEA

The production of DHEA in the adrenal cortex and ovaries is regulated by adrenocorticotropic hormone and gonadotropin, respectively. DHEA is mainly produced in the adrenal cortex, only 10% of DHEA is produced in the gonads, and the brain also produces a small amount of DHEA (*21*). Approximately 6-8 mg of DHEA are produced per day in humans (*22*). In the blood, DHEA is mainly bound to albumin, a small amount will also bind to sex hormone-binding globulin (SHBG), and the remaining amount is free.

The level of DHEA changes during aging. The fetal adrenal gland produces a large amount of DHEA, but the level decreases rapidly after birth. The level of DHEA increases rapidly in the first two years of puberty, reaching a peak at 20-30 years of age, and then decreases at a rate of 2 to 5% annually. In individuals ages 70-80 years, the level of DHEA in the blood is only 10 to 20% of the peak level (*23*). The downstream hormones of the HPA axis have inhibitory feedback action on the upstream hormones, but DHEA does not participate in negative feedback regulation of the HPA axis. Thus, when the serum DHEA level is low, the body is unable to increase output through an endogenous feedback mechanism. Therefore, the body is unable to compensate for the deficiency in DHEA levels alone.

2.2. Conversion of DHEA

In the adrenal gland, endogenous DHEA is translated into DHEA-S by sulfation at the C3β position. In addition, oral DHEA is converted into DHEA-S *via* the first pass effect of the liver and intestine. As mentioned above, DHEA-S is a circulating reservoir of DHEA. Circulating DHEA is transferred to related peripheral tissues (*e.g.*, the ovaries, prostate, bone, adipose tissue, and brain) and then converted into testosterone, androstenedione, estrone, dihydrotestosterone (DHT), and estradiol (E2).

DHEA has biological action locally and indirectly.

It is known to be a multi-directional "hormone buffer" and to supplement hormones in the body, which explains why it has been used to treat menopause-related diseases. At the same time, since only a small amount of DHEA is in free circulation and DHEA is only converted into estrogen in the peripheral tissues, systemic estrogen-like adverse effects, such as cholelithiasis (*24*) and venous thromboembolic and ischemic stroke events (*25*), can be avoided (*26*). In addition, when the level of DHEA in humans reaches 7 μg/L, the saturation of invertase occurs during the conversion of DHEA into active sex hormones, helping avoid a state of excess sex hormone levels in women.

2.3. Pathway of DHEA

DHEA and its oxidative metabolites have been found to activate some nuclear receptors, like the constitutive androgen receptor (AR), estrogen receptor (ER) alpha and beta, pregnane X receptor, peroxisome proliferator activated receptor (PPAR), and G protein-coupled ER (GPER1) (*27,28*). Since DHEA can be converted into androgen/estrogen, researchers have not clearly determined whether AR/ER are directly activated by DHEA or indirectly by the converted androgen/estrogen. However, the low affinity of ER for DHEA makes the latter a poor agonist of ER (*27*). In addition, the effects of DHEA on the proliferation and apoptosis of endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) are not associated with ER and AR (*29,30*). DHEA and its analogues are not converted to estrogen or androgen but nonetheless have beneficial action on CVD, suggesting that DHEA interacts with its own specific receptor.

3. Pathogenesis of AS

Many theories on the mechanism of AS have been proposed from different perspectives. In recent years, most scholars have supported the "endothelial injury response" theory (*31*), which posits that endothelial dysfunction is an initial step in the pathogenesis of AS. The major risk factor for this disease is damage to the arterial intima, and the formation of atherosclerotic lesions results from the inflammatory-fibrotic proliferative response of arteries to intimal injury (Figure 2). AS is commonly regarded as a chronic inflammatory disease of the arterial wall caused by an imbalance in lipid metabolism and changes in inflammatory responses, whereby the body is unable to prevent the recruitment of inflammatory cells alone. However, a recent hypothesis suggests that AS is not just an inflammatory reaction of the blood vessel wall. Neither inflammatory cells nor necrotic cells are removed, and thus effector cell proliferation and tissue regeneration are eventually induced (*32,33*).

Blood vessels begin to change in the early stages of

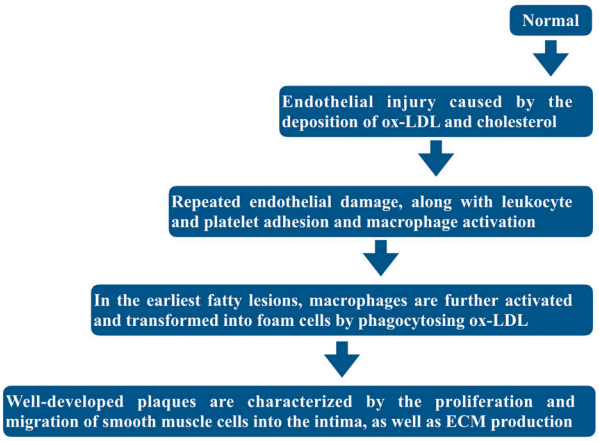

Figure 2. Pathogenesis of AS.

menopause. Endogenous estrogen and ERs decrease in postmenopausal women (*34,35*), resulting in the loss of inhibition of AS by estrogen, thus making them more vulnerable to AS. The mechanism may be an increase in the serum cholesterol level and high-density lipoprotein (HDL) particle size as well as interference with VSMC proliferation as a result of the decrease in endogenous estrogen and ERs (*36*).

4. The effects of DHEA on AS in postmenopausal women

4.1. DHEA alleviates dyslipidemia in postmenopausal women

Several early cross-sectional and prospective studies have revealed that the lipoprotein profile tends to worsen in postmenopausal women: plasma triglyceride (TG), total cholesterol (TC) low-density lipoprotein cholesterol (LDL-C), and lipoprotein levels increase and HDL cholesterol (HDL-C) levels decrease (*37,38*). In addition, studies (*39,40*) have indicated that age has more adverse effects on TC, LDL-C, TG, and non-HDL-C in postmenopausal women than BMI or smoking. This adverse change seems inevitable for postmenopausal women. However, dyslipidemia, which is mainly elevated LDL-C, is the most important factor for AS. Therefore, if AS in postmenopausal women is to be treated, then alleviating dyslipidemia is a very important aspect.

Substantial differences in the results of studies that have examined the effect of DHEA on blood lipid levels have been noted. Elevated plasma DHEA levels are reported to be correlated with HDL-C levels (*41*) but inversely correlated with LDL-C (*42*) and TC (*43,44*) levels. The correlation between plasma DHEA and TG levels was the most consistent. In a study by Jankowski *et al.*, treatment with DHEA resulted in a 17% reduction in serum TG levels (*48*). Lasco A *et al.* (*42*) reported that the serum TG levels of 20 postmenopausal women

decreased by about 20% after receiving DHEA (25 mg/d) for 12 months. A similar finding was noted in another study (*45*). However, one study (*46*) found that administration of DHEA does not change blood lipid parameters, which is consistent with the results of a previous study by the current authors (*47*). Use of lipid-lowering drugs may be a potential source of the inconsistency in the response of TG levels to DHEA (*48*). Therefore, whether DHEA can change blood lipid parameters or not needs to be studied more rigorously.

In addition to affecting blood lipid levels, DHEA can also directly inhibit lipid deposition (*49*). Fujioka *et al.* (*50*) found that DHEA can reduce the proliferation of adipocytes, which may be mediated by AR *via* an intracrine mechanism. DHEA also can promote lipid mobilization in adipose tissue by increasing the expression and activity of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) (*51*).

At present, there are conflicting results on improvement of blood lipid levels by DHEA. However, blood lipids are an important factor in the development and progression of AS, so the effect of DHEA on blood lipids needs to be studied further. Moreover, most of these studies involve normal people, and research needs to pay more attention to postmenopausal women.

4.2. DHEA corrects endothelial dysfunction in postmenopausal women

The loss of estradiol during postmenopausal may lead to a decline in endothelial function. For example, a decline in estradiol may alter the redox balance, thereby increasing oxidative stress and impairing endothelial function (*52*).

Endothelial dysfunction is involved in the pathogenesis of AS and CVD (*53*). One of the strategies for treating AS is to correct endothelial dysfunction (*54*). DHEA does not improve endothelial function through AR- or ER-mediated mechanisms (*55,56*). The effects of DHEA on ECs are shown in Figure 3.

4.2.1. DHEA inhibits EC oxidation

A study (*57*) has suggested that menopause is a risk factor for oxidative stress (OS). In postmenopausal women, not only progressive loss of estrogen and its protective effects (*58*), but also a further reduction in tocopherol and retinol levels as well as total antioxidant activity lead to OS (*59*). In a study by Taleb-Belkadi *et al.*, high levels of TBARS and carbonyl production and low levels of enzymatic defense found in

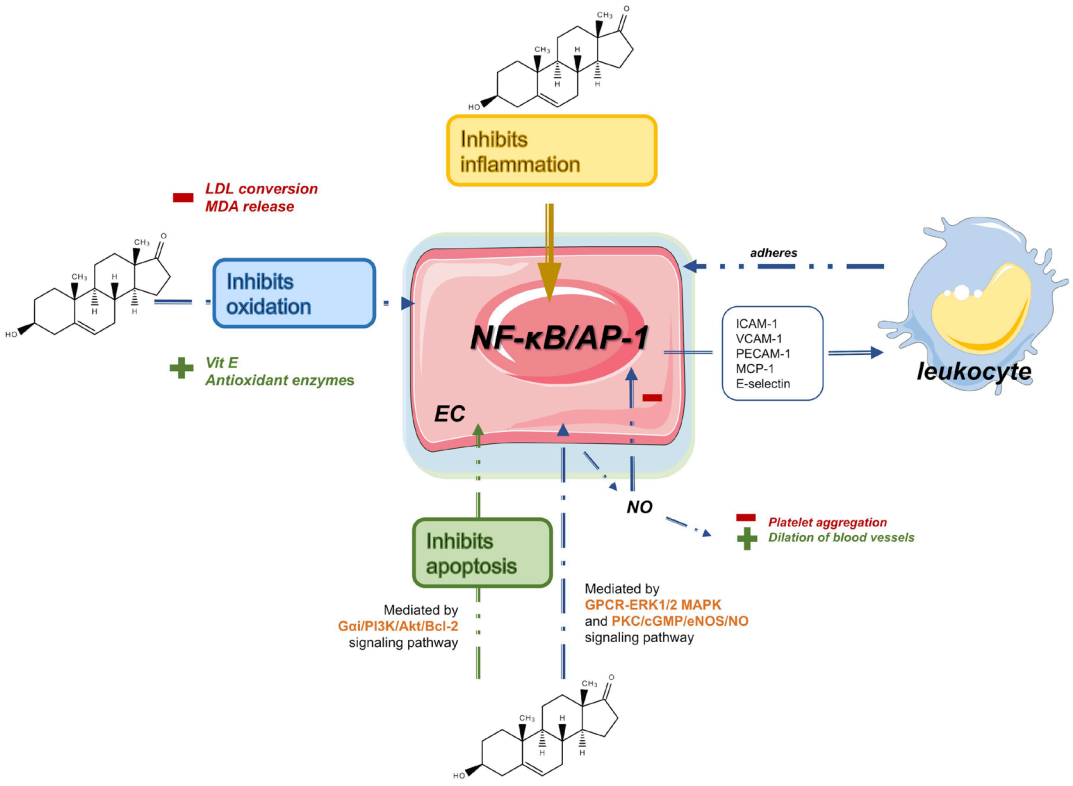

Figure 3. The effects of DHEA on ECs. First, DHEA inhibits EC oxidation by preventing the conversion of LDL to ox-LDL and the release of MDA, as well as by protecting endogenous vitamin E and the level and activity of antioxidant enzymes. Moreover, DHEA inhibits the production of MCP-1, ROS, ICAM-1, VCAM-1, PECAM-1, and E-selectin by ECs to prevent leukocytes from adhering to ECs, which involves NF-kB and AP-1. In addition, DHEA promotes NO production through the activation of eNOS *via* a GPCR-ERK1/2 MAPK cascade and the PKC/cGMP/eNOS/NO signalling pathway. NO subsequently inhibits platelet aggregation and the invasion and adhesion of leukocytes and it promotes the dilation of blood vessels. Moreover, DHEA protects ECs from apoptosis by activating the DHEAR/Gαi/PI3K/Akt/Bcl-2 signalling pathway.

postmenopausal women indicated that the women were exposed to OS. OS, and especially the oxidation of LDL in the arterial wall, can lead to worse AS through the stages of the menopausal transition in healthy women (*60*). In addition, the production of the superoxide anion O^{2-} and an increase in the levels of peroxynitrite are also characteristics of atherosclerotic lesions (*61,62*).

According to a previous study (*63*), the synthesis of reactive oxygen species (ROS) promotes AS by increasing superoxide production and suppressing EC function. The production of large amounts of ROS overwhelms the antioxidant defenses in cells, causing neutrophil activation, protein modification, lipid peroxidation, and DNA damage, which are key factors that promote the development of AS and CVD (*64,65*) (Figure 3).

DHEA effectively inhibits the oxidation of low-density lipoprotein (LDL) to oxidized low-density lipoprotein (ox-LDL) (*47,66*), it inhibits ox-LDL-induced ROS production (*67*), it reduces superoxide production, it ameliorates endothelial dysfunction, and it prevents the development of AS.

In some experiments, DHEA increased the antioxidant capacity of LDL by protecting endogenous vitamin E (*68*) and by significantly reducing the chemotactic activity of monocytes (*69*), directly removing the free radicals produced by the lipoprotein oxidation process (*70*), and counteracting the cellular damage caused by LDL and ox-LDL, all of which enable DHEA to function as an antioxidant (*66,68*). In addition, DHEA restores the levels and activities of glutathione peroxidase, SOD, and catalase (*71,72*). Moreover, DHEA significantly inhibits the secretion of malondialdehyde (MDA) in ECs (*47*). As a cytotoxic end product of lipid peroxidation, MDA causes cross-linking polymerization of macromolecules such as proteins and nucleic acids and it affects the respiratory function of the mitochondria *in vitro*. At the same time, DHEA also increases the antioxidant capacity of certain subcellular structures (*73*).

In summary, DHEA has antioxidant action by inhibiting the production of ox-LDL and MDA, removing free radicals, reducing monocyte adhesion, and protecting antioxidant enzymes.

4.2.2. DHEA inhibits EC inflammation

The level of inflammation is higher in postmenopausal women, which is evident in higher levels of TNF-α, IL-1 α, and CRP (*74,75*). Novella *et al.* suggested that this may be due to the change in estrogen-mediated regulation of female inflammatory biomarkers (*76*) which were identified as independent risk factors for CVD in postmenopausal women (*77*). DHEA can reduce inflammation, and especially in ECs, and ECs are closely related to AS.

DHEA alleviates inflammation of ECs independent of the ERα or ERβ pathway. *In vitro*, DHEA significantly inhibits monocyte chemoattractant protein-1 (MCP-1) secretion, ROS production, and expression of intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), platelet and EC adhesion molecule 1 (PECAM-1), and E-selectin (*78*). Moreover, DHEA also reduces the expression of adhesion molecule receptors in the U937 monocyte-like cell line, which suppresses the adhesion of monocytes to injured ECs (*47*). In one study (*79*), DHEA significantly reduced the LPS-induced transcription of nuclear factor kappa B (NF-κB). Moreover, DHEA impairs monocyte adhesion by suppressing the activity of NF-κB, thereby inhibiting the development of AS (*47*). A recent study (*80*) also indicated that DHEA restrains neutrophil recruitment and adhesion to ECs by reversing inflammation-induced down-regulation of developmental endothelial locus 1 (a secreted homeostasis factor) expression.

4.2.3. DHEA protects ECs by inducing NO production

The ability of vascular ECs to resist AS and antithrombotic factors largely relies on the production and release of active substances such as NO. NO blocks the expression of pro-inflammatory molecules as well as adhesion molecules in ECs. NO also inhibits the infiltration and adhesion of leukocytes (*81*).

Healthy endothelium, which normally produces NO, avoids the development and complications of AS (*82*). Nevertheless, the production of estrogen is reduced in postmenopausal women, and thus the activity of NO synthase decreases (*83,84*), which leads to a decrease in NO synthesis in ECs. A study found that a lack of NO and damaged endothelial progenitor cells resulted in vasodilation dysfunction in postmenopausal women, who are more prone to CVD, and especially AS.

DHEA activates eNOS through genomic and non-genomic mechanisms, and DHEA directly regulates human vascular walls by controlling the synthesis and stability of the eNOS protein in ECs (*85*). DHEA also effectively increases serum NO levels by activating PKC/cGMP/eNOS/NO pathways to prevent platelet aggregation, improve EC function, and alleviate early pathological changes associated with AS (*44,47,86*).

4.2.4. DHEA promotes EC proliferation and inhibits EC apoptosis

During aging, EC apoptosis increases, which affects the development of AS (*87,88*). The production of TNF-α induced by LPS and testosterone promotes apoptosis of ECs, whereas DHEA has the opposite effect on ECs. DHEA increases EC proliferation *in vitro* (*44*) and protects ECs from apoptosis (*89*). This anti-apoptotic effect of DHEA does not rely on ER or conversion into E2, but it is associated with the GTP-binding protein (Gαi) and the downstream phosphatidylinositol 3-kinase

(PI3K)/Akt signalling cascade (*90*).

4.3. DHEA inhibits the proliferation and migration of VSMCs

Lee *et al.* (*91*) noted marked proliferation of aortic VSMCs in ovariectomized mice. During aging, the level of sirtuin 1, a novel modulator of neointima formation caused by arterial injury, decreased (*92*). The reduction in this protein indirectly promotes the proliferation and migration of VSMCs (*93*). VSMC proliferation and migration of surrounding extracellular matrix (ECM) are the main reasons for thickening of the intimal wall, which will lead to AS (*94*).

DHEA is involved in relaxing VSMCs and inhibiting the proliferation and migration of VSMCs (*30,95*). DHEA does not have a significant effect on the phenotypic transition of VSMCs but rather reduces OS and inflammation in VSMCs by directly interrupting the ROS-dependent ERK1/2 signalling and p38 mitogen-activated protein kinase (MAPK)/NF-κB signalling pathways, thereby inhibiting the proliferation of VSMCs (*95*). Regardless of whether VSMCs undergo a phenotypic shift, DHEA can have a beneficial effect on these cells. DHEA-specific receptors are present in human VSMCs, and DHEA regulates the proliferation and apoptosis of VSMCs *via* a mechanism independent of ER and AR (*30,44*).

All of the aforementioned effects of DHEA on AS are shown in Figures 4 and 5 and Table 1.

5. Use of DHEA in the treatment of AS

As early as 1996, one study (*102*) proposed that DHEA is the source of youth, but the clinical use of DHEA is still hotly debated.

Several of the aforementioned studies have indicated that DHEA has anti-atherosclerotic action in animal models. DHEA improves cardiovascular risk-related parameters (*42*) and can be used as a drug for primary prevention of CVD (*103*). However, some studies have indicated that DHEA has no effect on CVD risk (*104-106*) and no effect on endothelial function (*92,107-109*). A meta-analysis (*110*) by Wu *et al.* noted no correlation between the level of DHEA-S and AS. However, other meta-analyses (*15,111*) noted that the lower the level of DHEA, the worse the prognosis for patients with CVD.

Qin *et al.* (*38*) suggested that DHEA had no effect on the blood lipid profile, and especially that of healthy postmenopausal women (*112*). This finding is consistent with the results of a previous study by the current authors (*47*). Nevertheless, there may be health benefits for women with adrenal insufficiency (*113,114*).

There are many factors responsible for the differing results of those studies. At present, many studies are based on rats and other rodents as models, but they are not the best model because they have almost no endogenous DHEA (*115*). In addition, the dosage of DHEA in those experiments is usually too high and it differs (*112,113*). Moreover, DHEA is rapidly metabolized, leading to somewhat differing results of many studies (*43*). A recent meta-analysis (*116*) suggested that publication bias and small flawed studies may also explain the discrepancy.

Therefore, whether postmenopausal women should

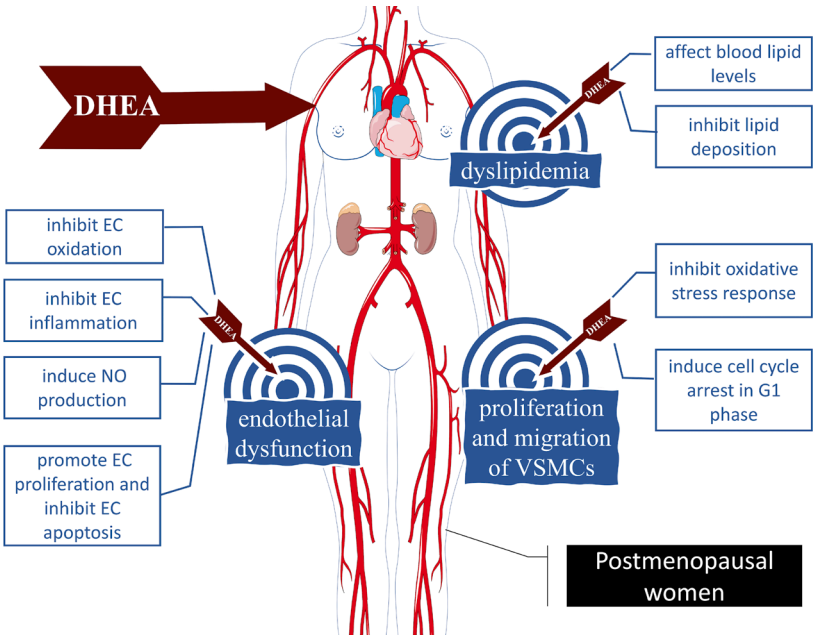

Figure 4. DHEA has specific action against aspects of AS in postmenopausal women. Postmenopausal women have dyslipidemia, abnormal proliferation and migration of VSMCs, and endothelial dysfunction. DHEA can play a role in alleviating these adverse aspects. First, it can improve dyslipidemia by affecting blood lipid levels and inhibiting lipid deposition. Second, DHEA can inhibit the oxidative stress response and induce cell cycle arrest in the G1 phase. In addition, DHEA can inhibit the oxidation and inflammation of ECs, induce NO protection, promote the proliferation of ECs, and inhibit the apoptosis of ECs.

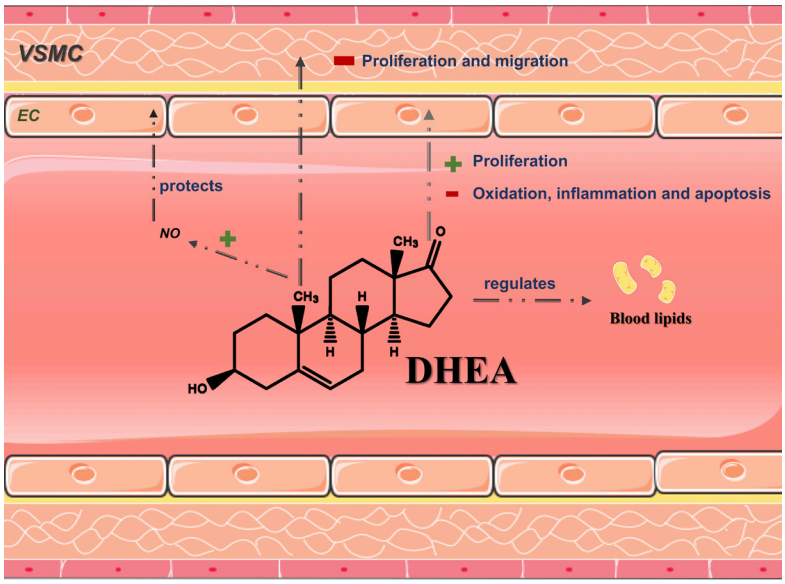

Figure 5. The effects of DHEA on atherosclerosis. First, DHEA affects the development and progression of AS by regulating blood lipid parameters, but substantial differences in results have been noted. Moreover, DHEA preserves EC function by inhibiting the oxidation and inflammation of ECs through NO production and promoting EC proliferation and inhibiting EC apoptosis. In addition, DHEA inhibits the progression of AS by inhibiting the proliferation and migration of VSMCs.

Table 1. The effects of DHEA on AS

Pathophysiological role of DHEA	Specific changes/mechanisms
Effects on blood lipids	A subject of debate
Effects on endothelial function	
Inhibition of EC oxidation	Prevention of LDL conversion to ox-LDL (*47,66,67*) Protection of endogenous vitamin E (*68*) Inhibition of leukocyte adhesion to ECs (*69*) Restoring the level and activity of antioxidant enzymes (*70,72*) Inhibition of MDA release by ECs (*47,70*)
Inhibition of EC inflammation	Inhibition of leukocyte adhesion to ECs: inhibiting the production of MCP-1, ROS, ICAM-1, VCAM-1, PECAM-1, and E-selectin by ECs; decreasing the expression of CCR2, LFA-1, and VLA-4 in the U937 monocyte-like cell line (*47*) Inhibition of IL-8, ICAM-1 and VCAM-1 production induced by TNF-α by blocking the LPS/TNF-α/PPARα/NF-κB signalling pathway (*47,96*) Inhibiting EC adhesion and oxidative stress by blocking AP-1 activity (*67,97,98*)
Protecting ECs through NO production	Inhibitory effect of NO on platelet aggregation and dilation of blood vessels (*86*) Inhibitory effect of NO on the expression of NF-κB, ICAM-1 and VCAM-1; prevention of the invasion and adhesion of leukocytes (*99*) Activation of eNOS *via* a GPCR-ERK1/2 MAPK cascade (*85*) Increasing NO production through the PKC/cGMP/eNOS/NO signalling pathway (*44,86*)
Promotion of EC proliferation and inhibition of EC apoptosis	EC proliferation (*85*) Protecting ECs from apoptosis by activating the DHEAR/Gαi/PI3K/Akt/Bcl-2 signalling pathway (*89*)
Inhibition of VSMC proliferation and migration	Promoting relaxation and inhibiting the proliferation of VSMCs by directly interrupting ROS-dependent ERK1/2 signalling and the p38 MAPK/NF-κB signalling pathway (*30,95*) Inhibiting the phenotypic transition and proliferation of VSMCs by blocking platelet-derived growth factor receptor-β (PDGFR-β) and regulating glutathione/glutathione (GSH/GRX) and low molecular weight protein tyrosine phosphatase (LMW-PTP) (*100*) Causing apoptosis: inducing cell cycle arrest in the G1 phase; upregulating the expression of the cyclin-dependent kinase (CDK) inhibitor $p16^{INK4a}$, activating caspase-3, and inducing PPARα expression in VSMCs (*101*)

take DHEA to treat or prevent forms of CVD such as AS is unclear. In addition, there is no clear standard for its indications and dosage (*117*).

DHEA causes adverse reactions such as hirsutism and acne. DHEA is believed to increase the risk of breast cancer in postmenopausal women (*118,119*). That said, experiments have indicated that the use of DHEA for 52 weeks has no effect on the endometrium (*94*). Evaluating the appropriate dose for patients is difficult because of the possibility of those adverse reactions, and indications for DHEA need to be carefully evaluated (*120*). Timing of use is also important. Treatment should start during menopausal transition, that is, within six years after menopause (*93,121*).

At present, studies on the clinical use of DHEA are still lacking. Therefore, use of DHEA should be carefully considered, the patient's eligibility should be determined, the patient's adrenal function should be considered, and whether the patient can tolerate the drug's adverse effects should be considered.

6. Conclusion

As a hormone precursor, DHEA is an endogenous steroid hormone and important source of estrogen and androgen in postmenopausal women. In addition, DHEA itself has a variety of biological actions that are independent of ER/AR and its conversion into estrogen/androgen, and it functions in almost all systems of the body (*43*). The current review has analyzed the mechanisms of postmenopausal women's susceptibility to AS. It has also discussed how DHEA plays a role in combating AS by countering these mechanisms, which include regulating the blood lipid status, protecting ECs (including coping with OS and inflammatory reactions of the vascular endothelium, inhibiting apoptosis of ECs, and inducing NO production) and inhibiting the proliferation and migration of VSMCs. In addition to its activity against AS, DHEA might have other protective effects on the cardiovascular system, such as preventing and reversing pulmonary hypertension (*55*) and reducing insulin resistance (*122*). However, further studies need to examine the mechanism and long-term effects of DHEA and additional clinical trials need to examine DHEA supplements. DHEA may serve as a better treatment for postmenopausal women and the entire population in the near future.

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Review

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Neoadjuvant therapy *vs*. upfront surgery for resectable pancreatic cancer: An update on a systematic review and meta-analysis

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SUMMARY The effectiveness of neoadjuvant therapy (NAT) remains controversial in the treatment of pancreatic cancer (PC). Therefore, this meta-analysis aimed to investigate the clinical differences between NAT and upfront surgery (US) in resectable pancreatic cancer (RPC). Eligible studies were retrieved from PubMed, Embase, and Cochrane Library. The endpoints assessed were R0 resection rate, pathological T stage < 2 rate, positive lymph node rate, and overall survival. A total of 4,588 potentially relevant studies were identified, and 13 studies were included in this study. In patients with RPC, this meta-analysis showed that NAT presented an increased R0 resection rate, pathological T stage < 2 rate, and a remarkably reduced positive lymph node rate compared to US. However, patients receiving NAT did not result in a significantly increased overall survival. These findings supported the application of NAT, especially as a patient selection strategy, in the management of RPC. Additional large clinical studies are needed to determine whether NAT is superior to US.

Keywords neoadjuvant therapy, resectable, pancreatic, neoplasm, prognosis

1. Introduction

Pancreatic cancer (PC) is the fourth-largest cause of cancer-related mortality in the USA and exhibits poor prognosis and low resection rate among aggressive malignancies (*1*). Although improvements in surgical techniques and postoperative management have expanded the spectrum of patients eligible for surgical resection, only 15-20% of the patients fall within resectable pancreatic cancer (RPC) (*2*). Simultaneously, due to exocrine and endocrine pancreatic dysfunction, the wasting syndrome of cachexia occurs in > 80% of the patients with PC during diagnosis (*3,4*). In 2021, National Comprehensive Cancer Network (NCCN) guidelines recommended surgery with adjuvant therapy (SFadj) as the first choice and neoadjuvant therapy (NAT) only for RPC patients with high-risk factors (*5*). In many high-volume centers worldwide, the mortality rate from pancreatectomy is < 2% (*6*). Adjuvant chemotherapy has a survival benefit for RPC (*7*). Strikingly, < 40% of patients undergo pancreatectomy because they cannot obtain a chance for scheduled treatment (*8-11*). Regardless of advances in surgical technique and adjuvant therapy, 5-year overall survival (OS) rates of only 25-50% in patients undergoing SFadj was measured due to high systemic recurrence rates (*12,13*).

In 1992, NAT was first proposed for patients with RPC (*14*). In recent years, NAT has presented several advantages in borderline resectable pancreatic cancer (BRPC), including early treatment of micrometastases, increased likelihood that a high percentage of patients with RPC will receive postoperative chemotherapy, potentially downsized tumors, and selection of patients suitable for surgery (*15-20*). Notably, the outcomes from a study further supported the neoadjuvant gemcitabine and oxaliplatin treatment for RPC because R0 resection rate is 52% and the OS is 27.2 months (*21*). Typically, an increasing number of retrospective studies revealed beneficial effects with NAT (*9,22-26*). However, the first randomized controlled trial (RCT) of NAT *vs*. upfront surgery (US) in RPC explained that the data were not statistically significant (*27*). Moreover, a meta-analysis reported that the overall survival between the NAT and US groups did not differ significantly (*28*). Several studies revealed that NAT might carry the risk of disease progression that was initially resectable to unresectable PC (*29,30*); whether NAT can improve the prognosis in RPC is yet unclear.

Furthermore, whether NAT or US is optimal for

patients with RPC is still controversial. Accordingly, the present study aimed to investigate the differences between NAT and US in RPC. The treatment prognosis included the R0 resection rate, pathological T stage < 2, positive lymph nodes rate (8th edition American Joint Committee on Cancer), and OS.

2. Materials and Methods

This meta-analysis followed the PRISMA guidelines (*31*).

2.1. Literature search

The literature was reviewed systematically by searching PubMed, Embase, and the Cochrane Library for studies published before October 2021. The search strategy included the following domains of medical subject headings (MeSH) terms: "Neoadjuvant", "Resectable", and "Pancreatic". These terms were combined with "AND". No language and publication time restrictions were applied. The search is described in Table S1 (*http://www.biosciencetrends.com/action/getSupplementalData.php?ID=85*).

2.2. Inclusion and exclusion criteria

Inclusion criteria: 1) Study type: randomized controlled trial and retrospective cohort study; 2) Participants: patients conformed to the diagnostic criteria of RPC; 3) Intervention: NAT and US groups; 4) Outcomes: R0 resection rate, pathological T stage <2 rate, positive lymph nodes rate or OS; 5) Language: published in English language. Exclusion criteria: 1) repeated publications; 2) review articles, letters, case reports, and animal studies; 3) unable to obtain full-text outcome; 4) no R0 resection rate, pathological T stage < 2 rate, positive lymph nodes rate, or OS; 5) unable to extract data from the literature; 6) patients conform to the diagnostic criteria of BRPC or unresectable PC.

2.3. Data extraction and quality assessment

Two researchers (Yuhua Zhang and Youyao Xu) screened the titles and abstracts for eligibility and then screened the full-text independently. A third researcher (Yizhen Chen) extracted relevant data after further review. Any disagreement was resolved by discussion, and a consensus was achieved between the researchers. The following data were extracted from each study: R0 resection rate, pathological T stage < 2 rate, positive lymph nodes rate, and OS. The hazard ratio (HR) and the 95% confidence interval (CI) were extracted directly from each study. When the HR and the 95% CI were not reported, they were obtained from the Kaplan-Meier survival curves using the Engauge Digitizer 11.1 software (Markmitch, Boston, MA, USA).

All studies used the Newcastle-Ottawa scale (NOS) for quality assessment. The NOS assigns a score of 0-9, with points assigned based on selection, comparability, and exposure. In this meta-analysis, we noted that a score > 6 was defined as acceptable.

2.4. Statistical analysis

The data were extracted and input into an Excel spreadsheet. The statistical analyses were performed using RevMan software (version 5.3, Nordic Cochrane Center, Copenhagen, Denmark). The heterogeneity of the studies was assessed using the chi-square-based *Q*-test and I^2 statistics test. Statistically, significant heterogeneity was considered if P was < 0.1 or the I^2 statistic was > 50%. Estimates were summarized applying fixed-effects or random-effects models according to the heterogeneity. Sources of heterogeneity were investigated *via* sensitivity analysis. A funnel plot was drawn to assess publication bias.

3. Results

3.1. Study characteristics

A total of 4,588 potentially relevant studies were identified, among which 2,432 were excluded as irrelevant after screening the titles and abstracts. Subsequently, 132 studies were included for full-text screening, and 13 studies were included in the final data synthesis (Figure. 1).

The demographics of the included studies are summarized in Table. 1. In this study, 2 RCTs and 11 retrospective cohort studies (RCSs) were included. These 13 studies (*8-10,25,27,32-39*) encompassed a total of 10,060 patients, among which 2,587 (26%) were assigned to NAT and 7,473 (74%) received US. Of these, 5 studies were conducted in Europe, 4 in the USA, 3 in Asia and 1 in Australia. Table 2 and Table 3 summarize the characteristics of patients who underwent NAT and US, respectively. However, the commonly used NAT regimens included 5-fluorouracil (5 studies, N = 311) and gemcitabine (8 studies, N = 446). Based on the methodology, a NOS score of ≥ 6 was defined as acceptable. All the included studies scored > 6. A full description of the score of NOS is available in Table S2 (*http://www.biosciencetrends.com/action/getSupplementalData.php?ID=85*).

3.1.1. R0 resection difference between the NAT and US groups

A meta-analysis of 10 studies was conducted using a random-effects model; the NAT and US groups included 2,501 and 7,009 patients, respectively. The data showed that NAT presented an increased R0 resection rate for RPC (OR = 1.59, 95% CI = 1.41-1.80). A slight

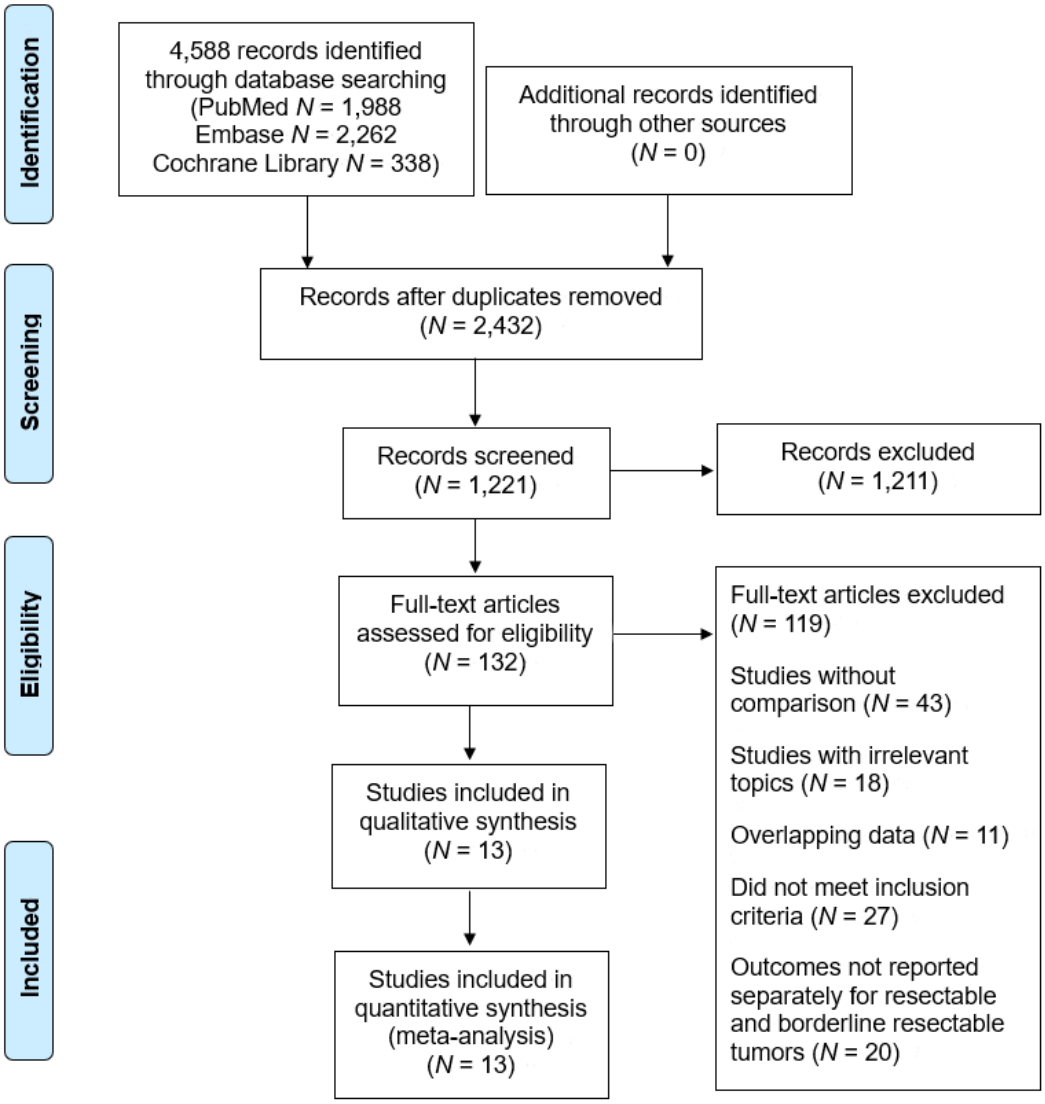

Figure 1. Flowchart of the evidence search and study selection process.

Table 1. Demographics of included studies

First author (*Ref.*)	Country	Publication year	Study period	Study design	Tumor	Patients (*n*)	Male (%)	R0 criteria (mm)	Quality score
Fujii (*35*)	Japan	2017	2001-2013	RCS	RPC	273	NA	> 1	7
Casadei (*33*)	Italy	2015	2003-2009	RCT	RPC	38	57.9	> 1	7
Tzeng (*32*)	USA	2014	2002-2007	RCS	RPC	167	54.5	NA	7
Motoi (*10*)	Japan	2014	2007-2009	RCS	RPC	582	55.0	NA	7
Papalezova (*34*)	USA	2012	1999-2007	RCS	RPC	236	53.5	NA	7
Golcher (*27*)	Germany	2015	1999-2003	RCT	RPC	66	53.0	NA	8
Mokdad (*9*)	USA	2017	2006-2012	RCS	RPC	8,020	52.0	NA	7
Vento (*8*)	Finland	2007	1999-2002	RCS	RPC	47	53.2	NA	7
Artinyan (*25*)	USA	2011	1987-2006	RCS	RPC	458	46.9	NA	7
Barbier (*37*)	France	2011	1997-2006	RCS	RPC	173	NA	> 1	7
Moutardier (*36*)	France	2004	1997-2002	RCS	RPC	56	58.9	NA	7
Tajima (*38*)	Japan	2011	2006-2009	RCS	RPC	34	61.8	NA	7
Maloney (*39*)	Australia	2021	2013-2019	RCS	RPC	126	42.1	> 1	7

RCS, retrospective cohort study; RCT, randomized controlled trial.

heterogeneity was observed in 10 studies ($Chi^2 = 17.31$, $p = 0.04$, $I^2 = 48\%$) (Figure. 2A). Therefore, subgroup analysis evaluated the impact of the analytical method, which was divided into intention-to-treat (ITT) analysis and per-protocol (PP) studies. As a result, studies with ITT analysis did not show any heterogeneity using a

Table 2. Characteristics of NAT included studies

First author (*Ref.*)	No. of patients	Median age (years)	Regimen	Median OS (months)	Resection rate ITT (%)	R0 rate (%)	patients with positive lymph nodes (%)	Pathological T stage < 2 (%)
Fujii (*35*)	40	65	5-FU+oteracil and gimeracil+RT (45 Gy) + S-1	24.9	90	86	39	NA
Casadei (*33*)	18	71.5	Gem+Gem with RT (45 Gy)	NA	61.1	64	55	55.6
Tzeng (*32*)	115	65.5	Gem+Cis+RT (45 Gy)	28	82.6	89.4	51.6	23.2
Motoi (*10*)	185	68	Gem+S-1+RT (35.2–54 Gy)	NA	92.4	95.9	30.6	25.3
Papalezova (*34*)	144	64	5-FU+RT (30–50.4 Gy)	20	52.8	78	25	NA
Golcher (*27*)	33	62.5	Gem+Cis+RT (55.8 Gy)	25	57.6	90	32	21.1
Mokdad (*9*)	2,005	64	NA	26	100	83.2	48	27.4
Vento (*8*)	22	65	Gem+RT (50.4 Gy)	30.2	59.1	NA	32	NA
Artinyan (*25*)	39	61.7	5-FU+RT	34	NA	NA	45	NA
Barbier (*37*)	88	65	5-FU+Cis+RT (45 Gy)	17	43	74	29	NA
Moutardier (*36*)	39	65	5-FU+Cis+RT (30/45 Gy)	13.7	58.9	NA	13	NA
Tajima (*38*)	13	62.6	Gem+S-1	NA	NA	84.6	76.9	NA
Maloney (*39*)	40	71	Gem/Gem+Cap/Gem+Nab/ FOLFIRINOX	21	95	63.2	NA	NA

5-FU, 5-fluorouracil; Cis, cisplatin; Gem, gemcitabine; S-1, s-1; Meiji Combination Capsules, T20/25; Nab, Nab-Paclitaxel; RT, radiation therapy; NA, not available.

Table 3. Characteristics of US included studies

First author (*Ref.*)	No. of patients	Median age (years)	Median OS (months)	Resection rate ITT (%)	R0 rate (%)	Resection rate ITT (%)	patients with positive lymph nodes (%)	Pathological T stage < 2 (%)
Fujii (*35*)	233	67	23.5	88	70	90	71	NA
Casadei (*33*)	20	67.5	NA	75	33	61.1	87	0
Tzeng (*32*)	52	61.9	25.3	92.3	81.2	82.6	81	6.3
Motoi (*10*)	397	68	NA	94.5	81.3	92.4	55.2	18.4
Papalezova (*34*)	92	65	17	74	79	52.8	62	NA
Golcher (*27*)	33	65.1	18.9	70	70	57.6	57	8.7
Mokdad (*9*)	6,015	65	23	100	77.9	100	74	14.3
Vento (*8*)	25	63	35.9	100	NA	59.1	44	NA
Artinyan (*25*)	419	61.8	19	NA	NA	NA	65	NA
Barbier (*37*)	85	64	15	79	67	43	64	NA
Moutardier (*36*)	17	65	26.6	100	NA	58.9	65	NA
Tajima (*38*)	21	66	NA	NA	85.7	NA	54.1	NA
Maloney (*39*)	86	69	24	98.8	57.6	95	NA	NA

NA, not available.

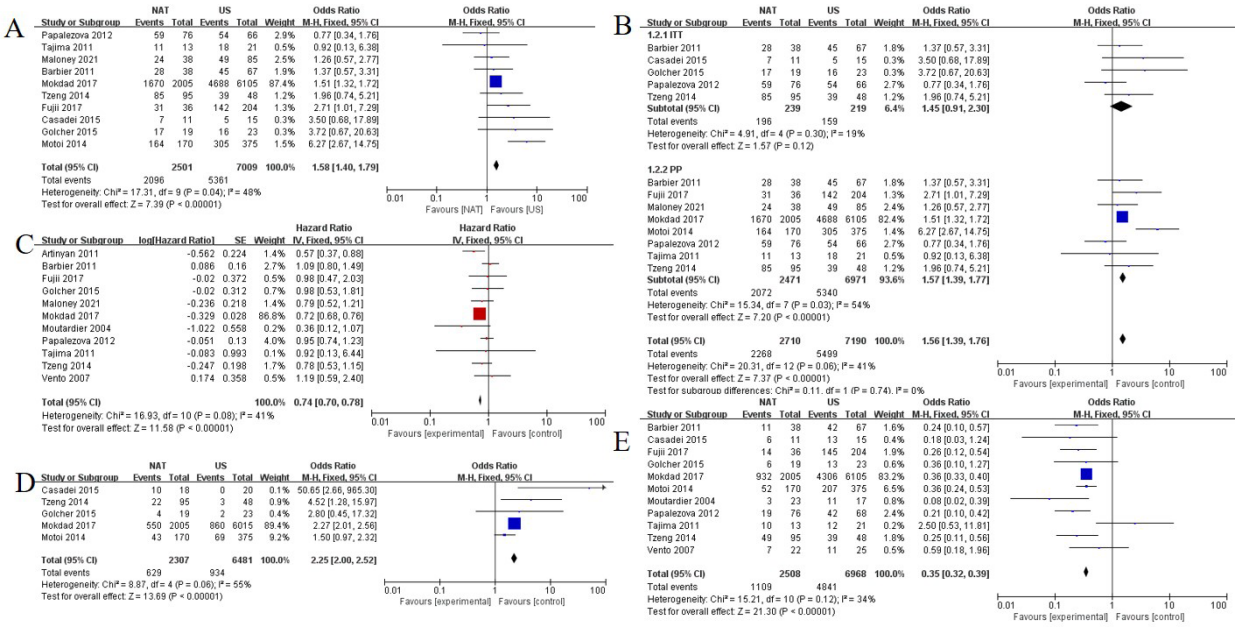

Figure 2. Forest plots of NAT *vs.* US A, R0 resection rate; **B**, Subgroup analysis based on the analytic method of R0 resection rate (ITT or PP analysis); **C**, OS; **D**, pathological T; **E**, positive lymph nodes.

fixed-effects model (Chi^2 = 0.11, p = 0.74, I^2 = 0%) (Figure. 2B).

3.1.2. OS difference between NAT and US groups

A meta-analysis of 11 studies was conducted using a fixed-effects model; the NAT and US groups included 2,578 and 7,078 patients, respectively. The data showed that patients receiving NAT did not result in a significantly increased OS for RPC (HR = 0.74, 95% CI = 0.70-0.78) (Figure. 2C).

3.1.3. Pathological T difference between NAT and US groups

A meta-analysis of 5 studies was conducted using a random-effects model; the NAT and US groups included 2,307 and 6,481 patients, respectively. A slight heterogeneity was detected in 5 studies (Chi^2 = 8.87, p = 0.06, I^2 = 55%). Furthermore, sensitivity analysis demonstrated that the study by Casadei *et al*. had a profound influence on heterogeneity. The heterogeneity decreased after removing this study (Chi^2 = 4.52, p = 0.21, I^2 = 34%). The data revealed that NAT presented an increased pathological T < 2 rate for RPC using a fixed-effects model (OR = 2.22, 95% CI = 1.97-2.49) (Figure. 2D).

3.1.4. Positive lymph nodes between NAT and US groups

A meta-analysis of 11 studies was conducted using a fixed-effects model on NAT and the US groups, including 2,508 and 6,968 patients, respectively (OR = 0.35, 95% CI = 0.32-0.39). No heterogeneity was detected in 11 studies (Chi^2 = 15.21, p = 0.12, I^2 = 34%). The data demonstrated that the NAT group had a distinctly reduced rate compared to the US group in positive lymph nodes for RPC (Figure. 2E).

3.2. Publication bias

A funnel plot was constructed, which showed that the risk of publication bias was low between R0 resection rate, OS, pathological T stage, and positive lymph nodes (Figure. 3).

4. Discussion

Presently, the standard treatment for RPC is SFadj. Surgical resection is the only potentially curative treatment for managing PC. First, NAT requires a cytological or histological diagnosis (*40*). However, the diagnostic sensitivity of endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) in patients with suspected PC is approximately 11% (*41*). A study of 583 patients with histopathologically confirmed

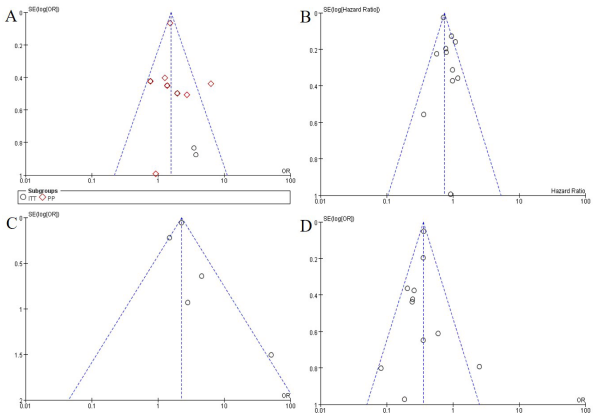

Figure 3. Funnel plot for outcomes; A, R0 resection rate; **B**, OS; **C,** pathological T; **D**, positive lymph node.

PC demonstrated that the major pathological response was detected in 77 (13.2%) patients encompassing only 23 (3.9%) patients with a complete pathological response (histopathologically, < 5% viable cancer cells were noted in the surgical specimen) (*42*). Typically, surgery can avoid the treatment delay caused by negative biopsy and the progress of NAT. Second, postoperative adjuvant therapy can achieve positive results with respect to survival time (*12,13,43-47*). Therefore, although SFadj is the recommended option, recurrences are both locally and systemically common after the therapy, and 5-year OS is rare (*48*). Moreover, during surgery, estimation of the negative lateral margin involving vessels for the surgeon is difficult (*49*), which might increase postoperative complications.

Recently, comprehensive treatment for NAT has gradually attracted widespread attention. Many studies showed that NAT improved R0 resection rate and OS (*9,30,40,50,51*). Several reasons could be ascribed to the preference of NAT in RPC. First, distant metastases arose before treatment. The probability of micrometastasis was 28%, 73%, and 94% for tumors with a primary lesion size of 1 cm, 2 cm, and 3 cm, respectively (*52*). NAT increases the proportion of patients receiving systematic treatment because this method may allow time for postoperative chemotherapy (*30*). Second, NAT might improve the prognosis because the technique has a regional downstaging effect. It also can reduce tumor cell viability, making it less likely to spread during surgery (*30,40,53*). Third, patients treated with NAT usually completed multimodality therapy and were affected less severely by occurrence of pancreas fistula after resection than patients treated with US. This phenomenon could be related to pancreatic fibrosis (*54,55*). NAT could improve the intensity of systemic treatment, usually delayed for 2-3 months if surgery is conducted first. Some patients fail to receive adjuvant treatment due to various reasons (*8-11*). Typically, patients have a better tolerance for preoperative than postoperative systemic therapy. The improvement in tolerance ensured completion of

treatment. In 2015, the results of multicenter RCTs indicated that NAT was feasible, safe, and efficacious in approximately 77.8% of the patients with RPC (*33*). At present, Prep-02/JSAP-05 study is underway to help solve this question. A RCT comparing US *vs*. NAT using gemcitabine + S-1, including 364 patients showed an advantage for OS in patients with NAT (36.7 months *vs* 26.6 months, HR 0.72, $p = 0.015$) (*56*). However, NAT also has some limitations. First, NAT might transform the tumors that can achieve R0 resection into those that cannot achieve resection and also show distant metastasis. Second, NAT affects the patient's general condition and reduces tolerance to surgery. This method might decrease resectability due to tumor progression during the preoperative treatment. Third, the operative time and intraoperative blood loss volume were significantly increased in NAT, indicating technical difficulty for the surgeon (*10,35*).

For patients with RPC, the biggest obstacle is the type of treatment regimens to adopt. Surgery has been applied to treat RPC for > 100 years, but no significant improvement has been observed in survival time. In recent years, the promotion of treatment at high-volume pancreatic medical centers and popularization of artery-first approaches have improved the R0 resection rate. However, advances in the prognosis of RPC by improving surgical treatment were limited. As a result, the clinical research direction of RPC has gradually shifted from improving surgical techniques to the selection of treatment strategies, and the clinical treatment model has gradually shifted from surgery-first to multiple disciplinary treatments (MDT). Indubitably, NAT is the focus of research during this transition, which is embodied in the strategic selection of "US or NAT for RPC." In the absence of clear guidelines, three indicators are used for evaluation (*57*). First, the tumor size and degree of vascular invasion. Second, the patient's general condition and nutritional status were assessed to determine their tolerance for surgery. Third, tumor biological condition. The common detection indicators include Computed Tomography (CT), Magnetic Resonance Imaging (MRI), carbohydrate antigen 19-9 (CA19-9), and miRNA. CA19-9 has been utilized for diagnosis, prognosis, and monitoring for recurrence, and the response should be considered when distinguishing treatment regimens for an individual patient (*58*). In addition, we can decide to continue or change chemotherapy regimens. Strikingly, the level of CA19-9 was not assessed. Thus, how to obtain the CA19-9 cut-off to distinguish treatment regimens needs to be investigated in future studies.

The present meta-analysis clarified the difference between NAT and US for RPC. The data from 13 included studies involving 10,060 patients provided an accurate conclusion than a single study. The R0 resection rate is a known prognostic indicator for patients with RPC. To date, the NAT group is found to be superior to the US group in the aspect of R0 resection rate with respect to the hypothesis mentioned above. However, the definitions of R0 can vary among included studies, which could interfere with the final reported outcomes. These findings proposed that NAT can achieve locoregional control of RPC by increasing the R0 resection rate. In terms of pathological T stage < 2, we found that the NAT group was marginally superior to the US group; indeed, the NAT group had an obviously reduced positive lymph node rate compared to the US group. The pathological data indicated that NAT was more frequently observed in pT1-2 and N0 categories than in the US group. Therefore, NAT has a satisfactory regional downstaging effect and reduced lymph node involvement. However, patients receiving NAT did not show a significantly increased OS. The survival time is one of the most important prognostic indicators for patients with RPC, which is influenced by many factors. These results differed between patients with BRPC and local advanced pancreatic cancer (LAPC), which could be attributed to varied tissue and cell characteristics. In addition, this meta-analysis included literature spanning a prolonged period, with advances in surgical techniques, imaging techniques, specimen staining, and standardization of histopathological reports that affect the criteria for resectability (*37*). Another explanation for this result might be the variation in NAT regimens during studies.

Nevertheless, our meta-analysis has some limitations. First, most of the included studies were retrospective in design, which led to unmeasured confounding. Moreover, the number of included studies and the sample size was small. Second, multiple neoadjuvant regimens were included in this meta-analysis. However, subgroup analyses were not applicable for the different regimens because of the complexity of specific treatment strategies. Third, patients receiving NAT represent only those who tolerated treatment and underwent resection. However, we could not identify all patients who received NAT and intended to be resected later but did not proceed with resection. Fourth, this study extracted the HR and 95% CI from one of the included studies utilizing the Engauge Digitizer, which may have caused a bias. Fifth, the quality of the included studies needs to be assessed using the Cochrane collaboration's risk of bias tool for RCTs.

5. Conclusions

This meta-analysis represented a comprehensive review regarding the difference between NAT and US. Overall, it revealed a significant advantage in R0 resection rate, pathological T stage < 2 rate, and positive lymph node rates. Based on the above results, US was recommended for patients who have a high possibility of R0 resection. Tumor progression during NAT was prevented, which lead to the loss of the chance of radical resection. On

the other hand, it was worth trying to administer NAT to patients with a lower chance of radical resection. In summary, large trials should be conducted to elucidate the NAT approach for RPC and draw accurate conclusions.

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Original Article

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Dysfunction of peripheral regulatory T cells predicts lung injury after cardiopulmonary bypass

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SUMMARY Lung injury caused by cardiopulmonary bypass (CPB) increases the mortality after cardiac surgery. Previous studies have shown that regulatory T cells (Tregs) play a protective role during CPB, but the correlation between Tregs and CPB-induced lung injury remains unclear. Here, we conducted a prospective study about Treg cells in patient receiving CPB. Treg cells were collected from patients before the CPB operation (pre-CPB Tregs), and the effect of pre-CPB Tregs on the occurrence of CPB-induced lung injury was evaluated. Data showed that the baseline level of Treg cells in peripheral blood were lower in patients who developed lung injury after CPB, compared to those who did not develop lung injury after CPB. Function analyses revealed that pre-CPB Tregs from CPB-induced lung injury patients presented decreased ability in suppressing the proliferation and IFN-γ production of CD4 and CD8 T cell. Also, pre-surgery levels of TGF- β and IL-10 were markedly lower in lung injury patients than in non-lung injury patients. In addition, PD-1 and Tim-3 expression on pre-CPB Tregs were significantly lower in CPB-induced lung injury patients than the CPB patients without lung injury. Above all, we found impaired peripheral Treg responses in CPB-induced lung injury patients, indicating a potential role of Treg cells in the early diagnosis of CPB-induced lung injury.

Keywords Regulatory T cell, lung injury, cardiopulmonary bypass

1. Introduction

Cardiopulmonary bypass (CPB) procedure is a basilic component of conventional cardiac surgeries (*1*). Pulmonary dysfunction after CPB was described almost 40 years ago (*2*), and lung injury is one of the most common complications with non-cardiogenic refractory hypoxemia as the main clinical manifestation. About 2% patient with lung injury could develop into acute respiratory distress syndrome (ARDS) with about 15.5% mortality. Therefore, identifying specific warning biomarkers is critical for preventing morbidity and mortality of lung injury after CPB.

Lung injury caused by CPB might be attributed to the disorder and unbalance of immune response and different $CD4^+$ T cells are involved in the development of lung injury (*3,4*). Regulatory T cell (Treg) is a subset of $CD4^+$ T cells, which is significant for immune homeostasis and maintaining self-tolerance. These cells were termed suppressor cells originally (*5*), expressing CD25 as well as Foxp3. It has been reported that Treg cells play a critical role in resolution of acute lung injury (ALI) in mice. Data showed that Rag-1 mice exhibited a profound impairment in resolution of lung injury, which could be reversed by administration of Treg cells, while depletion of Tregs in wild type mice delayed recovery (*6*). Besides, the recovery of ALI was also attributed by upregulation of Tim-3 on Tregs. However, the effects of Tregs on lung injury patients after CPB are still not clear.

Here, we showed that the dysfunction of Treg cells in patients with CPB-induced lung injury appeared before the surgery, which suggests a new biomarker of CPB-induced lung injury and a therapeutic approach through increasing Treg population and function.

2. Materials and Methods

2.1. Ethics statement

Ethical approval (YXLL-KY-2020-56) was obtained from the Research Ethics Committee of Qianfoshan Hospital. Informed consent was obtained from every patient and the study protocols were approved by the Research Ethic committee of the Shandong Province Qianfoshan Hospital. The ethical consideration for

this study followed the principles of the Declaration of Helsinki.

2.2. Patients

Patients scheduled for cardiac surgery under cardiopulmonary bypass between October 2020 and January 2021 at our hospital were enrolled in the study. Exclusion criteria included heart failure, severe pulmonary hypertension, preoperative presence of respiratory distress syndrome, age > 75, and without providing consent form. They were grouped into non-lung injury and lung injury groups based on the presence or absence of lung injury after CPB. The oxygenation index was calculated according to the results of blood gas analysis and ventilator parameters within 24 hours after operation. Lung injury group was defined if the oxygenation index was less than 200, otherwise it belonged to non-lung injury group.

2.3. Isolation of peripheral blood mononuclear cells (PBMCs) and cell culture

PBMC were obtained by Ficoll-Hypaque (Thermo Fisher, Waltham, MA, USA) centrifugation (Eppendorf, Hamburg, Germany) of heparinized blood and resuspended in RPMI (GIBCO, Grand Island, NY, USA) tissue culture medium containing 10% fetal calf serum (GIBCO, Grand Island, USA), streptomycin (GIBCO, Grand Island, USA), and penicillin (GIBCO, Grand Island, USA).

2.4. Serum

Ten mL of whole-blood samples were collected from patients. Serum samples were obtained after centrifugation at 800× g for 10 min, aliquoted and stored at –80°C until assayed.

2.5. Enzyme-linked immunosorbent assay

Cytokines in the sera were (IL-10, TGF-β, IFN-γ and IL-17A) measured by Bio-Plex Pro™ Human Cytokine Assays® (Bio-Rad Laboratories, Inc., Hercules, CA, USA). In brief, the serum samples were diluted 4-fold with the diluting solution, and centrifuged at 10,000× g for 5 minutes. Fifty μL of the supernatant was used for the cytokine assay in accordance with the manufacturer's instruction.

2.6. Flow cytometry

PBMCs were stained with different antibodies according to the manufacturer's instruction, in which antibodies used for the experiments included APC/CY7-CD8, BV605-CD4, PE/CY7-CD25, FITC-PD-1, PE-Tim-3 or isotype-matched control IgG. All the antibodies and isotype controls were purchased from BD PharMingen San Diego, CA, USA. After staining, cells were washed twice with PBS and were subjected to flow cytometry analysis using a FACS Foterassa (BD, San Diego, CA, USA). Analyses of flow cytometry were performed by FlowJo software.

2.7. Intracellular cytokine staining

PBMCs were stimulated with 20 ng/mL phorbol myristate acetate and 1 μg/mL ionomycin (Sigma-Aldrich, St. Louis, MO, USA) for 6 h to detect IFN-γ- or IL-17A-producing T cell frequencies in patients with lung injury or non-lung injury. Brefeldin A (10 μg/mL; Sigma-Aldrich, St. Louis, MO, USA) was added to cultured PBMCs for 4 h. Stimulated PBMCs were washed in phosphate-buffered saline (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM disodium hydrogen phosphate, 2 mM potassium dihydrogen phosphate, pH 7.4, Sigma-Aldrich, St. Louis, MO, USA) and incubated with PC/CY7-CD4, APC/CY7-CD8a or matched isotype (Thermo Fisher, Waltham, MA, USA) for 30 min in dark at 4°C. PBMCs were then fixed in 4% formaldehyde, permeabilized with 0.1% saponin (Sigma-Aldrich, St. Louis, MO, USA) and stained v450-INF-γ or FITC-IL-17A (Thermo Fisher, Waltham, MA, USA) or matched isotype control monoclonal antibody (Thermo Fisher, Waltham, MA, USA). Cells were analyzed using a FACS Foterassa (BD, San Diego, CA, USA).Analyses of flow cytometry were performed by FlowJo software.

2.8. Foxp3 staining

For intracellular staining of Foxp3, cells were fixed and permeabilized with Foxp3 staining buffer (Thermo Fisher, Waltham, MA, USA), then stained with allophycocyanin-conjugatedanti-humanFoxp3 mAbs (0.5 μg per 10^6 cells; Thermo Fisher, Waltham, MA, USA). Lymphocytes were gated with characteristic low forward scatter/side scatter, using a FACS Foterassa instrument. Analyses of flow cytometry were performed by FlowJo software.

2.9. Isolation of $CD4^+CD25^+$ T cells and $CD4^+CD25^-$ T cells

$CD4^+$ T cells were isolated from PBMC using magnetic bead separation. To isolate $CD4^+CD25^+$ and $CD4^+CD25^-$ T cells, purified $CD4^+$ T cell population were incubated with PE-labeled anti-CD25 Ab (BD PharMingen San Diego, CA, USA) and were isolated by BD Arial (BD, San Diego, CA, USA).

2.10. *In vitro* proliferation assays

$CD4^+CD25^-$ cells (5×10^4) from healthy donor were

Table 1. Staining Intensity of ALM and SSM lesions

Items	Non-lung injury	Lung injury	*p* value
Number	26	14	
Age	62.46 ± 2.03	62.35 ± 2.33	0.97
Gender (male/female)	16/10	5/9	0.19
BMI	24.71 ± 0.58	24.74 ± 0.90	0.98
NYHA (I/II/III/V)	0/0/22/4	0/0/13/1	0.64
During operation			
Cardiopulmonary bypass time (min)	148.31 ± 6.81	151.64 ± 8.41	0.77
Auxiliary circulation time (min)	76.19 ± 6.65	67.21 ± 7.55	0.40
Lowest Hct	28.53 ± 0.75	31.21 ± 1.23	0.06
Oxygenation index (before surgery)			
PaO_2/FiO_2 (mmHg)	363.88 ± 7.26	362.31 ± 12.56	0.91

BMI, body mass index; NYHA, New York heart association. Continuous variables with a normal distribution were represented in mean ± SEM, continuous variable with a non-normal distribution were presented as the median. Categorical variables were presented as number (%).

cultured in 96-well plates (0.2 mL) with 5×10^4 $CD4^+CD25^+$ (from patients) for 3 days. Proliferation was measured in triplicates by the expression of CFSE (Sigma-Aldrich, St. Louis, MO, USA).

2.11. Statistical analysis

All data were analyzed using SPSS 22.0 software. Data were presented as mean ± standard error of the mean (SEM). Percent detectable were compared by Pearson's chi-squared test. The differences among the two different groups were evaluated with student *t* test. Graphs were prepared with Prism version 8 (GraphPad Software Inc., La Jolla, CA). For all tests, p values less than 0.05 were considered significantly different. n.s. not significant, * represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$.

3. Results

3.1. Patient characteristics

According to the inclusion/exclusion protocol, 40 patients (21 male and 19 female) who underwent cardiovascular surgery between October 2020 and January 2021 were enrolled in this study. Among those patients, 26 did not develop lung injury after CPB, and 14 developed injury after the surgery (Table 1). The average age, gender, BMI, and NYHA class between these two groups before surgery were similar. In addition, CPB time, auxiliary circulation time, and lowest Hct during surgery were similar between those two groups.

3.2. Low level of peripheral Treg cells in lung injury patients before surgery

Considering a certain correlation between Treg cells and progression of lung injury after cardiopulmonary bypass, it would be interesting to clarify the difference of Tregs between non-lung injury and lung injury patients before surgery. We examined Treg cells in PBMCs from 40 patients before the CPB operation (pre-CPB Tregs), in which 14 developed lung injury after the surgery. As shown in Figure 1, the proportion (Figures 1b and 1c) of pre-CPB Tregs was lower in lung injury patients compared with non-lung injury patients, while little difference was found between lung injury and non-lung injury patients in Th1 (Figure 1d, e and Th17 (Figures 1f and 1g) cells, indicating there was already difference in Treg cells but not in other T helper subsets between lung injury and non-lung injury patient even before cardiopulmonary bypass.

3.3. Suppressive role of Tregs in the proliferation of effector T lymphocytes

Treg cells were originally described as suppressing proliferation of other lymphocyte subsets. Thus, we speculated that the dysfunction of peripheral pre-CPB Treg cells may actively participate in the onset of lung injury after CPB. We isolated pre-CPB Treg cells from patients and co-cultured with $CD4^+CD25^-$ (Figures 2a and 2b) or with $CD8^+CD25^-$ (Figures 2c and 2d) cells from healthy donors. Both effector CD4 and CD8 T cells cocultured with pre-CPB Treg cells from lung injury patients showed higher proliferation ability compared to those with pre-CPB Tregs from non-lung injury patients. These results suggested that pre-CPB Tregs from lung injury patients presented reduced ability to suppress the proliferation of effector CD4 and CD8 T cells.

3.4. Inhibition of Tregs on function of effector T lymphocytes

As Treg cells were reported to inhibit the function of other effector T lymphocytes, next we examined the cytokine produced by effector T lymphocytes that cocultured with the pre-CPB Treg cells from patients. Results showed that more IFN-γ^+ CD4 T cells were found in $CD4^+CD25^-$ T cells co-cultured with pre-CPB Tregs from lung injury patients than with those from non-lung injury patients (Figure 3b). Similarly, IFN-γ-

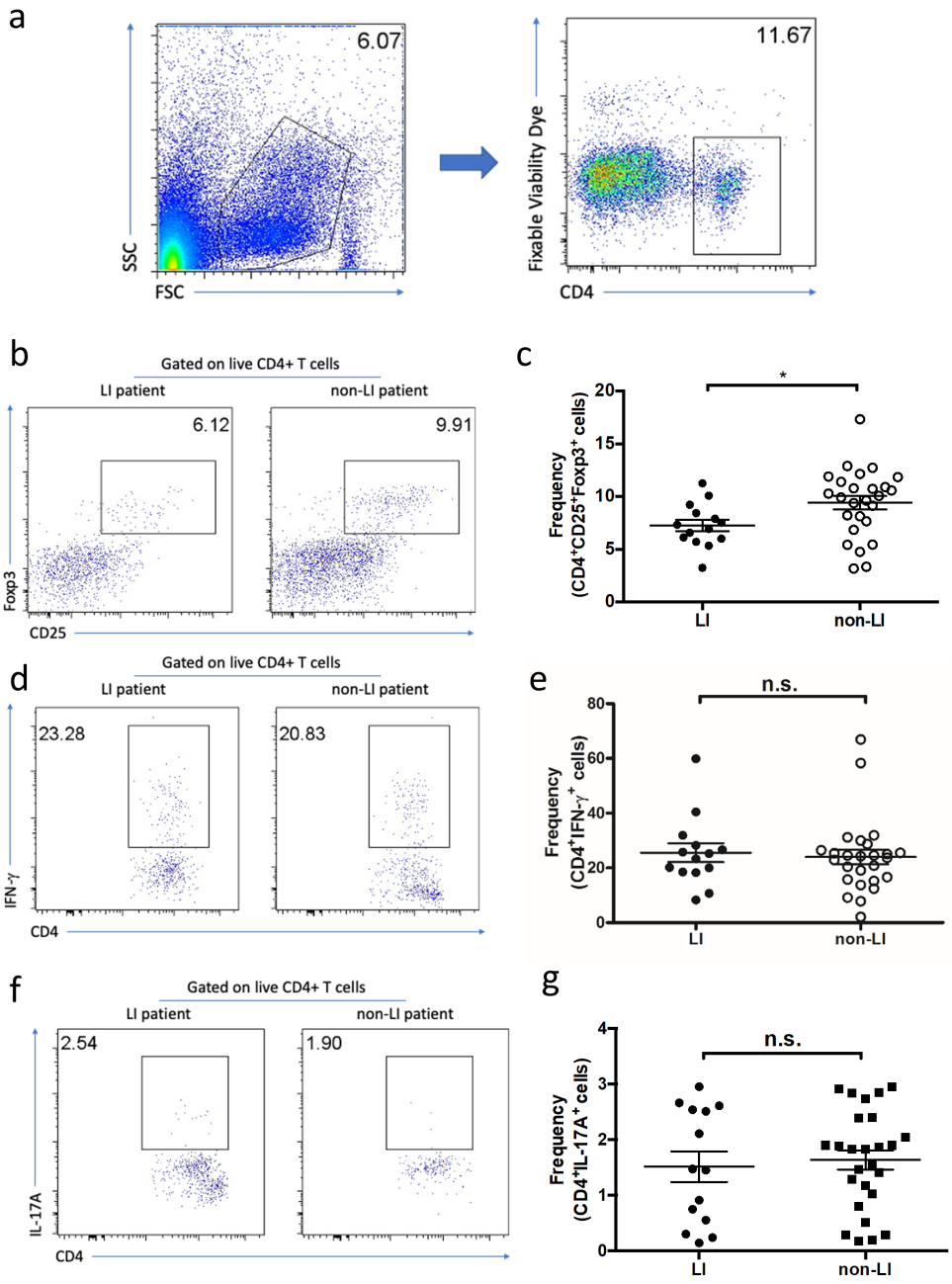

Figure 1. Treg cells in lung injury and non-lung injury patients. PBMC from lung injury and non-lung injury patients before CPB respectively were analyzed for Treg, Th1 and Th17 cells by flow cytometry. **a**. The flow cytometry gating strategy for live $CD4^+$ T cells (applied for all gating strategy in this study). **b**. Representative plots for flow cytometry data of Treg cells ($CD4^+CD25^+Foxp3^+$) in PBMC. **c**. The frequency of Treg cells in **(a)**. **d**. Representative plots for flow cytometry data of Th1 cells ($CD4^+IFN\text{-}\gamma^+$) in PBMC. **e**. The frequency of Th1 cells in **(d)**. **f**. Representative plots for flow cytometry data of Th17 cells ($CD4^+IL\text{-}17A^+$) in PBMC. **g**. The frequency of Th1 cells in **(f)**. Error bars denote mean ± SEM. Each dot in **c**, **e** and **g** represented an individual.Statistical analysis was performed using t test analysis, * $p < 0.05$.

producing CD8 T cells increased when cocultured with pre-CPB Tregs from lung injury patients compared to those with pre-CPB Tregs from non-lung injury patients (Figure 3d). These data suggested that pre-CPB Tregs from lung injury patients presented defect in inhibiting cytokine secreting by effector T lymphocytes.

3.5. Altered TGF-β and IL-10 levels in serum

Treg cells can play immunosuppressive roles by excreting TGF-β and IL-10. Considering lower amount of pre-CPB Treg cells in LI patients, we examined levels of TGF-β, IL-10, IFN-γ, and IL-17A in serum of the patients before surgery. Data showed that the pre-surgery levels of TGF-β (Figure 4a) and IL-10 (Figure 4b) were markedly lower in lung injury patients than in non-lung injury patients meanwhile IFN-γ (Figure 4c) and IL-17A (Figure 4d) had similar levels between serum from Lung injury and non-lung injury patients, indicating the defect of Treg cells in cytokine

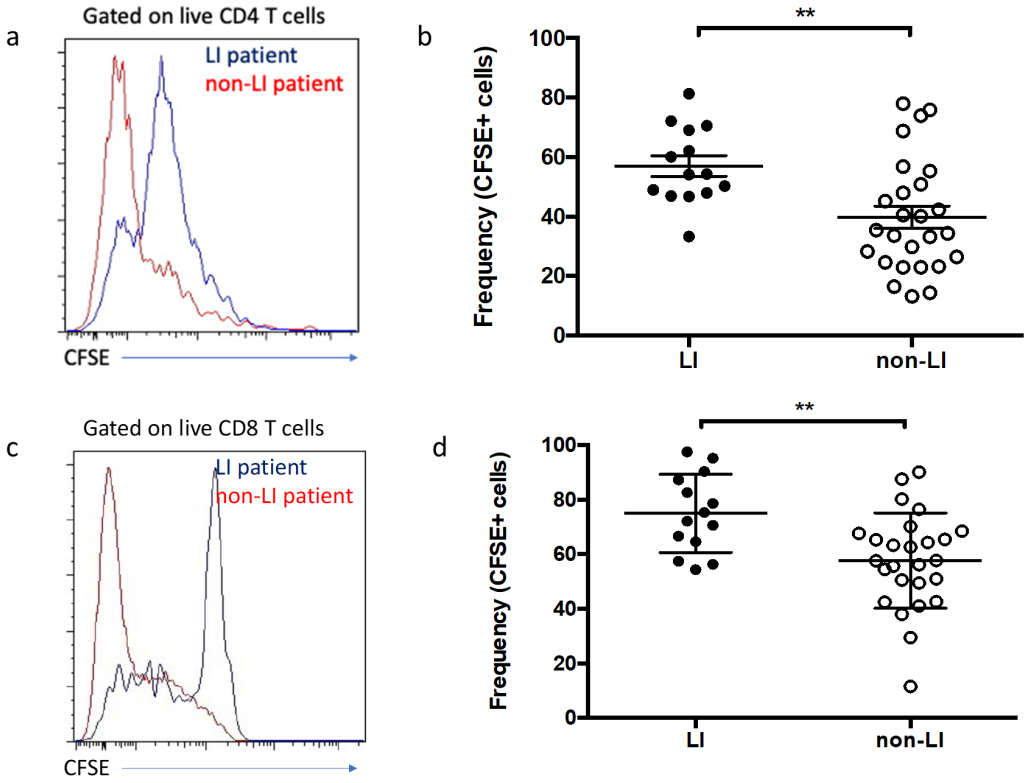

Figure 2. Inhibition of Treg cells on CD4 and CD8 T cells. Treg cells ($CD4^+CD25^+$) were isolated from periphery blood of lung injury or non-lung injury patients before CPB, then co-cultured with $CD4^+CD25^-$ or $CD8^+CD25^-$ from healthy donor. **a**. Representative histogram for flow data of CFSE expression in $CD4^+CD25^-$ T lymphocytes post co-cultured with Treg cells. **b**. Frequency of $CFSE^+$ cells in **a**. **c**. Representative histogram for flow data of CFSE expression in $CD8^+CD25^-$ T lymphocytes post co-cultured with Treg cells. **d**. Frequency of $CFSE^+$ cells in **c**. Each dot in **b** and **d** represented an individual. Statistical analysis was performed using *t* test analysis, * $p < 0.05$.

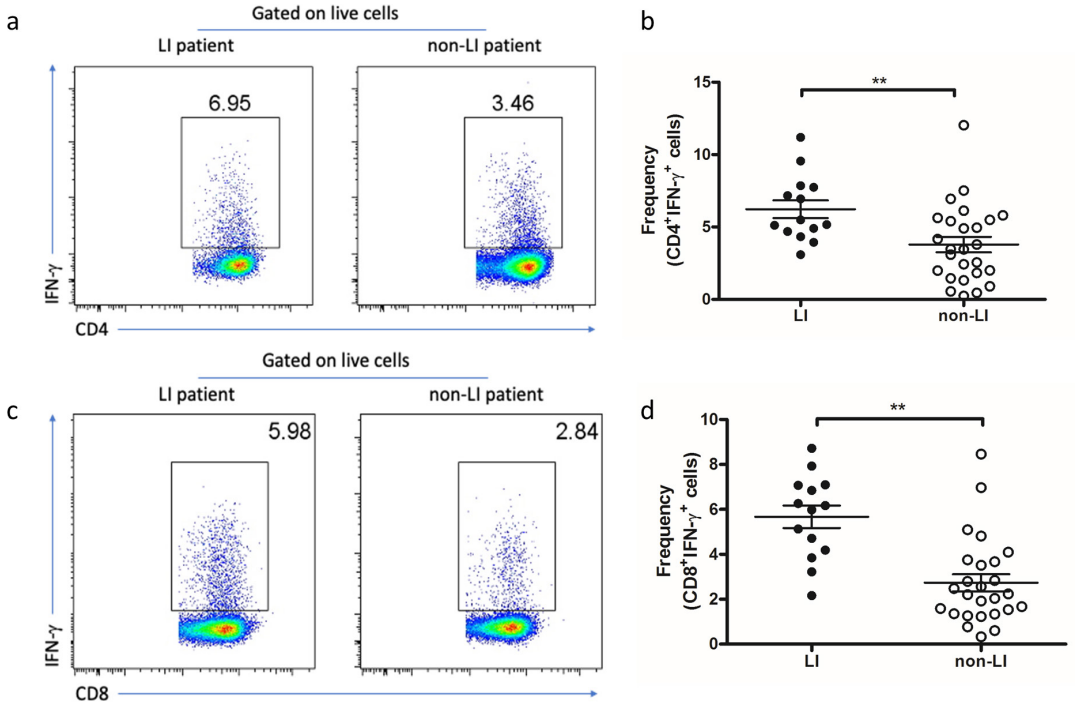

Figure 3. IFN-γ-producing T cells in lung injury and non-lung injury patients. Treg cells ($CD4^+CD25^+$) were isolated from periphery blood of lung injury or non-lung injury patients before CPB, then co-cultured with PBMC from healthy donor. **a**. Representative plotsfor flow data of $CD4^+IFN\text{-}\gamma^+$ cells post co-cultured with Treg cells. **b**. The frequency of $CD4^+IFN\text{-}\gamma^+$ cells in **(a)**. **c**. Representative plots for flow data of $CD8^+IFN\text{-}\gamma^+$ cells post co-cultured with Treg cells. **d**. The frequency of $CD8^+IFN\text{-}\gamma^+$ cells in **(c)**. Each dot in **b** and **d** represented an individual. Statistical analysis was performed using *t* test analysis, * $p < 0.05$.

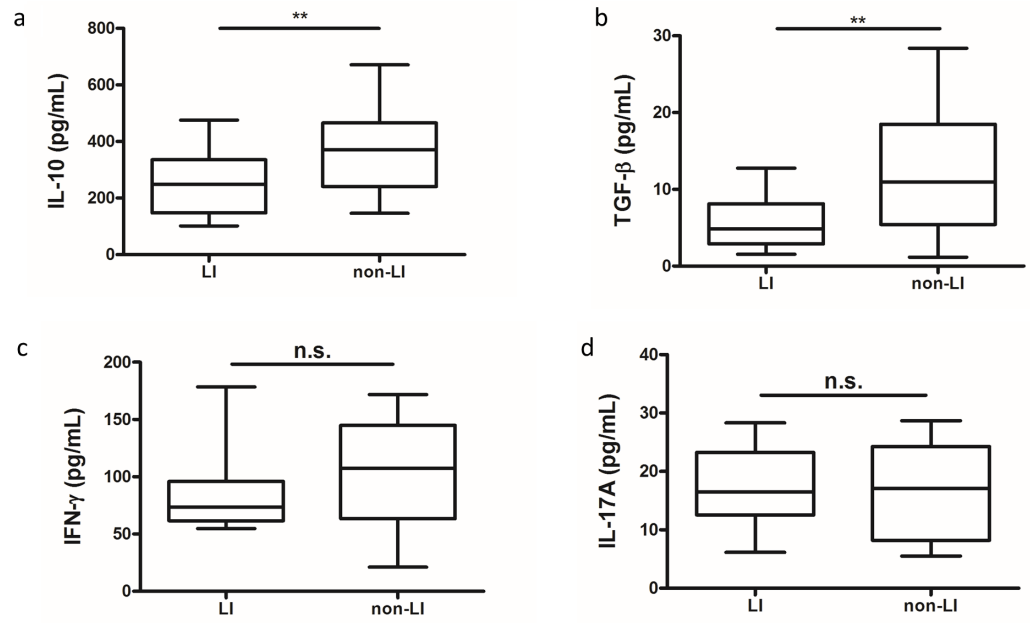

Figure 4. Levels of cytokines from serum in lung injury and non-lung injury patients. The levels of TGF-β **(a)**, IL-10 **(b)**, IFN-γ **(c)** and IL-17A **(d)** in serum from lung injury and non-lung injury patients were detected by ELISA. Each dot represented an individual. Statistical analysis was performed using *t* test analysis, * $p < 0.05$.

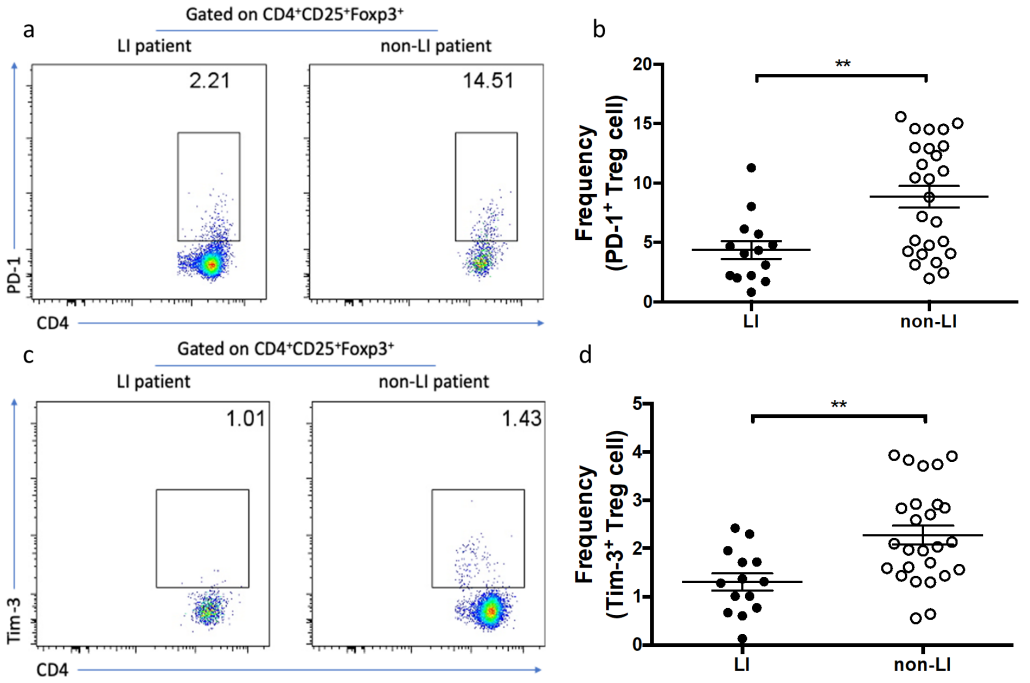

Figure 5. Expression of PD-1 and Tim-3 on Treg cells. Inhibitory immune checkpoint expression on Tregs in PBMC from lung injury and non-lung injury patients were analyzed by flow cytometry. **a**. Representative plots for flow data of $CD4^+CD25^+Foxp3^+PD\text{-}1^+$ cells in peripheral blood. **b**. The frequency of $CD4^+CD25^+Foxp3^+PD\text{-}1^+$ cells in **(a)**. **c**. Representative plots for flow data of $CD4^+CD25^+Foxp3^+Tim\text{-}3^+$ cells in peripheral blood. **d**. The frequency of $CD4^+CD25^+Foxp3^+Tim\text{-}3^+$ cells in **(c)**. Each dot in **b** and **d** represented an individual. Statistical analysis was performed using *t* test analysis, * $p < 0.05$.

production in lung injury patients.

3.6. Expression of PD-1 and Tim-3 on Tregs

PD-1 and Tim-3 could be expressed on Tregs and modulate function of these cells. Also, previous studies demonstrated that PD-1-PD/L1 pathway plays a central role in lung protection during acute lung injury. In addition, higher expression of Tim-3 on Tregs was associated with better clinical outcome in acute lung injury patients. Thus, we further examined the expression of PD-1 and Tim-3 on peripheral pre-CPB Tregs from lung injury and non-lung injury patients. We observed less $PD\text{-}1^+$ Treg cells among PBMC from

lung injury patients than from non-lung injury patients (Figure 5b). Similarly, lower level of Tim-3$^+$ Tregs was also found in pre-CPB Tregs from lung injury patients compared with non-lung injury patients (Figure 5d).

4. Discussion

In this study, we showed a decrease in peripheral Treg cells both in population and in function from lung injury patients. Less pre-CPB Tregs were detected in lung injury patients than in non-lung injury patients, and these cells were with lower suppressive ability to the proliferation and cytokine secretion of effector T cells. Also, declined serum levels of TGF-β and IL-10 indicated impaired Treg function in cytokine production in lung injury patients. In addition, expression of PD-1 and Tim-3 on pre-CPB Tregs were lower in lung injury patients compared with those in non-lung injury patients. All these data indicate there is a difference in Treg cells between lung injury and non-lung injury patients before cardiac surgery, and propose Tregs as a potential biomarker for lung injury diagnosis during CPB.

Previous studies have suggested that circulating humoral and inflammatory factors mediate the pulmonary injury associated with CPB due to exposure to foreign material (*7*). Other factors such as complement activation, ischemia-reperfusion injury, proteases, arachidonic acid metabolites, endotoxin, and bacterial translocation also contribute to this process (*8*). Apoptosis, also known as programmed cell death, plays important roles in disease states (*9*). Some supporting treatment can decrease the occurrence of cardiomyocyte apoptosis in the CPB process (*10,11*) and protect cardiomyocyte (*12,13*). But the exact mechanism underlying the process is unclear. Treg cells, either natural or induced, suppress a variety of physiologic and pathological immune responses (*14,15*). These cells have been considered as a potential target for treating several lung injuries. Previous research mainly focused on Tregs in mice model. However, research about Tregs in human lung injury after CPB remains unclear. Interestingly, in our study, lower level with impaired function of Treg cells was found in lung injury patients before surgery than in non-lung injury patients, suggesting patients with better immunosuppressive function before surgery may have less lung injury after CPB. This could explain why patients with similar pre-operation conditions underwent same surgical process could develop into different degree of lung injury post-operation.

Mechanism of Treg regulation in lung injury remains unclear (*16,17*). Programmed cell death receptor 1 (PD-1) pathway is critical to maintain the intricate balance between positive and negative signals to ensure adequate immune protection against pathogens and yet prevent over activation of lymphocytes. Similar to PD-1, compelling evidence is emerging for the role of Tim-3 in peripheral immune tolerance, autoimmune response, and antitumor and antiviral immune evasion (*18*). Studies have shown that Tim-3 is constitutively expressed on natural Tregs and has been identified as a regulatory molecule of alloimmunity through its ability to modulate CD4$^+$ T cell differentiation in mice. In humans, Tim-3 expression on Treg cells identifies a population highly effective in inhibiting pathogenic Th1- and Th17-cell responses. Here, we observed decreased expression of PD-1 and Tim-3 on Treg cells in lung injury patients before surgery, indicating the involvement of these molecules through Treg cells and may play an important role in the development of CPB-induced lung injury.

This study reported the difference of Treg cells between lung injury and non-lung injury patients before cardiac surgery, and proposed Tregs as a potential biomarker for lung injury diagnosis during CPB. However, the sample size of this current study is relatively small, and population-based studies with large sample size are required to further verify the findings in this present work. Additionally, we only compared the population and function of pre-CPB Tregs between lung injury and non-lung injury patient. Further studies to identify specific molecules of Treg cells in CPB-induced lung injury and investigate the mechanism for Treg cells on lung injury development after CPB are required.

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Original Article

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Macroscopically complete excision is a beneficial strategy for selected patients with peritoneal sarcomatosis

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SUMMARY The occurrence of peritoneal sarcomatosis (PS) in patients with retroperitoneal sarcoma (RPS) indicates a poor prognosis. However, the appropriate treatment modality remains unclear. This study aimed to identify its prognostic factors and further explore the role of macroscopically complete excision (CE) in the management of PS. A retrospective database was established to evaluate patients with RPS who underwent resection between January 2011 and January 2019. Univariate and multivariate survival analyses were performed to analyze the prognostic factors and identify the population that will optimally benefit from CE. This study included a total of 49 patients with PS from 211 patients with RPS, and 34 (69.4%) patients of whom with PS underwent CE successfully. The median follow-up time was 36.0 months. There were 8 patients excluded because of loss to follow-up (n = 4) or death from complications within 90 days postoperatively (n = 4). The CE group had a marginally better prognosis compared to the macroscopically incomplete excision (IE) group (median disease-specific survival: 20 months *vs.* 8 months). Multivariate survival analysis demonstrated that completeness of operation (CE *vs.* IE) was the only independent prognostic factor in PS patients (P = 0.042). There was no significant difference in the overall complications between the CE and IE groups (P = 0.205). In conclusion, completeness of macroscopical excision is an independent prognostic predictor of PS. If technically possible, CE is a feasible strategy to improve the prognosis of selected patients with PS.

Keywords peritoneal sarcomatosis, prognostic factors, macroscopically complete excision, survival benefit, appropriate patients

1. Introduction

Retroperitoneal sarcoma (RPS) refers to several categories of rare tumors that originate from the retroperitoneal mesenchymal tissue. RPS accounts for approximately 16% of all soft tissue sarcoma cases (*1,2*).

Peritoneal sarcomatosis (PS) is a state of intraperitoneal dissemination of sarcomas. PS is rare and occurs only in approximately 10% of patients with primary RPS disease (*3*). However, PS is highly common in patients with recurrent RPS disease, occurring in 35-82% of patients (*4,5*). The presence of pathologically confirmed lesions on the surface of the peritoneum or intraperitoneal viscera is considered as PS. It can be a spontaneous phenomenon or caused by iatrogenic factors (*6*). Traditionally, PS often indicates end-stage disease, with a median survival of less than 1 year (*7*).

However, data on PS are limited owing to its rarity and complexity, and thus the management of PS remains controversial. Most pathological subtypes of RPS are not sensitive to radiotherapy and chemotherapy (*8*), and thus, surgical resection is the primary treatment modality. However, the role of complete resection for PS is still controversial. Some researchers believe that surgery should be restricted to palliative intervention according to the symptoms, with the goal of avoiding complications and preserving function (*9*), while others support that macroscopically complete resection can significantly improve the prognosis of patients (*6,10*).

Therefore, this study aimed to analyze the prognostic factors, explore the benefits of macroscopically complete excision (CE) for patients with PS, and identify the population who will optimally benefit from CE.

2. Patients and Methods

2.1. Study design and patients

Using the RPS database at Peking University Cancer

Hospital Sarcoma Center, we identified 211 patients who underwent surgery for RPS between January 2011 and January 2019. Among these, the clinicopathological data of 49 patients with PS were collected and analyzed. The patients were defined as primary and recurrent patients according to whether they had undergone RPS-associated surgical treatment before admission or not. The RPS pathological subtypes were classified according to the 2020 World Health Organization criteria for soft tissue tumors (*11*). PS nodules were carefully assessed through intraoperative exploration and postoperative pathological examination. Pathological diagnoses were reviewed by two experienced pathologists specializing in sarcomas. Disagreements were resolved by discussion between them.

The study variables included sex, age, body mass index (BMI), pathological subtypes, operation history (primary or recurrent), the sum of the largest diameter (SLD) of tumors with a diameter more than 1 cm on imaging, ascites, number of nodules, the Federation Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grade (*12*).

This study was approved by the ethics committee of the Peking University Cancer Hospital and was conducted according to the tenets of the Declaration of Helsinki. All patients in our study provided written informed consent for data collection.

2.2. Surgical outcomes and follow-up

Current surgeries were classified into complete excision (CE) and incomplete excision (IE). CE was defined as the status of removal of all macroscopic disease (R0/R1). IE was defined as the status of macroscopic residual disease (R2) (*13*). Additionally, piecemeal excision and/or tumor rupture intraoperatively were also considered as IE owing to their high risk for residual disease. Operation reports, immediate postoperative imaging and pathological reports were carefully reviewed and checked to confirm the completeness according to above definition.

Postoperative complications occurring within 90 days (POD 90) of the procedure were graded according to Clavien-Dindo classification (*13*). Postoperative pancreatic fistula was diagnosed according to the criterion of 2016 International Study Group on Pancreatic Surgery (*14*). The patients were prospectively followed with regular telephone follow-up and outpatient follow-up including physical examination, ultrasonography, abdominopelvic contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) every 3 months for the first 2 years, every 6 months for the subsequent three years, and yearly thereafter.

2.3. Statistical analysis

The primary endpoint was disease-specific survival (DSS), defined as death due to tumor progression. Survival time was calculated from the time of operation to the concerning event. Survival curves were generated using the Kaplan-Meier method and compared using the log-rank test. The optimal cut-points for continuous variables were determined by using the maximally selected rank statistics (*15,16*). The Cox proportional hazards regression model was used for the univariate and multivariate prognostic analysis (enter method). Variables with $P < 0.1$ in the univariate analysis and clinically significant variables were included in the multivariate analysis. Comparisons of continuous variables were performed by Wilcoxon's rank-sum test, and the comparisons of categorical variables were performed by Pearson's Chi-square test, continuity correction Chi-square test, or Fisher's exact test, as appropriate. To identify the PS patients who will optimally benefit from CE, subgroup analysis and *P* for interaction were also calculated (*17*). All statistical analyses were performed using SPSS version 26.0 (IBM) and R version 4.0.5 *(http://www.r-project.org/)* with packages of "survival", "surminer", "maxstat", , and "maxstat". Statistical significance was set at a two-sided $P < 0.05$.

3. Results

Among the 211 screened patients with RPS, 49 (23.2%) patients with PS (29 men and 20 women; median age, 50 years; range, 16-86 years) were included in this study (Figure S1, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=86*). Of these patients, 15 (30.6%) and 34 (69.4%) were identified as primary and recurrent patients, respectively. The incidence rate of PS was 13.0% (15/115) and 35.4% (34/96) in the primary and recurrent patients, respectively. The clinicopathological characteristics are shown in Table 1. The most common pathological subtypes were dedifferentiated liposarcoma, well-differentiated liposarcoma, leiomyosarcoma, undifferentiated pleomorphic sarcoma, and synovial sarcoma. All pathological subtypes are listed in Table S1 (*http://www.biosciencetrends.com/action/getSupplementalData.php?ID=86*). The optimal cut-off values for "age", "BMI", and "SLD" were 66 years old, 27 kg/m^2, and 20 cm, respectively.

In total, 34 of the 49 patients (69.4%) with PS underwent CE successfully. Reasons resulting in IE included wide dissemination ($n = 6$), superior mesenteric artery invasion ($n = 3$), abdominal aorta invasion ($n = 2$), bilateral kidney invasion ($n = 1$), piecemeal resection ($n = 2$), and tumor rupture ($n = 1$). All the patients underwent multivisceral resection (MVR) with at least one involved organ resection. The detailed information including initial and current surgeries is summarized in Table 2.

Table 1. Patient characteristics

	Total (n = 49)	CE (n = 34)	IE (n = 15)	P
Sex				0.236[a]
Female	20	12	8	
Male	29	22	7	
Age (median [range], years)	50.0 [16.0-86.0]	48.0 [16.0-78.0]	51.0 [25.0-86.0]	0.737[c]
≥ 66	8	6	2	1[b]
< 66	41	28	13	
Operation history				0.951[b]
Primary patients	15	11	4	
Recurrent patients	34	23	11	
BMI (median [range], kg/m^2)	22.6 [16.9-39.0]	22.3 [17.8-35.5]	24.5 [16.9-39.0]	0.233[c]
≥ 27	10	6	4	0.736[b]
< 27	39	28	11	
SLD (median [range], cm)	27.0 [6.0-69.0]	21.0 [10.0-36.0]	29.0 [6.0-69.0]	0.068[c]
≥ 20	35	22	13	0.220[b]
< 20	14	12	2	
Ascites				1[b]
Yes	10	7	3	
No	39	27	12	
LPS				0.371[a]
Yes	28	18	10	
No	21	16	5	
FNCLCC grade				0.743[a]
G1, G2	18	13	5	
G3	31	21	10	
Number of nodules				0.667[b]
≥ 7	10	8	2	
< 7	39	26	13	

[a]Pearson's Chi-square test; [b]continuity correction Chi-square test; [c]Wilcoxon's rank-sum test. CE, macroscopically complete excision; IE, macroscopically incomplete excision; SLD, the sum of the largest diameter; LPS, liposarcoma; FNCLCC, Federation Nationale des Centres de Lutte Contre le Cancer.

Table 2. Surgical results in patients with peritoneal sarcomatosis

	CE (n = 34)	IE (n = 15)	P
History of surgery			0.951[b]
Primary surgery	11 (32.4%)	4 (26.7%)	
Recurrent surgery	23 (67.6%)	11 (73.3%)	
Initial surgical method of recurrent patients[a]			0.825[c]
En-bloc resection	3 (13.0%)	1 (6.7%)	
Simple tumor resection	18 (78.3%)	8 (53.3%)	
Macroscopically incomplete resection	2 (8.7%)	2 (13.3%)	
Multivisceral resection	34 (100%)	15 (100%)	
Number of resected organs of the current surgery (median [range])	4.5 [1-13]	4 [1-10]	0.371[d]
Common resected organs of the current surgery			0.002[c]
Colon	27 (79.4%)	14 (93.3%)	
Kidney	18 (52.9%)	2 (13.3%)	
Small bowl	10 (29.4%)	6 (40.0%)	
Pancreas	11 (32.4%)	5 (33.3%)	
Abdominal wall	9 (26.5%)	3 (20.0%)	
Resection of major vessels and revascularization	5 (14.7%)	0 (0%)	

[a]Only the recurrent patients need to be considered for their initial surgery; [b]continuity correction Chi-square test; [c]Fisher's exact test; [d]Wilcoxon's rank-sum test. CE, macroscopically complete excision; IE, macroscopically incomplete excision.

There were 8 patients excluded from survival analysis because of loss to follow-up (n = 4) or death from complications within 90 days after surgery (n = 4). The median follow-up time was 36.0 months. Overall, the median DSS of the patients with PS was 15 months (95% confidence interval [CI], 9-34). The 3- and 5-year DSS rates were 19.9% and 0, respectively. The median DSS was 20 months and 8 months in patients with PS in whom CE and IE were performed, respectively. The 3-year DSS was 25.1% in the CE group, and 10.3% in the IE group (log-rank P = 0.077, Figure 1). Besides, the multivariate analysis showed that completeness of operation (CE *vs.* IE) was the only independent prognostic factor in the patients with PS (P = 0.042)

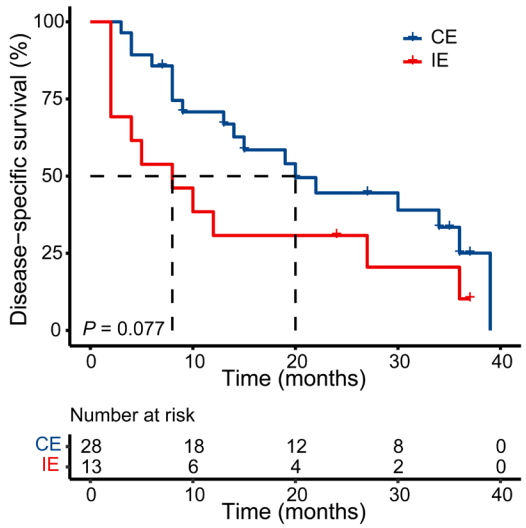

Figure 1. Survival of patients with peritoneal sarcomatosis and survival benefit from macroscopically complete excision.

(Table 3).

We further investigated the prognosis of the subgroups and interactions between treatment and sex, age, operations, BMI, SLD, ascites, LPS, and FNCLCC grade. Overall, CE lowered the risk of DSS by 48% (hazard ratio [HR], 0.52; 95% CI, 0.24-1.11). This effect was significant in patients with recurrent disease (HR, 0.28; 95% CI, 0.10-0.74), non-LPS (HR, 0.15; 95% CI, 0.04-0.57), and FNCLCC grade 3 (HR, 0.34; 95% CI, 0.13-0.88). Furthermore, differences by interaction were particularly robust in the covariates of operation history type (*P* for interaction = 0.026), and FNCLCC grade (*P* for interaction = 0.005) (Figure 2).

To evaluate the perioperative safety of CE, we analyzed the outcomes of patients with PS within 90 days postoperatively. Overall, the rate of 90 days postoperative mortality was 8.2% (4/49), and the rate of severe morbidity (Clavien-Dindo ≥ III) was 28.6% (14/49). Common complications included abdominal

Table 3. Univariate and multivariate survival analysis in patients with peritoneal sarcomatosis

	Univariate analysis		Multivariate analysis	
	P	HR (95% CI)	*P*	HR (95% CI)
Age (≥ 66 *vs.* < 66, years)	0.771	1.14 (0.46-2.84)	0.814	1.16 (0.33-4.13)
Male *vs.* Female	0.576	1.25 (0.57-2.71)	0.605	1.31 (0.48-3.58)
BMI (< 27 *vs.* ≥ 27, kg/m^2)	0.986	0.99 (0.42-2.34)	0.933	0.95 (0.32-2.85)
Completeness of excision (IE *vs.* CE)	0.077	1.99 (0.93-4.25)	0.042	2.66 (1.04-6.83)
Operation history (recurrent *vs.* primary)	0.938	0.97 (0.44-2.15)	0.616	1.34 (0.43-4.13)
Ascites (yes *vs.* no)	0.242	1.68 (0.71-3.98)	0.606	0.72 (0.21-2.49)
FNCLCC grade				
G2 *vs.* G1	0.232	3.56 (0.44-28.44)	0.282	3.69 (0.34-39.89)
G3 *vs.* G1	0.102	5.46 (0.71-41.78)	0.13	5.46 (0.61-49.05)
SLD (≥ 20 *vs.* < 20, cm)	0.209	1.79 (0.72-4.44)	0.412	1.66 (0.50-5.54)
Pathology (LPS *vs.* Non-LPS)	0.210	1.61 (0.77-3.38)	0.428	1.55 (0.52-4.61)
Number of nodules (≥ 7 *vs.* < 7)	0.223	1.67 (0.7-3.8)	0.523	1.59 (0.38-6.55)

HR, hazard ratio; CI, confidence interval; CE, macroscopically complete excision; IE, macroscopically incomplete excision; FNCLCC, Federation Nationale des Centres de Lutte Contre le Cancer; SLD, the sum of the largest diameter; LPS, liposarcoma.

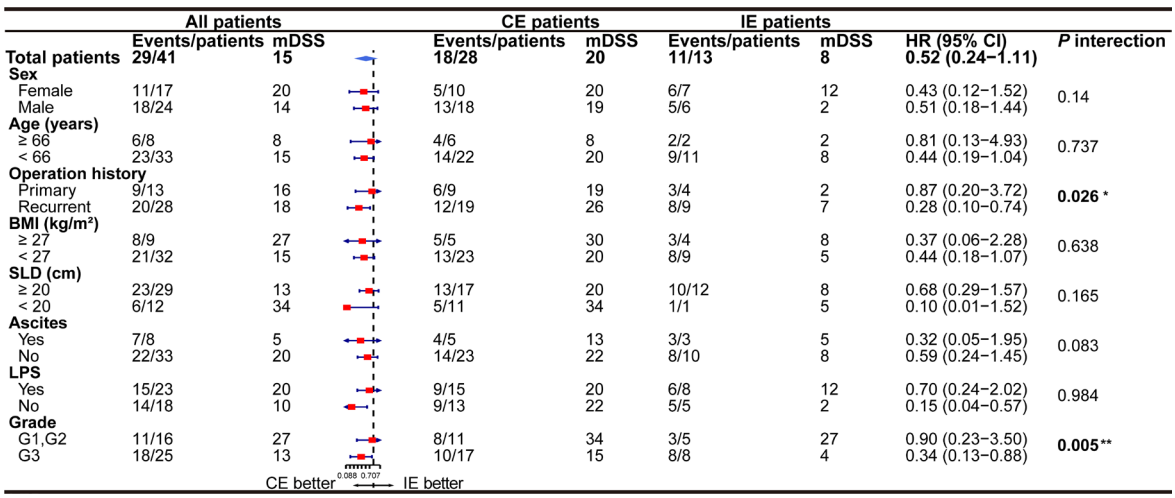

Figure 2. Disease-specific survival by the completeness of macroscopical excision in each subgroup. *P for interaction < 0.05; **P for interaction < 0.01; CE, macroscopically complete excision; IE, macroscopically incomplete excision; BMI, body mass index; SLD, the sum of the largest diameter; LPS, liposarcoma; HR, hazard ratio; CI, confidence interval; mDSS, median disease-specific survival.

Table 4. Postoperative outcomes

	Total (*n* = 49)	CE (*n* = 34)	IE (*n* = 15)	*P*
POD 90 complications				0.205
Yes	26 (53.1%)	16 (47.1%)	10 (66.7%)	
No	23 (46.9%)	18 (52.9%)	5 (33.3%)	
POD 90 complications grade				0.556
Clavien-Dindo I-II	12 (46.2%)	6 (37.5%)	6 (60.0%)	
Clavien-Dindo III-IV	10 (38.5%)	7 (43.8%)	3 (30.0%)	
Clavien-Dindo V	4 (15.4%)	3 (18.8%)	1 (10.0%)	
Classification of major complications				
Hemorrhage	1 (2.0%)	1 (2.9%)	0 (0%)	
Abdominal infection	4 (8.2%)	3 (8.8%)	1 (6.7%)	
Urinary retention/urinary tract irritation	2 (4.1%)	2 (5.9%)	0 (0%)	
Coagulation disorder	1 (2.0%)	0 (0%)	1 (6.7%)	
Lower limb dysfunction	1 (2.0%)	1 (2.9%)	0 (0%)	
Incision infection	4 (8.2%)	1 (2.9%)	3 (20.0%)	
Delayed gastric emptying	1 (2.0%)	1 (2.9%)	0 (0%)	
Anastomotic fistula (grade B)	2 (4.1%)	0 (0%)	2 (13.3%)	
Pancreatic fistula (biochemical leakage)	1 (2.0%)	0 (0%)	1 (6.7%)	
Pancreatic fistula (grade B)	4 (8.2%)	4 (11.8%)	0 (0%)	
Pancreatic fistula (grade C)	1 (2.0%)	0 (0%)	1 (6.7%)	
Organ dysfunction (heart, lung, kidney)	4 (8.2%)	3 (8.8%)	1 (6.7%)	

CE, macroscopically complete excision; IE, macroscopically incomplete excision; POD, postoperative day.

infection (*n* = 4, 8.2%), incision infection (*n* = 4, 8.2%), grade B pancreatic fistula (*n* = 4, 8.2%) and organ dysfunction (*n* = 4, 8.2%), *etc.* The overall complication rate was 47.1% in the CE group and 66.7% in the IE group. There was no significant difference between the two groups (*P* = 0.205). Specifically, the main complications in the CE group were grade III-IV complications, while grade I-II complications were more commonly seen in the IE group. However, there was no significant difference in the complication rate between the two groups (*P* = 0.556). The occurrence of postoperative complications in PS patients is shown in Table 4.

4. Discussion

The onset of RPS is usually insidious with a large volume and obvious heterogeneity in histology. Theoretically, RPS originates from and should be restricted to the retroperitoneal space. However, sometimes the anatomical boundary is broken through spontaneously or iatrogenically, and lesions occur on the surface of the peritoneum or intraperitoneal viscera. Such phenomenon is regarded as PS (*20*).

PS is not a very rare phenomenon in patients with RPS. According to our data, its incidence rate was 13.0% (15/115) and 35.4% (34/96) in the primary and recurrent RPS patients, respectively. Traditionally, as peritoneal carcinomatosis of gastric cancer or colon cancer, PS is regarded as metastasis of RPS, indicating end-stage disease, with a poor outcome. In this case, systemic therapy is usually the first choice and surgery is perceived as conferring little survival benefit. However, differences of biological behavior between RPS and carcinoma should not be ignored. Unlike carcinoma, the intra-abdominal recurrence, rather than lymph node metastasis and distant metastasis, is the main cause of treatment failure in RPS (*18,19*). Unlike the diffusely miliary distribution of peritoneal carcinomatosis, the distribution of PS is often nodular and limited. In addition, unlike the overall high response rate to systemic therapy of carcinoma, few subtypes of RPS are sensitive to systemic therapy. Therefore, the treatment modality of PS remains worth exploring.

The effect of systemic therapy for advanced RPS including PS is unsatisfactory. The response rate is in the 5-25% range, and median progression-free survival is in the 2~6 months range (*20*). The combination of cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) is an effective treatment of peritoneal carcinomatosis. However, Baratti *et al.* reported that the combination of CRS and HIPEC does not compare favorably to those of CRS alone in PS (*21*). Another systematic review in 2011, which included eight prospective and one randomized trial representing 240 patients, concluded that there is a lack of adequate evidence to support that the combination of intraperitoneal chemotherapy and CRS is superior to CRS alone in PS (*22*). Contrarily, several studies reported that the completeness of CRS predicted prognosis in multivariate analysis, indicating that local disease control affected the survival significantly (*4,23*).

Surgical treatment is the mainstay of RPS. It seems that if technically possible, CE remains the most effective treatment of PS. Considering the large anatomical relations with adjacent organs within the abdominal cavity, it is challenging to accurately confirm

negative microscopic margins in RPS. Therefore, in many studies as well as the present study, the status of "removal of all macroscopic disease" was regarded as CE (*24,25*). Technically, CE is feasible to RPS with PS, while its effect remains controversial. One of the controversies is whether PS patients can benefit from CE, and the other one is its safety.

Karakousis *et al.* reported a CE rate of 64% in patients with PS, and patients with CE had a better prognosis than those with IE (median OS: 22.8 *vs.* 8.6) (*10*). A similar result was observed in the study by Sugarbaker *et al.*, with a CE rate of 62.8%. CE improved the prognosis significantly ($P = 0.005$), with the mortality and morbidity of 7% and 19%, respectively (*4*). In the study by Baratti *et al.*, the rate of CE was 75.7%, with an operative mortality of 3.7% and a morbidity of 21.6% (POD 30, National Cancer Institute Common Terminology Criteria) (*21*). Our results also revealed that a large proportion of patients with PS could achieve CE (69.4%). The survival benefit of CE was observed (median DSS: 20 months *vs.* 8 months), and CE is the only independent prognostic factor of PS in the multivariate analysis. Exploring further, recurrent patients and patients with FNCLCC grade 3 disease may benefit more from CE. As for the safety, the mortality and major morbidity of the present study was 8.2% and 28.6%, respectively, similar to literature reports.

According to the consensus from Trans-Atlantic Retroperitoneal Sarcoma Working Group (TARPSWG), the role of surgery for multifocal intra-abdominal metastases is limited to palliative intervention as dictated by symptoms (*20*). However, a series of studies as well as the present study revealed that aggressive surgery may provide survival benefits for selected patients. Considering the complexity, it is recommended that the indication of surgery was discussed by a multidisciplinary team of sarcoma specialists, based on comprehensive consideration. A number of disease- and patient-specific factors should be considered during the decision-making, including if distant metastases exist, if life-threatening conditions exist, if macroscopically complete resection is possible, the pathology, the interval between previous surgery and recurrence, the effect of systemic treatment, *etc.* Generally speaking, if there is no complete resection possibility or rapid recurrence from previous complete resection, surgery should be considered cautiously and limited to palliate severe symptoms. Otherwise, "CE-intent surgery" could be considered aggressively.

The number of lesions was also one of the factors that should be considered, combined with others. Anaya *et al.* found that patients with more than 7 tumors have the worst prognosis (*26*). However, we did not reproduce this significant difference in multivariate analysis ($P = 0.523$). It is worth noting that sometimes it was difficult to confirm the number of lesions (especially small lesions) before surgery. According to our data, only in 44.9% (22/49) of patients, the status of PS could be detected by CT/MRI preoperatively. Therefore, more often, the accurate number of lesions had to be confirmed during the surgery. The result of the present study indicated that when PS was found incidentally during the surgery, it is preferred to pursue CE rather than immediately giving up to palliative resection, if technically possible.

Surgical procedures of RPS are challenging. No matter curative-intent surgery or palliative-intent surgery frequently requires a complex, multivisceral resection (*27*). In the present study, all patients of RPS with PS underwent MVR, referring to multiple specialties including gastrointestinal surgery, hepatopancreatobiliary surgery, urinary surgery, and vascular surgery, *etc.* The goal of the surgical cytoreduction was to remove all visible tumors. However, due to various reasons, sometimes it was impossible to achieve CE. In the present study, the commonest reasons resulting in IE were widespread tumors, anatomical restriction, and piecemeal resection (or tumor rupture). Surgeons had to balance the completeness and security as well as the quality of life individually. Owing to the rarity and complexity, a great number of studies have validated the volume- outcome relationship in RPS, thus patients were strongly recommended to be admitted to a specialized sarcoma center for treatment (*8,28,29*).

This study had certain limitations. First, the retrospective design may have produced biases and resulted in weaker evidence. However, owing to the rarity and the complexity of PS, it is difficult to carry out prospective randomized controlled studies with a large sample size. Second, to minimize the interference of surgery-related factors on statistical analysis, we used data from a single center to ensure the consistency of selection criteria and surgical techniques. However, this also limited the sample size. Nonetheless, through rigorous quality control and analysis, we minimized the influence of bias and confounding factors as much as possible. Thus, we believe that our findings can be helpful for patient assessment and decision-making for treatment. Further studies with a prospective design and a larger sample size are required to validate our findings.

In conclusion, the completeness of macroscopical excision is an independent prognostic factor of PS. If technically possible, CE is a feasible surgical strategy that can improve the prognosis of peritoneal sarcomatosis safely in professional sarcoma centers.

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Association of angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists use with risk of atrial fibrillation after pacemaker implantation among very old patients

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SUMMARY It remains unknown whether and to what extend the angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) can play a role in the development of atrial fibrillation (AF) after pacemaker implantation in very old patients. Therefore, we aimed to investigate the association between oral ACEIs or ARBs and the risk of developing AF in very old patients after pacemaker implantation. Patients above 80 years old with pacemaker implantation and without baseline history of AF were included and their real-world information about ACEIs or ARBs use was extracted from electronic medical records. New AF cases were confirmed *via* the records of outpatient visits. The multivariable Cox proportional-hazards model was used to evaluate the associations between oral ACEIs or ARBs and risk of AF after pacemaker implantation. Among a total of 388 identified patients aged 80 to 98 years, 118 used ACEIs, 174 had ARBs therapy, and 115 AF were identified after pacemaker implantation during a median follow-up time of 3.1 years. After adjustment for potential confounders, patients with daily use of ARBs had a relatively lower risk of AF after pacemaker implantation (HR: 0.627, 95% CI: 0.425, 0.926; $P = 0.019$) compared with those non-users, whereas ACEIs therapy didn't show a significant relation with AF risk (HR: 1.335, 95% CI: 0.894, 1.995; $P = 0.157$). In conclusion, for very old patients with a permanent pacemaker, daily use of oral ARBs was associated with a relative lower risk of AF after pacemaker implantation, however, daily use of ACEIs was not related with AF risk.

Keywords atrial fibrillation, pacemaker implantation, very old patients, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists

1. Introduction

Pacemaker implantation is a common and effective treatment to control all kinds of bradycardia arrhythmias (*1*). In recent years, an augmented life expectancy and enhanced therapeutic options for heart diseases have increased the proportion of the elderly (≥ 80 years) requiring pacemaker implantation. However, a significant amount of researches have described that the incidence of atrial fibrillation (AF) in pacemaker implanted population increased from 3% to 15-30%, and very old patients were associated with a higher risk of AF (*2-5*). Indeed, AF induced by artificial pacing is widely considered to be directly related to atrial pathological remodeling, which includes structural pathological remodeling and electrical remodeling. In addition, sympathetic activation, inflammation, and pacing mode are associated with AF development after pacemaker implantation (*6-8*). Factors influencing the occurrence of AF after pacemaker implantation remain elusive, especially among elderly patients, and related evidence is limited.

Previous studies have revealed that angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are primarily related to alleviating cardiac structural pathological remodeling and electrical remodeling. Besides, a certain proportion of very old patients take ACEIs or ARBs daily to regulate blood pressure, alleviate heart failure and other diseases. Therefore, the potential association between ACEIs or ARBs use and the occurrence of AF after pacemaker implantation in the elderly patients warrants further investigation.

The mechanisms of action of ACEIs and ARBs are slightly distinct. Previous studies have demonstrated that ARBs essentially inhibited the local systemic

renin-angiotensin-aldosterone system (RAAS) activity and alleviated the atrial remodeling. In contrast, ACEIs mainly play a role of RAAS in circulation but with limited function in the regional heart. Therefore, it is reasonable to hypothesis that ARBs and ACEIs might have an effect on the occurrence of AF after pacemaker implantation. Thus, we conducted a real-world study to explore the effect of ACEIs and ARBs on the occurrence of AF after pacemaker implantation among very old patients, thereby providing evidence for cardiovascular drugs selection.

2. Methods

2.1. Study subject

After de-identification of personal information, patients who received pacemaker implantation in Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine from 2012 to 2018, aged more than 80 years old and without a known baseline history of AF, were included in this study. Real-world information of daily oral ACEIs or ARBs use was extracted from the prescribed medication information. Exclusion criteria were as follows: (*i*) Previous history of cardiac surgery, (*ii*) Abnormal thyroid function (hyperthyroidism), (*iii*) Congenital heart disease, (*iv*) Severe liver and renal dysfunction, (*v*) Severe valvar heart disease, (*vi*) Patients with AF (either paroxysmal or permanent) before the study, (*vii*) Patients who were diagnosed with AF within 3 months after pacemaker implantation were also excluded since it has been widely accepted that at least 3 months were needed for cardiac remodeling in inducing AF, and related studies also commonly excluded such individuals (*2,9*).

2.2. Medication information collection

The main exposure of interest was the use of ACEIs or ARBs, which was identified from the prescription records. We collected detailed information about the prescribed ACEIs and ARBs. The main purpose of this study was to compare the risk of subsequent development of AF after pacemaker implantation in patients with or without ACEIs/ARBs use, as well as the contrasting roles they played in subjects.

2.3. Primary outcomes

New AF cases were defined by EMR checks and confirmed by professional physicians during either an ambulatory visit, discharge diagnosis, or during post-operation follow up appointments. AF cases were further diagnosed based on the International Classification of Diseases, 10th Revision, with the diagnosis code of (ICD-10: I48).

2.4. Covariates

For each patient, we conducted a retrospective review of their EMR and database information, including hypertension, diabetes mellitus, heart failure, left ventricular ejection fraction (LVEF), and NYHA Functional Classification as covariates. Data on other related prescribed medications, including β-blockers, calcium-channel blockers (CCBs), statins, and diuretics were also abstracted.

2.5. Statistical Analyses

Continuous variables were expressed as mean ± standard deviation, whereas categorical variables were expressed as frequencies. The Kolmogorov-Smirnov test was used to examine whether the continuous variables were normally distributed. Student's *t*-tests or otherwise the Mann-Whitney *U* tests were used to compare the normal distributed continuous data in two groups. Categorical variables were compared by using the chi-square test. Cox proportional models were applied to calculate the hazard ratio (HR) and 95% confidence interval (CI) to evaluate the association between oral ACEIs or ARBs and AF risk after pacemaker implantation, after adjustment for age, gender, documented comorbidities and concomitant medications. Kaplan-Meier curve and log-rank test was employed to compare the AF-free survival in ACEIs/ARBs users and non-users.

All statistical analyses were performed by using the STATA 15.1 software. The *P*-value less than 0.05 was considered as statistical significance.

3. Results

3.1. Patient characteristics

A total of 388 patients aged over 80 years (80-98 years) were included for analyses. 115 AF cases were identified after pacemaker implantation during a median follow-up time of 3.1 years. Among them, 118 (30.4%) patients received ACEIs therapy and 42 AF cases were identified, whereas 174 (44.9%) took ARBs, and 43 AF cases were observed. Baseline characteristics of all subjects are summarized in Table 1 and Table 2. Compared with ACEIs non-users, ACEIs users were more likely to receive cardioprotective medications including β-blocker (73.7% *vs*. 63.0%, $P = 0.039$), statins (74.6% *vs*. 54.8%, $P < 0.001$). Meanwhile, the ACEIs users had higher prevalence of hypertension (83.1% *vs*. 70.4%, $P = 0.009$) and heart failure (61.0% *vs*. 44.8%, $P = 0.003$) (Table 1). ARBs users presented with an increased use of CCB (75.1% *vs*. 56.1%, $P < 0.001$), and were more likely to suffer from hypertension (79.3% *vs*. 70.1%, $P = 0.039$) and

Table 1. Baseline characteristics of ACEIs users and non-users

Characteristics	ACEIs users $n = 118$	Non-users $n = 270$	*P* value
Male	64 (54.2)	145 (53.7)	0.92
Age (year)	85.9 ± 3.6	86.1 ± 4.0	0.63
Current smokers	35 (29.7)	44 (16.3)	0.003
Current drinkers	13 (11.0)	12 (4.4)	0.015
History of hypertension	98 (83.1)	190 (70.4)	0.009
History of heart failure	72 (61.0)	121 (44.8)	0.003
NYHA functional class			
1	46 (39.0)	149 (55.2)	< 0.001
2	28 (23.7)	75 (27.8)	
3	33 (28.0)	40 (14.8)	
4	11 (9.3)	6 (2.2)	
History of diabetes	28 (23.7)	65 (24.1)	0.94
β-blocker users	87 (73.7)	170 (63.0)	0.039
CCB users	84 (71.2)	168 (62.2)	0.089
Diuretics users	98 (83.1)	185 (68.5)	0.003
Statins users	88 (74.6)	148 (54.8)	< 0.001
LVEF (%)	60.36 ± 9.55	62.52 ± 8.86	0.095
LAD, mm	40.29 ± 5.37	38.56 ± 4.62	0.019
AF	42 (35.6)	73 (27.0)	0.090
TFPAF (month)	27.9 ± 17.9	29.6 ± 15.0	0.59
Follow-up months	35.9 ± 18.2	37.0 ± 18.0	0.58

Abbreviation: NYHA, New York Heart Association; ACEI, angiotensin-converting enzyme inhibitor; CCB, calcium channel blocker; AF, atrial fibrillation; TFPAF, time from pacemaker implantation to atrial fibrillation occurrence. Values were given as mean ± standard deviation, or frequency (percentage).

Table 2. Baseline characteristics of ARBs users and non-users

Characteristics	ARBs users $n = 174$	Non-users $n = 214$	*P* value
Male	94 (50.4)	115 (53.7)	0.96
Age (year)	86.3 ± 4.0	85.9 ± 3.8	0.33
Current smokers	33 (19.0)	46 (21.5)	0.54
Current drinkers	10 (5.7)	15 (7.0)	0.61
History of hypertension	138 (79.3)	150 (70.1)	0.039
History of heart failure	82 (47.1)	111 (51.9)	0.35
NYHA functional class			
1	92 (52.9)	103 (48.1)	0.22
2	50 (28.7)	53 (24.8)	
3	27 (15.5)	46 (21.5)	
4	5 (2.9)	12 (5.6)	
History of diabetes	50 (23.7)	43 (20.1)	0.047
β-blocker users	123 (70.7)	134 (62.6)	0.094
CCB users	129 (74.1)	123 (57.5)	< 0.001
Diuretics users	132 (75.9)	151 (70.6)	0.24
Statins users	111 (63.8)	125 (58.4)	0.28
LVEF (%)	62.66 ± 8.07	61.10 ± 9.94	0.20
LAD, mm	38.53 ± 4.43	39.59 ± 5.29	0.12
AF	43 (24.7)	72 (33.6)	0.055
TFPAF (month)	32.3 ± 15.4	27.0 ± 16.2	0.086
Follow-up months	40.1 ± 18.2	33.9 ± 17.4	< 0.001

Abbreviation: NYHA, New York Heart Association; ARB, angiotensin-receptor blocker; CCB, calcium channel blocker; AF, Atrial fibrillation; TFPAP, time from pacemaker implantation to atrial fibrillation occurrence. Values were given as mean ± standard deviation or frequency (percentage).

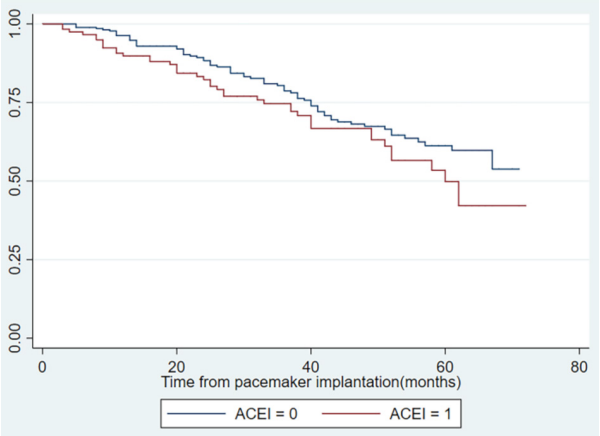

Figure 1. Kaplan-Meier curve of AF-free survival for 72-month follow-up after pacemaker implantation in ACEIs users and non-users.

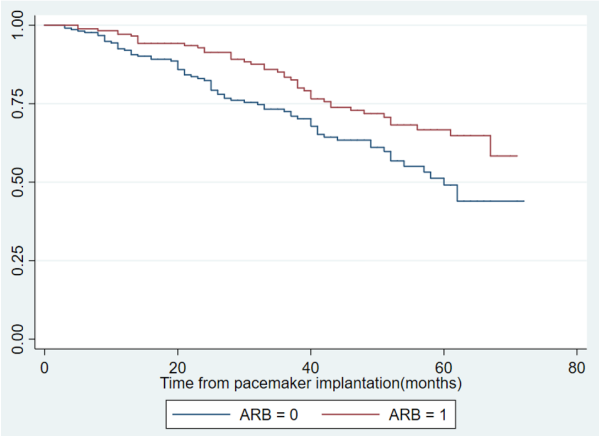

Figure 2. Kaplan-Meier curve of AF-free survival for 72-month follow-up after pacemaker implantation in ARBs users and non-users.

diabetes (23.7% *vs*. 20.1%, $P = 0.047$) (Table 2).

3.2. Primary outcome: AF after pacemaker implantation

Among a total of 388 patients aged more than 80 years old, 115 AF cases were identified after pacemaker implantation during a median follow-up time of 3.1 years. Among the enrolled individuals, 292 patients ($n = 388$) used ACEIs or ARBs. Kaplan-Meier curve indicated that the ACEIs users related with a non-significant increased risk of developing AF after pacemaker implantation ($P = 0.157$) (Figure 1), whereas patients taking ARBs had a benefit on AF-free survival after pacemaker implantation ($P = 0.019$) (Figure 2).

Consistent results were detected by using a multivariable Cox regression. Patients with daily use of ARBs had a relatively lower risk of AF after pacemaker implantation (HR: 0.627, 95% CI: 0.425, 0.926; $P = 0.019$), which remained statistically significant after adjusting for other clinical confounders: age, gender, smoking status and drinking status, medication use (β-blocker, CCBs, statins, and diuretics), and history

Table 3. Associations between ACEIs or ARBs users and risk of atrial fibrillation after pacing in Chinese very old patients

Variables	Case/N	HR (95% CI)	*P* value
ACEIs users	42/118	1.335 (0.894, 1.995)	0.157
Non-users	73/270	Referent	
ARBs users	43/174	0.627 (0.425, 0.926)	0.019
Non-users	72/214	Referent	

Model adjustment: age, gender, current smoking status, current drinking status, medication use (β-blocker, CCB, statins, diuretics), history of chronic diseases (hypertension, NYHA functional class, and ischemic stroke). Abbreviations: HR, hazard ratio; NYHA, New York Heart Association; ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin-receptor blocker; CCB, calcium channel blocker.

of chronic diseases (hypertension and ischemic stroke), New York Heart Association functional classification for HF. ACEIs use shown to be non-related with AF risk (HR: 1.335, 95% CI: 0.894, 1.995, $P = 0.157$) (Table 3).

4. Discussion

Pacemaker implantation is currently a common and effective method to treat all kinds of bradycardia arrhythmias. Due to the progressive increase of life expectancy in recent years, the trend has remained stable over the past few years that the patient's age of receiving a permanent pacemaker implantation has been increasing. For example, the mean age of pacemaker implantation has increased from 77 years in 2012 to 77.8 years in 2016, and about 50% of total pacemaker implantation individuals are above 80 years old in Spain (*10*). Moreover, the incidence of AF has been observed in the pacemaker implanted population, especially in the geriatric patients, which accounted for 30% of total AF patients and was nearly twice higher than the young patients (*11*). However, most available studies on AF developing after pacemaker implantation are usually conducted on middle-aged patients, especially those below the age of 80 years old. Some of these trials even exclude this subgroup of patients. Therefore, among very old patients, ways to decrease the risk of AF after pacemaker implantation merits further investigation.

Basic studies have demonstrated that AF induced by artificial pacing is directly related to atrial remodeling, including structural pathological remodeling and electrical remodeling. On the other hand, as an independent risk factor for the development of AF, aging may inevitably lead to myocardial hypertrophy and fibrosis for their excitable state of RAAS. Thus, factors that inhibit the local RAAS activity induced by pacemaker implantation in very old patients may decrease the risk of AF. This study mainly focused on potential factors that decrease AF risk in very old patients with a permanent pacemaker.

A high proportion of very old patients take daily ACEIs/ARBs for heart diseases. Previous studies have corroborated that ACEIs and ARBs are related to alleviating cardiac structural pathological and electrical remodeling. Interestingly, ACEIs/ARBs have been reported to be more effective among the elderly than in young patients since they can inhibit the excitable state of RAAS in the regional heart in those very old patients. Furthermore, multiple studies have confirmed that ARBs and ACEIs can prevent AF episodes. For example, ARBs are more effective than ACEIs in reducing the risk of AF in old patients with a history of hypertension (*12*); Olmesartan can reverse moderate myocardial hypertrophy, reduce structural remodeling caused by AF, and prevent recurrence of AF (*13*); Enalapril can also reduce the sensitive index of AF through inhibition of atrial remodeling to reduce the occurrence of AF (*14*). Therefore, ACEIs/ARBs may affect AF development in patients with a permanent pacemaker, especially in very old individuals.

Compared to ACEIs in preventing AF episodes after pacemaker implantation in oldest old patients, ARBs might be more effective as they can alleviate cardiac pathological structural and electrical remodeling by functioning on cardiac local angiotensin receptors (activating the AT2 receptor and antagonizing AT1 receptor). By inhibiting the activation of the AT1 receptor, a feedback mechanism increases angiotensin II synthesis, leading to AT2 activation. The latter counter-regulates the effects of the AT1. In addition, AT2 receptor stimulation attenuates the progression of myocardial fibrosis and advances antifibrotic process, mediating vasodilation and inhibiting cellular growth and connective tissue deposition. Past studies have established that ACE2 expression and activity increased in hearts with losartan treatment, and contributes to the increase of Ang-(1-7) activity, while higher Ang1-7 expression levels in left atrial myocardial tissues may lead to lower expression levels of TGF-β1, which then inhibit myocardial fibrosis. In addition, a serine protease with an extremely high affinity for the angiotensin-producing enzyme AngI, which is sensitive to hemagglutinin, was identified in the human heart (*15*). Thymidine has higher specificity and catalytic activity for the conversion of AngI to AngII than ACE, accounting for 6% of captopril in normal human heart and animal tissue extracts. The heart of dogs with chronic volume overload hypertrophy suggests that chymotrypsin, rather than ACE, is the main source of AngII *in vitro* (*15,16*). However, subsequent studies have shown that intracoronary infusion can inhibit 60% of the formation of AngII in the entire myocardial circulation of the body.

ARBs was reported to inhibit chymase function, which is typically increased in the pacing heart. Therefore, ARBs may be more effective in decreasing Ang II induced by pacemaker implantation, which then

attenuates pathological remodeling of heart structure, finally decreasing the risk of AF in elderly pacing patients. ARBs may also prevent AF developing in very old patients with a permanent pacemaker implantation through attenuating alterations in electrical remodeling. The shortening of the atrial effective refractory period (AERP) caused by pacing may promote atrial fibrillation, which is subsequently attributed to the reduction of the action potential duration (APD), resulting in a gradual decrease in the transient outward current (Ito) and L-type Ca^{2+} current (ICa, L). This implies that the direct arrhythmic effect of AngII is predominantly mediated through AT1R signaling. The possible cellular mechanisms of this direct arrhythmic effect of AngII may be due to the alteration of sarcoplasmic reticulum (SR) Ca^{2+} release. In isolated human atrial myocytes, AngII increases the frequency of spontaneous ryanodine receptors (RyR) that mediate basic Ca^{2+} release events (*17*). That effect is mediated by AT1Rs since it is blocked by candesartan. While effects of ACEIs on the ICa,L remains controversial. Recently, a rabbit model recently demonstrated that ACEIs increases the ICa,L current density but do not prevent its down-regulation from tachy-pacing. In addition, the remodeling of connexin 43 (*CX43*) and connexin 40 (*Cx40*) induced by pacemaker implantation may serve as a potential mechanism for the occurrence of AF in the elderly pacing population. For instance, Shyu *et al*. (*18*) observed an increase in *CX43* mediated by the activation of AT1 in cultured rat cardiomyocytes. Additionally, an earlier study described the crucial role of AT1 in gap-junctional remodeling. Connexins are intercellular channels and may form ascendant coupling regions, therefore they are instrumental in the electrical and structural remodeling of the atrium. Interestingly, studies have pointed out that *Cx43* and *Cx40* gene therapies can inhibit the electrical remodeling of the atrium to prevent AF. While dephosphorylation of *Cx43* caused by ARBs use is associated with the downward remodeling of *Cx43* (*19*), thus ARBs may function through reducing *Cx43*. Generally, ARBs may attenuate pathological remodeling of the cardiac structure and electrical remodeling and decrease the risk of AF in the oldest old pacing patients. Consequently, they may seem more effective, since they act on cardiac local angiotensin receptors. These findings are consistent with the results of our study in the sense that with ARBs use, there would be a lower risk of AF after pacemaker implantation in very old population.

Multiple guidelines have recommended ACEIs/ARBs use for very old patients suffering from hypertension or heart failure, while ignoring the fact that these RAAS-activity inhibiting drugs which may also be effective for treating AF by alleviating atrial remodeling. Lately, attention to treating AF has been transformed from anti-arrhythmic drugs to upstream therapy. Owing to the growing experimental evidence demonstrating the impact of Ang II on the atrial myocardium, several studies have been published on the possible therapeutic effect of ACEIs and ARBs in patients with AF (*20*). However, there are no studies about ACEIs or ARBs use in elderly pacing individuals. Therefore, whether ACEIs or ARBs use can reduce AF occurrence after pacemaker implantation in oldest old patients remain controversial, and it should be resolved. The results of our present study suggested that in very old patients with a permanent pacemaker, ARBs may be preferred over ACEIs, given that they reduce the risk of AF developing after pacemaker implantation.

Overall, our results demonstrated that very old individuals with daily oral ARBs consumption after pacemaker implantation were associated with a lower risk of developing new-onset AF than those without ARBs using, which remained statistically significant after adjusting for related clinical covariates. In contrast, individuals receiving ACEIs therapy did not show a protective effect against AF development. Our findings might contribute to the idea that the daily use of ARBs is associated with a less risk of inducing subsequent AF in very old pacing patients, while ACEIs users related with a non-significant increased risk of developing AF after pacemaker implantation. Therefore, it might provide evidence for the selection of the clinical drugs for very old pacing individuals.

Our study has some limitations. Firstly, we only transferred the retrospective data in a single center based on case records, so the final sample size of the group is small. Secondly, the definition of the onset time of AF may have a small range of bias because it is generally difficult to accurately define the onset time of AF by inquiring about symptoms, and asymptomatic AF may have been missed and are likely underrepresented in this study. Thirdly, the factors leading to the occurrence of AF are very complex. We have considered the correction factors as far as possible, but inevitably, there would still be some omissions. Fourthly, the effect of ACEIs therapy is related to dose, while according to electronic medical records, ACEIs were prescribed at conventional dose. Thus, the does weren't classified clearly. In addition, the pacing mode may also affect the incidence rate of AF after pacemaker implantation. We did not categorize different pacing modes or conduct subgroup analyses, because most of the included individuals received ventricular single chamber pacing (VVI mode).

In conclusion, our real-world data found that among very old pacing patients, daily oral ARBs might be associated with a lower risk of AF after pacemaker implantation but ACEIs use was not related with AF. However, our findings need to be reconfirmed by further well-designed randomized controlled trials.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. All data generated and analyzed during this study are included in this published article.

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Elevated serum CA19-9 indicates severe liver inflammation and worse survival after curative resection in hepatitis B-related hepatocellular carcinoma

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SUMMARY We explored the prognostic value of preoperative CA19-9 in α-fetoprotein (AFP)-positive and -negative HCC with hepatitis B virus (HBV) background (HBV-HCC), and explored the underlying mechanism. Recurrence-free survival (RFS) and overall survival (OS) were assessed in HBV-HCC patients who underwent curative resection (Cohort 1). Immunohistochemical staining of CA19-9 in HCC and liver parenchyma were quantified in another cohort of 216 patients with resected HCC (Cohort 2). Immunohistochemical staining of CA19-9 and serum CA19-9 level was also compared between patients with HCC and intrahepatic cholangiocarcinoma (ICC) (Cohort 3). In Cohort 1, CA19-9 ≥ 39 U/mL was an independent risk factor for RFS (HR = 1.507, 95% CI = 1.087-2.091, $p = 0.014$) and OS (HR = 1.646, 95% CI = 1.146-2.366, $p = 0.007$). CA19-9 ≥ 39 U/mL was also associated with significantly higher incidence of macrovascular invasion (MaVI) compared with CA19-9 < 39 U/mL (23.0% *vs*. 7.2%, $p = 0.002$), and elevated aminotransferase and aspartate aminotransferase to platelet ratio index (APRI), and lower albumin. Immunohistochemical staining of CA19-9 revealed that CA19-9 expression was found exclusively in the background liver but not in HCC tumor cells. In contrast, tumor tissue was the main source of CA19-9 in ICC patients. CA19-9 ≥ 39 U/mL was associated with worse OS and RFS in both AFP-positive and negative HCC patients. CA19-9 indicated more severe inflammation and cirrhosis in the liver of HCC patients.

Keywords carbohydrate antigen 19-9, hepatocellular carcinoma, α-fetoprotein, survival, EpCAM

1. Introduction

Primary liver cancer is the sixth most commonly diagnosed cancer and the fourth leading cause of cancer-related death worldwide (*1,2*). In general, primary liver cancer is classified into two types as hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC), with HCC being more common, accounting for 75-85% of all cases. However, mixed HCC-ICC and other rare types have also been reported. Alpha-fetoprotein (AFP) and carbohydrate antigen 19-9 (CA19-9) are the most commonly used biomarkers for HCC and ICC. A higher AFP level is associated with poor outcome after curative resection or liver transplantation (*3*). CA19-9, also known as Sialyl-Lewis-a, is mainly used as a biomarker for malignancies of the hepatobiliary tract and pancreas (*4*). However, serum CA19-9 levels may also be elevated in gastric, esophageal, and colonic cancers and in a number of non-malignant conditions including jaundice (*5*). Meanwhile, the serum CA19-9 level is elevated in approximately 60% of cholangiocarcinoma patients and in 30% of HCC patients (*6*). It is also frequently elevated in patients with combined HCC-cholangiocarcinoma. Elevated preoperative serum CA19-9 levels have been reported to be associated with worse survival in HCC patients

who had undergone resection (> 27U/mL) or liver transplantation (> 100 U/mL) (*7-9*). However, most of these studies mainly included patients with HCV-related HCC who underwent resection or transplantation, and the underlying mechanism by which CA19-9 influences prognosis remains unclear.

Thus, this study aimed to investigate the prognostic value of preoperative serum CA19-9 according to AFP status in HCC patients and in ICC patients with HBV background who underwent curative resection. And we will further explore the mechanism of CA19-9 by immunostaining EpCAM, a molecular marker for stem cells.

2. Methods

2.1. Patients and study design

We retrospectively evaluated three patient cohorts as follows. Cohort 1 involved 380 patients diagnosed with HCC at Tianjin Medical University Cancer Institute & Hospital (Tianjin, People's Republic of China) between 2012 and 2013. In this cohort, CA19-9 (+) was defined as serum CA19-9 ≥ 39 U/mL, whereas CA19-9 (-) was defined as serum CA19-9 < 39 U/mL, according to the upper limit of serum CA19-9 in our hospital. AFP (+) was defined as serum AFP > 20 ng/mL, whereas AFP (-) was AFP ≤ 20 ng/mL. Cohort 2 involved 216 patients with resected HCC in whom tissue microarray (TMA) samples were obtained. Patients with lymph node metastasis or distant metastasis were excluded to reduce confounding factors. Cohort 3 included 136 ICC patients who underwent radical resection.

All patients underwent curative resection for HCC, defined as complete macroscopic removal of the tumor. All tumors of HCC were staged according to the TNM classification system of International Union Against Cancer (8th edition) and the Barcelona Clinic Liver Cancer guidelines.

2.2. Demographic and clinicopathological factors

Demographic and clinicopathological factors including tumor factors, systemic inflammation factors, and liver factors were evaluated. Demographic factors included sex and age. Tumor factors included tumor size, number of tumor lesions, macroscopic vascular invasion (MaVI), microscopic vascular invasion (MiVI), intrahepatic metastasis, and tumor differentiation according to Edmondson's grade. Systemic factors included the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR). Liver factors included intraoperative detection of liver cirrhosis; alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (rGT), alkaline phosphatase (ALP), total bilirubin, and albumin levels; prothrombin time (PT); and the aspartate aminotransferase-to-platelet ratio index (APRI) as the parameter most closely related to liver cirrhosis and fibrosis in both chronic hepatitis B (*10,11*) and hepatitis C (*12,13*). NLR and PLR are both indicators of systemic inflammation and its relationship with the prognosis of several cancers has been identified (*14-16*).

2.3. TMA in ICC

We selected 158 consecutive patients with ICC who underwent surgical treatment at Tianjin Medical University Cancer Institute and Hospital between January 2012 and December 2017. Patients with combined HCC-CCA (*i.e.*, HCC and ICC) were excluded. The specimens of all patients were reviewed by two independent pathologists (Y.B. and Z.F.L.) to confirm the diagnosis of ICC and for restaging according to the 8th edition of the 2017 American Joint Committee on Cancer staging system. Of the 158 patients, we excluded 28 because of loss to follow-up (n = 11), non-R0 resection (n = 14), death from postoperative complications (n = 1), and death from non-tumor-related causes (n = 2). Thus, 130 patients (Cohort 3) were eventually included for comparison of clinical characteristics and survival analyses. The patients' formalin-fixed paraffin-embedded (FFPE) samples and hematoxylin-eosin (HE) staining slides from surgical specimens were then collected from the Department of Pathology in Tianjin Medical University Cancer Institute and Hospital. TMA samples comprising 2-mm cores of FFPE tumor tissue were prepared for various staining procedures by selecting representative tumor areas and a typical paratumoral region from each case. The Medical Ethics Committee of Tianjin Medical University Cancer Institute and Hospital approved this study, and informed consent was obtained from all patients.

2.4. Follow-up and postoperative treatment

All patients were monitored prospectively according to serum AFP and CA19-9 levels and using abdomen ultrasonography every 2 months in the first year and every 3 months after the first year. Recurrence was confirmed using computed tomography and/or magnetic resonance imaging based on typical imaging appearance in the imaging scan and an elevated AFP level. The treatment modality after relapse varied among individuals. Follow-up was concluded on July 10, 2019, with the patients followed up for a median of 56.6 months.

2.5. TMA and immunohistochemistry

TMAs were constructed as described previously (*17*). The mouse monoclonal antibodies used were anti-human CA19-9 (Zhongshan Company). Immunohistochemical

analysis was performed using a two-step protocol (Novolink Polymer Detection System, Novocastra) according to the manufacturer's instructions and as described previously (*17*). Briefly, paraffin sections were first deparaffinized and then hydrated. After microwave antigen retrieval, as required, endogenous peroxidase activity was blocked with incubation of the slides in 0.3% H_2O_2, and nonspecific binding sites were blocked with Protein Block (RE7102; Novocastra). After serial incubation with primary antibodies, Post Primary Block (RE7111; Novocastra), and secondary antibody (Novolink Polymer RE7112), the sections were developed in diaminobenzidine solution under a microscope and counterstained with hematoxylin. Negative control slides omitting the primary antibodies were included in all assays. CA19-9 immunoreactivity was evaluated in a semiquantitative manner on the basis of both labeling intensity and the percentage of immunopositive tumor cells for all antibodies. The score was calculated through multiplying staining intensity (0 = no staining, 1 = mild staining, 2 = moderate staining, and 3 = strong staining) by the percentage of immunoreactive tumor cells (0-100). The immunostaining result was considered negative (0) when the score was < 25; weak positive (1+) when the score was 26-100; moderate positive (2+) when the score was 101-200; or strong positive (3+) when the score was 201-300.

2.6. Statistical analysis

In univariate analyses of cumulative survival, survival curves were plotted using the Kaplan-Meier method and compared using the log-rank test. Multivariate analyses were based on the Cox proportional hazards regression model. For the comparison of individual variables, χ^2 tests, Fisher's exact tests, and Student's *t*-tests were used as appropriate. All statistical analyses were performed using SPSS software (SPSS v22.0, Chicago, IL). A two-tailed *P* value of < 0.05 was considered statistically significant.

3. Results

3.1. CA19-9 was an independent risk factor for RFS and OS

In Cohort 1, the 1-, 3-, 5-year overall survival (OS) was 80.3%, 37.7%, 34.4% for CA19-9 (+) patients and 90.3%, 62.7%, 51.4% for the CA19-9 (-) patients (Figure 1a). The 1-, 3-, 5-year RFS was 45.9%, 14.8%, 13.1% for the CA19-9 (+) patients, and 67.1%, 40.4%, 33.5%, respectively, for the CA19-9 (-) patients (Figure 1b). The 1-, 3-, 5-year OS was 84.0%, 47.1%, 38.3% for AFP (+) patients and 94.2%, 72.4%, 60.9% for the AFP (-) patients (Figure 1c). The 1-, 3-, 5-year RFS was 54.9%, 29.1%, 24.8% for the AFP (+) patients and 74.7%, 44.8%, 37.4% for the AFP (-) patients (Figure 1d).

In multivariate analysis, tumor size > 5 cm, presence of MaVI, AFP > 20 ng/mL, and CA19-9 ≥ 39 U/mL were independent risk factors for RFS (Table 1). Meanwhile, tumor size > 5 cm, presence of MaVI, AFP > 20 ng/mL, CA19-9 ≥ 39 U/mL, and albumin ≤ 35g/L were independent risk factors for OS (Table 2).

3.2. Positive CA19-9 predicted worse prognosis in both AFP (+) and AFP (-) HCC patients

The 1-, 3-, 5-year OS was 95.4%, 74.5%, 61.4% for patients with CA19-9 (-) and AFP (-), whereas they were 83.3%, 50.0%, 45.8% for patients with CA19-9 (+) and AFP (-) ($p < 0.05$, Figure 1e). The 1-, 3-, 5-year RFS was 77.1%, 47.1%, 39.2% for patients with CA19-9 (-) and AFP (-), whereas they were 45.8%, 20.8%, 16.7% in CA19-9 (+) and AFP (-) patients ($p < 0.05$, Figure 1f). These results showed that CA19-9 (+) predicted worse OS and RFS in AFP (-) patients.

The 1-, 3-, 5-year OS was 84.7%, 52.8%, and 42.9% for patients with CA19-9 (-) and AFP (+),and was 77.5%, 27.5%, 22.5% for patients with CA19-9 (+) and AFP (+) ($p < 0.05$, Figure 1e). The 1-, 3-, 5-year RFS was 58.9%, 34.4%, and 28.8% for patients with CA19-9 (-) and AFP (+) ($p < 0.05$, Figure 1f), whereas 40.0%, 10.0%, 7.5% for patients with CA19-9 (+) and AFP (+) ($p < 0.05$, Figure 1f). These results indicate that CA19-9 (+) predicted worse OS and RFS in AFP (+) patients. In summary, CA19-9 (+) predicted worse OS and RFS in both AFP (+) and AFP (-) HCC patients.

3.3. CA19-9 was associated with higher incidence of MaVI and a trend toward multiple tumors

CA19-9 was not associated with tumor size (6.1 ± 4.8 cm *vs*. 5.6 ± 3.8 cm, $p = 0.404$), MiVI (62.3% *vs*. 54.9%, $p = 0.225$) and AFP (5488.9 ± 28616.1 ng/mL *vs*. 5401.4 ± 40162.5 ng/mL, $p = 0.987$). However, CA19-9 was related to higher incidence of MaVI (23.0% *vs*. 7.2%, $p = 0.002$), and a trend toward more multiple tumors with marginal significance (23.0% *vs*. 13.8%, $p = 0.068$) (Table 3).

3.4. CA19-9 was associated with more severe liver cirrhosis and liver inflammation but not with systemic inflammation

Comparison of clinicopathological factors between CA19-9 (+) and CA19-9 (-) patients revealed that CA19-9 (+) patients tend to be older (mean age: 58.4 ± 10.4 years *vs*. 55.4 ± 10.6 years, $p = 0.048$), have higher incidence of liver cirrhosis (70.5% *vs*. 56.1%, $p = 0.037$), higher APRI (1.53 ± 1.61 *vs*. 0.72 ± 0.96, $p < 0.001$), elevated ALT (75.4 ± 77.3 U/L *vs*. 43.9 ± 59.5 U/L, $p = 0.004$), elevated AST (75.1 ± 69.0 U/L *vs*. 41.5 ± 46.0

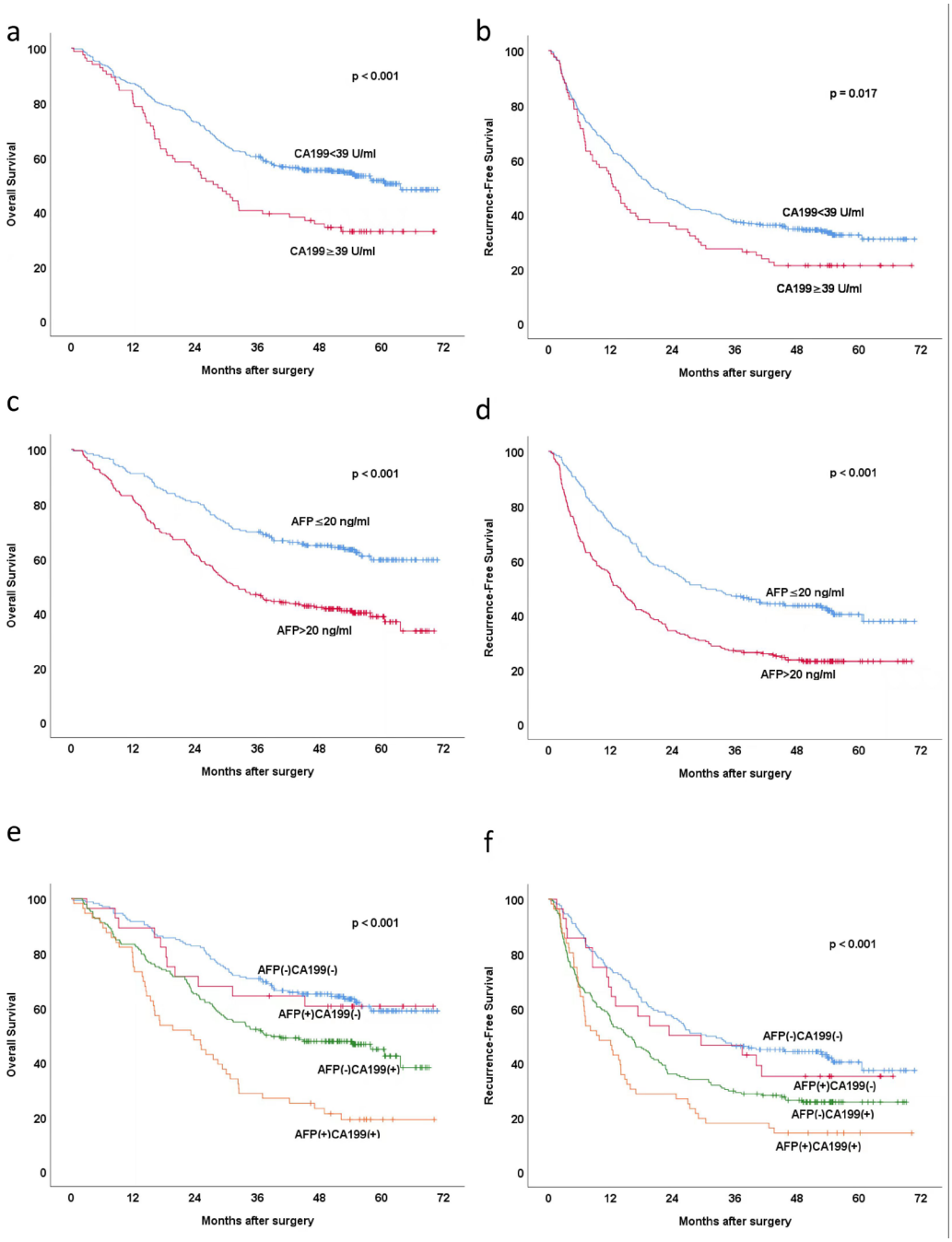


Figure 1. AFP, CA19-9 and combination to predict OS and RFS for HCC patients after curative resection.

U/L, $p < 0.001$), increased rGT (147.1 ± 162.4 U/L *vs*. 81.4 ± 99.4 U/L, $p = 0.003$), and lower level of albumin (39.8 ± 5.5 g/L *vs*. 42.0 ± 5.3 g/L, $p = 0.002$) (Table 3). All the factors except MaVI are related to liver cirrhosis.

To exclude the confounding effect of MaVI, we excluded patients with MaVI. The results showed that CA19-9 was still correlated with liver cirrhosis, APRI, ALT, AST, rGT and albumin (data not shown). Furthermore, multivariate analysis showed that CA19-9 (+) and MaVI (+) were both independent risk factors for RFS.

In the current study, CA19-9 was not correlated to NLR or PLR, indicating that CA19-9 was not correlated to systemic inflammation.

3.5. Immunohistochemical staining of CA19-9 in both HCC and ICC

To determine the source of CA19-9, we examined its expression in TMA samples of HCC patients. Immunohistochemical staining of CA19-9 in both tumor tissue and non-tumor liver parenchyma specimens from HCC patients was also assessed. The results showed that none of the HCC tumor cells express CA19-9, and CA19-9 was only expressed in non-tumor liver parenchyma (Figure 2).

Immunohistochemical staining of CA19-9 in both tumor and non-tumor liver parenchyma samples from Cohort 3 (Figure 2) revealed that CA19-9 was expressed

Table 1. Univariate and multivariate analysis for RFS in Cohort 1

Recurrence-free Survival, Variable	Comparison	Univariate, *P*-value	Multivariate, *P*-value	Hazard Ratio (95.0% CI)
Gender	Male *vs*. Female	0.268		
Age	≤ 50 *vs*. > 50 years	0.957		
Tumor size	≤ 5 *vs*. > 5 cm	< 0.001	0.001	1.569 (1.204-2.045)
Number	Solitary *vs*. Multiple	0.075		
MaVI	Yes *vs*. No	< 0.001	0.023	1.586 (1.065-2.361)
Differentiation	I/II *vs*. III/IV	0.482		
MiVI	Yes *vs*. No	0.025		
IHM	Yes *vs*. No	0.022		
Cirrhosis	Yes *vs*. No	0.588		
HBeAg	Yes *vs*. No	0.142		
AFP	≤ 20 *vs*. > 20 ng/mL	< 0.001	0.012	1.373 (1.071-1.759)
CA19-9	≥ 39 *vs*. < 39 U/ml	< 0.001	0.014	1.507 (1.087-2.091)
ALT	≤ 40 *vs*. > 40 U/L	0.046		
AST	≤ 40 *vs*. > 40 U/L	< 0.001		
Albumin	≤ 35 *vs*. > 35 g/L	0.114		
NLR	≤ 5 *vs*. > 5	0.019		
PLR	≤ 300 *vs*. > 300	0.072		
rGT	≤ 60 *vs*. > 60 U/L	< 0.001		
HKLC	0/1/2/3	< 0.001	NA	
BCLC	A/B/C	< 0.001	NA	

Table 2. Univariate and multivariate analysis for OS in Cohort 1

Overall Survival, Variable	Comparison	Univariate, *P*-value	Multivariate, *P*-value	Hazard Ratio (95.0% CI)
Gender	Male *vs*. Female	0.222		
Age	≤ 50 *vs*. > 50 years	0.246		
Tumor size	≤ 5 *vs*. > 5 cm	< 0.001	< 0.001	1.931 (1.430-2.607)
Number	Solitary *vs*. Multiple	0.029		
MaVI	Yes *vs*. No	< 0.001	0.003	1.871 (1.230-2.847)
Differentiation	I/II/III/IV	0.216		
MiVI	Yes *vs*. No	< 0.001		
IHM	Yes *vs*. No	0.002	0.009	1.483 (1.104-1.992)
Cirrhosis	Yes *vs*. No	0.158		
HBeAg	Yes *vs*. No	0.798		
AFP	≤ 20 *vs*. > 20 ng/mL	< 0.001	0.003	1.558 (1.163-2.089)
CA19-9	≥ 39 *vs*. < 39 U/ml	0.001	0.007	1.646 (1.146-2.366)
ALT	≤ 40 *vs*. > 40 U/L	0.142		
AST	≤ 40 *vs*. > 40 U/L	< 0.001		
NLR	≤ 5 *vs*. > 5	< 0.001		
PLR	≤ 300 *vs*. > 300	0.004	0.029	2.920 (1.118-7.624)
Albumin	≤ 35 *vs*. > 35 g/L	0.174		
rGT	≤ 60 *vs*. > 60 U/L	< 0.001		
TB	≤ 19 *vs*. > 19 μmol/L	0.056		
HKLC	0/1/2/3	0.011	NA	
BCLC	A/B/C	< 0.001	NA	

Table 3. Comparison of clinicopathological factors between patients with CA19-9 (+) and CA19-9 (-)

Cohort 1. Variable	CA19-9 < 39 U/mL (*n* = 319)	CA19-9 ≥ 39U/mL (*n* = 61)	*P*-value
Gender (Male/Female)	255/64 (78.0%)	48/13 (78.7%)	0.824
Age (year) (Mean ± SD)	55.4 ± 10.6	58.4 ± 10.4	0.048
Tumor size (Mean ± SD)	5.6 ± 3.8	6.1 ± 4.8	0.404
Number (Solitary *vs*. Multiple)	275/44 (13.8%)	47/14 (23.0%)	0.068
MaVI (Yes *vs*. No)	23/296 (7.2%)	12/49 (23.0%)	0.002
MiVI (Yes *vs*. No)	175/144 (54.9%)	38/22 (62.3%)	0.225
IHM (Yes *vs*. Bo)	111/208 (34.8%)	24/36 (39.3%)	0.440
AFP (ng/mL) (Mean ± SD)	5,401.4 ± 4,0162.5	5,488.9 ± 2,8616.1	0.987
HBeAg (Yes *vs*. no)	45/274	12/49	0.265
Cirrhosis (Yes *vs*. No)	179/140 (56.1%)	43/18 (70.5%)	0.037
APRI (Mean ± SD)	0.72 ± 0.96	1.53 ± 1.61	< 0.001
Ascites (Yes *vs*. No)	32/287	7/54	0.733
rGT (Mean ± SD)	81.4 ± 99.4	147.1 ± 162.4	0.003
ALT (U/L) (Mean±SD)	43.9 ± 59.5	75.4 ± 77.3	0.004
AST (U/L) (Mean ± SD)	41.5 ± 46.0	75.1 ± 69.0	< 0.001
Albumin (g/L) (Mean ± SD)	42.0 ± 5.3	39.8 ± 5.5	0.002
TB (μmol/L) (Mean ± SD)	18.5 ± 9.8	26.4 ± 37.8	0.109
NLR (Mean ± SD)	2.3 ± 1.5	2.1 ± 1.4	0.388
PLR (Mean ± SD)	134.3 ± 368.7	95.5 ± 47.8	0.412

in 64% (87/136) of ICC tumors and 4.4% (6/136) of non-tumor liver parenchyma. Serum CA19-9 was positive (≥ 39 U/mL) in 58.1% and negative (< 39 U/mL) in 41.9% of the patients with ICC. The results that immunohistochemical staining of CA19-9 was positive only in 4.4% of ICCs indicate that serum CA19-9 mainly derives from the tumor tissue of patients with ICC, which is distinct from the dominant expression of CA19-9 in the background liver in HCC patients (Figure 3).

3.6. relationship between EpCAM and serum CA19-9 and AFP

Positive and negative stain of EpCAM in tumor tissue was detected by immunohistochemistry (Figure 4a-b). The positive ratio of EpCAM staining was similar in patients with CA19-9 ≥ 39 U/mL and CA19-9 < 39 U/mL(Figure 4c). While more patients had elevated HBVDNA in patients with serum CA19-9 ≥ 39 U/mL than CA19-9 < 39 U/mL (Figure 4d).

It is quite the opposite for AFP. The proportion of

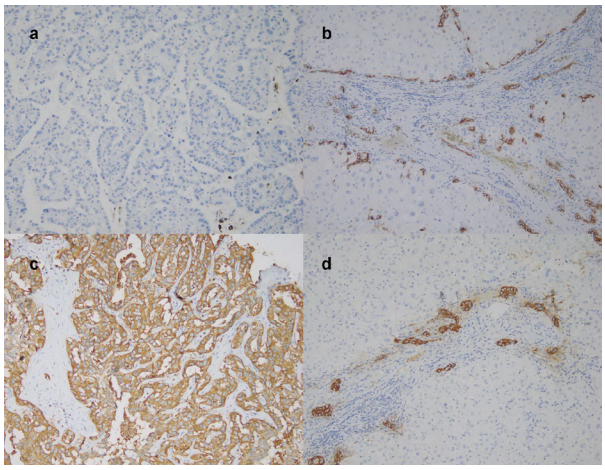


Figure 2. Immunohistochemical staining of CA19-9 in HCC and ICC in Cohort 2 and 3. (a), HCC tumor tissue was negative for CA19-9. **(b)**, HCC non-tumor liver parenchyma was positive for CA19-9 in the portal area. **(c)**, ICC tumor tissue was positive for CA19-9. **(d)**, ICC non-tumor liver parenchyma was positive for CA19-9 in the portal area.

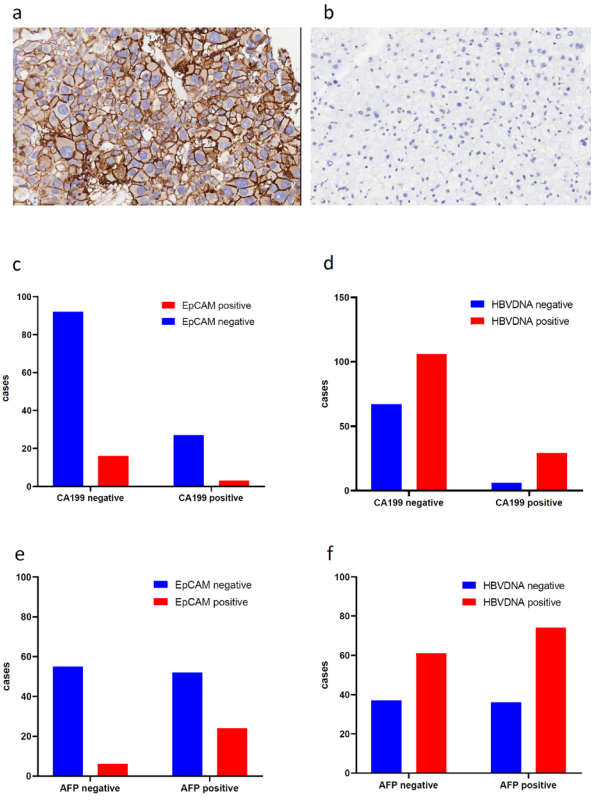
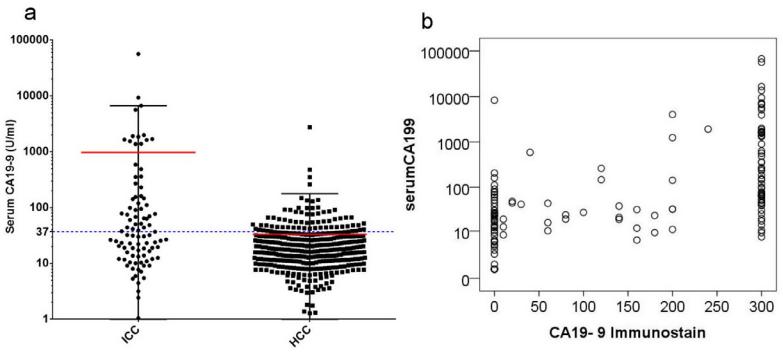


Figure 4. (a), positive staining for EpCAM. **(b)**, negative staining for EpCAM. **(c)**, the positive ratio of EpCAM staining were similar in patients with CA19-9 ≥ 39 U/mL and CA19-9 < 39 U/mL (10% *vs.* 14.8%, $p > 0.05$). **(d)**, more patients had elevated HBVDNA in patients with serum CA19-9 ≥ 39 U/mL than CA19-9 < 39 U/mL (82.9% *vs.* 61.3%, $p < 0.01$). **(e)**, the proportion of positive EpCAM staining in AFP positive group is much higher than that in AFP negative group (31.6% *vs.* 9.8%, $p < 0.001$). **(f)**, the proportion of positive elevated HBVDNA in AFP negative group is similar to that in AFP positive group (62.2% *vs.* 67.3%, $p > 0.05$).



c

ICC 136 cases	Tumor	Non-tumor Liver parenchyma
IHC CA19-9 (-)	49 (36%)	130 (96.3%)
IHC CA19-9 (+)	87 (64%)	6 (4.4%)

d

ICC 136 cases	IHC CA19-9 (-)	IHC CA19-9 (+)	
serum CA19-9 (-)	35	22	57 (41.9%)
serum CA19-9 (+)	14	65	79 (58.1%)

Figure 3. CA19-9 in HCC and ICC in Cohort 2 and 3. (a), comparison of serum CA19-9 level in ICC and HCC patients. **(b)**, Serum CA19-9 correlated with IHC CA19-9 ($p < 0.001$). **(c)**, Immunohistochemical staining of CA19-9 in ICC tumor and non-tumor liver parenchyma showed that CA19-9 is positive in 64% in tumor tissue and 4.4% in non-tumor liver parenchyma. **(d)**, comparison between serum CA19-9 and immunochemical staining of CA19-9.

positive EpCAM staining in AFP negative group is much more than that in AFP positive group (Figure 4e). While the proportion of positive elevated HBVDNA in AFP negative group is similar to that in AFP positive group (Figure 4f).

This indicated that AFP is related to the stemness of hepatocellular carcinoma as indicated by EpCAM. While CA19-9 is related to hepatitis and inflammation of the background liver as indicated by a higher proportion of elevated HBVDNA.

4. Discussion

Elevated serum CA19-9 levels had been reported to predict poor prognosis in HCC, even in AFP negative HCC. Chen *et al*. (*8*) found that a preoperative CA19-9 value of >27 U/mL was associated with poor prognosis after resection for HCC. Wan *et al*. (*9*) also showed that preoperative serum AFP levels of > 400 ng/mL and CA19-9 >100 U/mL predicted survival after liver transplantation in patients with HCC. Hsu *et al*. (*7*) found that an elevated serum CA19-9 level of ≥ 100 U/mL was an independent predictor of poor OS in HCV-related HCC. Lu *et al*. (*18*) found that a preoperative CA19-9 level of > 32.6 U/mL predicted poor prognosis and can be used as a prognostic marker in AFP-negative HCC. However, only one study evaluated patients with only HBV-related HCC. The current study included patients with exclusively HBV-related HCC. At a cut-off value of 39 U/mL, 16.1% of patients (61/380) were found to be CA19-9 positive, and CA19-9 ≥ 39 U/mL predicted worse OS and RFS in both AFP (-) and AFP (+) patients. CA19-9 positivity was revealed to be closely related to more severe liver cirrhosis and liver inflammation, as indicated by elevated rGT, ALT, AST and APRI (*19*).

CA19-9 is synthesized by normal biliary epithelium or by malignant tumors (*20*), and it is frequently elevated in biliary obstruction and biliary tract cancers (*21*). Furthermore, an elevated CA19-9 serum level is reported to be associated with mixed HCC-ICC, which tends to have more aggressive behavior than pure HCCs (*22*). In HCC, the source and implication of CA19-9 is still unclear. In the current study, we excluded the possibility of mixed HCC-ICC by two independent pathologists, and we applied immunohistology staining to confirm that the only source of serum CA19-9 in HCC patient is the background liver parenchyma. Furthermore, CA19-9 ≥ 39 U/mL was associated with elevated rGT, ALT, AST, APRI and higher incidence of MaVI. Previous reports have confirmed that elevated ALT, AST, and rGT levels are correlated to liver cirrhosis (*23,24*) and recurrence (*25,26*). Thus, we confirmed that CA19-9 is a liver biomarker, which indicated more severe liver inflammation and liver cirrhosis in HCC. To confirm this finding, we performed immunohistochemical staining of EpCAM, a biomarker for stemness of hepatocellular carcinoma (*27,28*). And we found that the positive ratio of EpCAM staining was similar between patients with CA19-9 ≥ 39 U/mL and CA19-9 < 39 U/mL. In contrast, elevated AFP was associated with positive EpCAM staining, indicating the stemness of tumor was associated with positive AFP rather than positive CA19-9. More impressively, more patients had elevated HBVDNA in patients with serum CA19-9 ≥ 39 U/mL than patients with CA19-9 < 39 U/mL. This finding confirms that CA19-9 is an indicator of hepatitis and liver inflammation.

Macrovascular invasion and multiple tumor nodules were also more common in CA19-9 (+) patients. These can be attributed to two reasons. First, chronic inflammation and cirrhosis of the liver are the key etiological risk factors for HCC (*29,30*), and an elevated ALT/AST/APRI in patients with elevated CA19-9 indicated more severe liver cirrhosis and an inflamed liver background (*31,32*), which is closely related to de novo tumor pathogenesis and multicentric recurrence (*33,34*). Second, liver inflammation has been reported as an independent risk factor for early tumor recurrence in patients with HCC (*35-37*), and preclinical studies have revealed that the inflammatory microenvironment of fibrotic liver promotes hepatocellular carcinoma metastasis by STAT3 activation (*38*).

Our study has several limitations. First, it is a single-center study of retrospective cohorts, and only the serum level and immunohistochemical expression of CA19-9 were evaluated. Second, the precise mechanism by which CA19-9 promotes macroscopic vascular invasion is still unclear and thus further studies are needed to elucidate the underlying mechanism.

5. Conclusions

In conclusion, CA19-9 is associated with lower OS and RFS in both AFP (+) and AFP (-) patients. Importantly, CA19-9 is secreted by the background liver, but not by tumor cells in patients with HCC. Thus, CA19-9 is not a tumor biomarker, but a biomarker for liver cirrhosis and inflammation and a risk factor for worse OS and RFS in HCC.

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Institute and Hospital, No.2017-1-35.

Conflict of Interest: The authors have no conflicts of interest to disclose.

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Original Article

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Cytomegalovirus viremia is associated with poor outcomes in AIDS patients with disseminated nontuberculous mycobacterial disease

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SUMMARY Both cytomegalovirus (CMV) viremia and disseminated nontuberculous mycobacterial (NTM) disease are common opportunistic infections in AIDS patients. Whether concurrent CMV viremia is associated with mortality in patients with AIDS and disseminated NTM disease is unknown. Subjects were patients with AIDS and disseminated NTM disease seen at a single center from January 2015 to April 2021. Data were retrospectively collected. Differences in demographics and clinical characteristics and hospitalization survival rates were compared between patients with disseminated NTM and with CMV viremia or not. Subjects were 113 AIDS patients with disseminated NTM who were seen at this Hospital from January 2015 to April 2021. Twenty-six of the patients had CMV viremia and 87 did not. The median age was 36 years (interquartile range [IQR] 29-42) and 108 patients were male (96%). The median CD4 count was 7 cells/μL (IQR 3-17). The median plasma CMV viral load was 9,245 IU/mL (IQR 3147-45725). The serum albumin of patients with CMV viremia was significantly lower than that of patients without CMV viremia (P = 0.03). Compared to patients without CMV viremia (81.6%), patients with CMV viremia had a significantly poorer prognosis (P = 0.01). Cox regression analysis indicated that the risk of a poor prognosis in patients with CMV viremia was 4.7 times higher than that in patients without CMV viremia (P = 0.003), and patients with CD8 more than 250/μL had a better prognosis (P = 0.02). CMV viremia increases the risk of a poor prognosis in patients with AIDS and a disseminated NTM infection. A routine CMV DNA test should be performed on patients with AIDS and disseminated NTM disease in order to reduce the risk of death.

Keywords AIDS, disseminated, nontuberculous Mycobacteria, Cytomegalovirus, clinical characteristics, outcomes

1. Introduction

Nontuberculous Mycobacteria (NTM) are ubiquitous in the environment and usually infect immunocompromised populations (*1,2*). A disseminated NTM infection mostly occurs in HIV-infected patients with a CD4 count below 50/μL (*3*). In the pre-antiretroviral therapy (ART) era, up to 43% of AIDS patients were reported to be infected with disseminated NTM, and especially those with severe immunodeficiency (*4*). The most common NTM is *Mycobacterium avium*-intracellulare complex, which accounted for 71% of pulmonary NTM infections in Australia, followed by 54% in Asia, 52% in North America, 51% in South Africa, 37% in Europe, and 31% in South America (*5*). In the post-ART era, the rate of NTM infections and their mortality have gradually decreased in AIDS patients in developed countries (*5*). In Asia, Africa, and Latin America, the rate of NTM infection in AIDS continues to rise, and NTM infection is a key reason for the increased hospitalization and mortality of AIDS patients (*6,7*). In Shanghai, China, the rate of NTM identification among AIDS patients is also increasing. A retrospective study by the current authors' team found that NTM accounted for 41 (41/101, 41%) of the isolates identified using 16S rDNA sequencing in 2014, 64 (64/137, 47%) in 2015, and 72 (72/162, 44%) in 2016 (*8*).

CMV is a double-stranded DNA virus belonging to the herpes virus family that can cause disseminated or localized end-organ disease in HIV-infected patients

with advanced immunosuppression (*9*). Positivity for CMV in HIV-infected people is much higher than that in HIV-negative people. CMV viremia, as a better indicator of active disease, has a prevalence of 2% to 23% in African cohorts living with HIV (*10*). Those with cell-mediated immunodeficiencies or undergoing cell-mediated immunosuppression cannot mount an adequate immune response to CMV and therefore are at the highest risk of CMV viremia (*11*). Ward *et al*. (*12*) found that CMV viremia was associated with a trend toward increased mortality in persons living with HIV who had tuberculosis, and particularly in older patients.

Few studies have examined co-infections with disseminated NTM and CMV, and most of the literature is in the form of case reports. Little is known regarding whether active CMV replication contributes to an increased risk of death in patients with AIDS and a disseminated NTM infection. The current study compiled serum CMV DNA results from patients with AIDS and a disseminated NTM infection at the Shanghai Public Health Clinical Center (SPHCC), it compared the differences in clinical characteristics and prognosis, and it retrospectively determined whether CMV viremia was a risk factor for a poor prognosis in patients with AIDS and a disseminated NTM infection.

2. Methods

2.1. Ethical statement

Ethical approval was granted by the Ethics Committee of the SPHCC (Ethics approval No. 2017-S022-04). The committee decided to waive the need for written informed consent from the participants in this study since the data were collected retrospectively and anonymous.

2.2. Study populations and clinical data

Data from January 1, 2015 to April 30, 2021 were retrospectively collected. Subjects were 113 patients with a disseminated NTM infection identified *via* blood culture (106), hydrothorax (2), lymph node aspiration (4), or ascites (1) in patients seen at the SPHCC. Electronic medical records were searched for HIV-positive patients diagnosed with a disseminated NTM infection. An NTM infection was identified based on testing negative for the MPT64 antigen or 16S rRNA (to identify the species of NTM). CMV DNA was detected in accordance with the method reported in the literature (*13*). Based on CMV DNA results, patients were divided into two groups: patients with CMV viremia (CMV DNA > 2,000 copies/mL) and patients without CMV (CMV DNA < 2,000 copies/mL). Laboratory tests (blood routine test, biochemistry, procalcitonin (PCT), CD4 and CD8 cell count, and C-reactive protein (CRP)) and outcomes during hospitalization were analyzed.

A blood culture or some other type of pathogen detection was performed along with a blood routine test, biochemistry, and cellular immunity as well as measurement of PCT and CRP. Mycobacterium was cultured and detected using the BACTEC MGIT 960 system, and it was operated in accordance with the manufacturer's instructions. In this study, a disseminated NTM infection was immediately treated with a combination of rifampicin (R), isoniazid (H), ethambutol (E) and pyrazinamide (Z) administered according to guidelines (*14,15*). For a severe infection, empirical treatment was given by adding macrolides and fluoroquinolones to the HREZ regimen. When the species was identified as NTM, H and Z were discontinued and amikacin were added to the regimen depending on the patient's clinical status. Patients were divided into those who survived and those with a poor prognosis (death or terminal discharge) depending on the final clinical outcome.

2.3. Statistical analysis

A Mann-Whitney test (for numeric variables) and Fisher's exact test (for categorical variables) were used to compare demographic and clinical characteristics between patients with CMV viremia and those without CMV viremia.

Cox regression analysis was used to analyze the risk factors for survival and define the outcome variables: 1 = death or terminal discharge, 0 = normal discharge; Time variable: duration of hospitalization. Independent variables: CMV viremia (0 = negative, 1 = positive), age (1 = < 35 years, 2 = ≥ 35 years), gender (1 = male, 2 = female), white blood cell count (1 = < 4.0×10^9/L, 2 = ≥ 4.0×10^9/L), neutrophil count (1 = < 3.5×10^9/L, 2 = ≥ 3.5×10^9/L), hemoglobin (Hb) level (1 = < 90 g/L, 2 = ≥ 90 g/L), serum albumin (Alb) level (1 = < 30 g/L, 2 = ≥ 30 g/L), CRP level (1 = < 50 mg/L, 2 = ≥ 50 mg/L), PCT level (1 = < 0.25 ng/mL, 2 = ≥ 0.25 ng/mL), erythrocyte sedimentation rate (ESR) (1 = < 75 mm/h, 2 = ≥ 75 mm/h), CD4 cell count (1 = < 10 cells/uL, 2 = ≥ 10 cells/uL), CD8 cell count (1 = < 250 cells/uL, 2 = ≥ 250 cells/uL), 1,3-β-D-glucose (BDG) level (1 = < 12pg/mL, 2 = ≥ 12 pg/mL), the time of ART (0 = within 30 days, 1 = more than 30 days). The variables included in the multivariate model were selected based on a significance level of $P < 0.1$ in univariate analysis. For survival analysis, patient survival was calculated in days from admission to death, terminal discharge, or normal discharge after admission, whichever occurred first. The difference was statistically significant when $P < 0.05$. Data were analyzed using IBM SPSS version 22.0 (IBM SPSS, Inc., Armonk, NY, USA), and survival was plotted using GraphPad Prism version 7.0.

3. Results

3.1. Patient selection and the demographic and clinical

characteristics of patients with AIDS and disseminated NTM disease

Data on 113 patients with a disseminated NTM infection seen at the SPHCC from January 2015 to April 2021 were analyzed. Twenty-six patients had CMV viremia and 87 did not. For details on patient selection, see Figure 1. The median age was 36 years (IQR 29-42) and 108 patients were male (96%). The median CD4 count was 7 cells/μL (IQR 3-17). Of the total patients, 89% (101/113) had CD4 < 50 cells/μL and 97% (110/113) had CD4 < 100 cells/μL. The median plasma CMV viral load was 9245 IU/mL (IQR 3147-45725). The serum albumin level in patients with CMV viremia was significantly lower than that in patients without CMV viremia (P = 0.03). The median CD4 count did not differ between patients with CMV viremia and patients without CMV viremia (P = 0.37). Compared to patients without CMV viremia, patients with CMV viremia had a significantly poorer prognosis (P = 0.01). Other clinical characteristics such as the CD8 cell count, the ratio of CD4/CD8 cells, blood culture conversion in days, the duration of hospitalization, and the proportion of patients receiving ART over 3 months later did not differ significantly between patients with CMV viremia and patients without CMV viremia (Table 1).

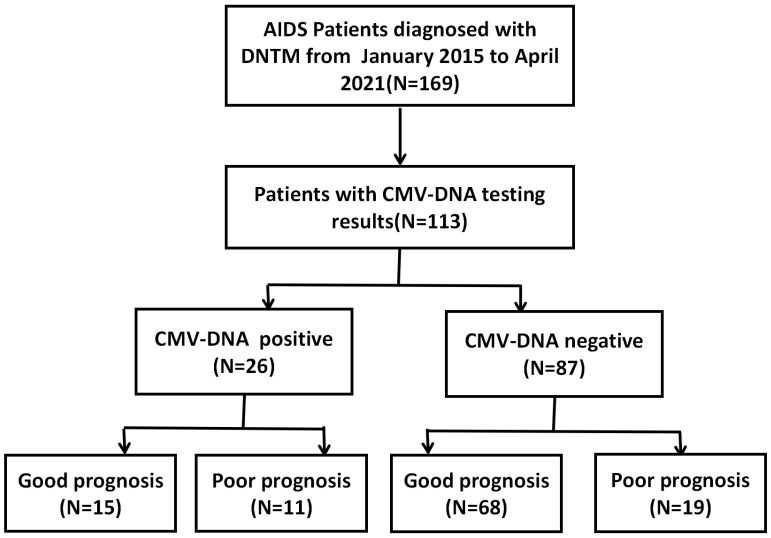

Figure 1. The flow chart for selection of eligible patients.

Table 1. The demographic and clinical characteristics of patients with AIDS and disseminated NTM disease

Demographic and clinical data	Normal range	Total cases (n = 113)	CMV viremia (n = 26)	No CMV viremia (n = 87)	P value
Age (years)	-	36 (29-42)	35 (29-49)	36 (29-41)	0.89
Male (%)	-	108 (95.6)	25 (96.2)	83 (95.4)	0.87※
WBC count (×10^9/L)	3.5-9.5	4.0 (2.7-5.4)	4.5 (3.1-5.8)	4.0 (2.5-5.3)	0.26
Neutrophil count (×10^9/L)	1.8-6.30	3.2 (1.9-4.5)	3.3 (2.4-4.7)	3.1 (1.8-4.2)	0.31
Hemoglobin (g/L)	115-150	87 (69-106)	85 (63-96)	91 (71-106)	0.15
CD4 (cells/uL)	41-1,590	7 (3-17)	6 (1-14)	7 (3-19)	0.37
CD8 (cells/uL)	19-1,140	258 (109-468), §N = 108	262 (67-384) §N = 25	254 (120-487) §N = 83	0.42
Ratio of CD4/CD8 cells	0.9-3.6	0.03 (0.01-0.09) §N = 108	0.03 (0.01-0.06) §N = 25	0.03 (0.12-0.09) §N = 83	0.95
Serum albumin (g/L)	40-55	30 (25-33)	27 (21-31)	31 (25-34)	0.03
CRP (mg/L)	0-10	50.7 (18.7-99.5) §N = 96	73.9 (22.1-117.2) §N = 22	39.9 (17.7-95.6) §N = 74	0.21
PCT (ng/mL)	0-0.05	0.25 (0.10-0.94) §N = 106	0.24 (0.09-3.80) §N = 24	0.26 (0.11-0.87) §N = 82	0.76
ESR (mm/h)	0-15	75 (57-94) §N = 67	79 (65-105) §N = 15	75 (56-94) §N = 52	0.56
BDG (pg/mL)	< 60	12 (10-120) §N = 108	26 (10-127) §N = 24	12 (10-114) §N = 84	0.64
Culture conversion (days)	-	14 (10-21)	14 (10-28)	14 (10-21)	0.57
Hospitalization (days)	-	19 (12-36)	16 (10-33)	21 (12-38)	0.27
ART > 30 days	-	24 (21.2)	3 (11.5)	21 (24.1)	0.17※
Poor prognosis	-	27 (23.9)	11 (42.3)	16 (18.4)	0.01※

※According to Fisher's exact test, otherwise according to the Mann-Whitney test; §N indicates the number of patients for whom clinical data were analyzed. WBC: white blood cell; CRP: C-reactive protein; PCT: procalcitonin; ESR: erythrocyte sedimentation rate; BDG:1,3-β-D-glucose; NTM: nontuberculous mycobacteria; CMV: cytomegalovirus; ART: antiretroviral therapy.

Table 2. COX regression analysis for patients with AIDS and disseminated NTM disease

Variables	Crude HR	95% CI	*P* value	Adjusted HR	95% CI	*P* value
Gender	Reference	--	--			
Female	1.63	0.38-6.88	0.51			
WBC	Reference	--	--			
≥ 4.0 × 10^9/L	0.64	0.30-1.38	0.26			
Neutrophil	Reference	--	--			
≥ 3.5 × 10^9/L	0.81	0.37-1.77	0.60			
PCT	Reference	--	--			
≥ 0.25 ng/mL	1.38	0.63-3.00	0.42			
ESR	Reference	--	--			
≥ 75 mm/h	1.10	0.36-3.44	0.86			
Albumin	Reference	--	--			
≥ 30 g/L	0.56	0.25-1.26	0.16			
CD4	Reference	--	--			
≥ 10/uL	0.49	0.20-1.23	0.13			
Age	Reference	--	--			
≥ 35 years	0.51	0.24-1.09	0.08	0.98	0.37-2.58	0.97
Hemoglobin	Reference	--	--	--	--	--
≥ 90 g/L	0.43	0.19-0.98	0.04	0.50	0.18-1.40	0.19
CRP	Reference	--	--	--	--	--
≥ 50 mg/L	2.41	0.99-5.88	0.05	1.62	0.58-4.51	0.36
BDG	Reference	--	--	--	--	--
≥ 12pg/mL	0.50	0.22-1.13	0.09	0.52	0.19-1.42	0.20
ART	Reference	--	--	--	--	--
≥ 30 days	0.27	0.06-1.13	0.07	0.57	0.12-2.79	0.49
CD8	Reference	--	--	--	--	--
≥ 250/uL	0.44	0.19-1.02	0.06	0.29	0.10-0.85	0.02
CMV viremia	Reference	--	--	--	--	--
YES	2.71	1.26-5.86	0.01	4.68	1.68-13.05	0.003

WBC: white blood cell; CRP: C-reactive protein; PCT: procalcitonin; ESR: erythrocyte sedimentation rate; BDG:1,3-β-D-glucose; NTM: nontuberculous mycobacteria; CMV: cytomegalovirus; ART: antiretroviral therapy.

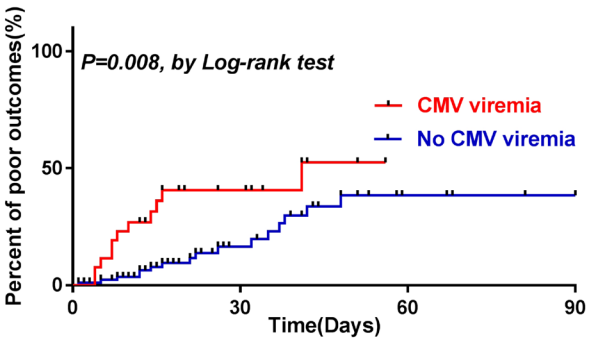

Figure 2. Survival analysis of patients with AIDS and disseminated NTM disease.

3.2. Comparison of the survival rate of patients with AIDS and disseminated NTM disease with and without CMV viremia

Of 113 patients with a disseminated NTM infection, 27 had a poor prognosis and 86 were discharged normally. Twelve patients died in hospital and 15 received a terminal discharge. In the patients with a disseminated NTM infection, the species was identified in 29 patients.

M. avium was detected in 25, M. intracellulare was detected in 2, *M. kansasii* was detected in 1, and *M. haemophilum* was detected in 1.

Cox regression analysis indicated that the risk of a poor prognosis in patients with CMV viremia was 4.7 times higher than that in patients without CMV viremia ($P = 0.003$), and patients with a CD8 cell count of more than 250 cells/uL had a better prognosis ($P = 0.02$), as shown in Table 2. Survival analysis indicated that patients with AIDS and a disseminated NTM infection had a longer survival and better prognosis than patients with AIDS, CMV viremia, and a disseminated NTM infection (Figure 2).

4. Discussion

The current results revealed that CMV viremia is associated with a nearly 5-fold increase in mortality in patients with AIDS and a disseminated NTM infection. This is a novel finding that corroborates CMV viremia as a detrimental biomarker in patients with AIDS and a disseminated NTM infection, irrespective of the presence or absence of end-organ CMV disease.

In China, population-based data indicate that the proportion of NTM among all mycobacterial isolates has increased from 11% to 23% (*16*). Isolates of NTM from patients with AIDS have also increased in recent years, and prognosis is poor without rapid identification and appropriate antibiotic therapy (*8*). Disseminated NTM disease, a key AIDS-defining opportunistic infection, is associated with significant morbidity and

mortality and with shorter survival. Both NTM disease and CMV disease are caused by intracellular pathogens requiring Type 1 T helper (Th1) cell immunity for protection (*17*). Viral infections can increase the production of Type 1 interferons (*e.g.*, interferon-alpha), which may subsequently impair Th1 cytokine (*e.g.*, interferon-gamma) responses (*18*).Thus, a concomitant infection with CMV could potentially counteract protective Th1 immune responses to a mycobacterial infection (*19*). The stimulation of peripheral blood mononuclear cells with CMV antigens results in lower levels of interferon-gamma and tumor necrosis factor alpha in CMV-seropositive versus CMV-seronegative patients (20).

CMV reactivation is associated with a higher mortality in AIDS patients with severe immunodeficiency (*21*). Two studies in different settings (Thailand (*22*) and South Africa (*23*)) confirmed that CMV viremia was associated with an increased risk of death despite prompt initiation of ART. Active CMV replication with viremia is associated with an over 2-fold increase in mortality in severely immunocompromised persons living with HIV who have cryptococcal meningitis (*24*). Looking specifically at patients with CMV viremia, those receiving preemptive anti-CMV therapy and in whom CMV DNA is no longer detectable have a better survival rate (*21*). Therefore, timely detection of a CMV infection and active anti-CMV treatment may improve patient prognosis. A routine CMV DNA test should be performed on patients with AIDS and disseminated NTM disease who have a low CD4 count.

Baseline serum albumin < 25g/L was an independent predictor of mortality in the competing risk model of patients with HIV and end-stage renal failure (*25*). The role of serum albumin in predicting illness and death is heavily influenced by inflammation (*26*). CMV replication have been shown to drive inflammation, and CMV coinfection may partially explain the inflammation noted in HIV-infected patients receiving ART (*27*). The current study found that the serum albumin level in patients with CMV viremia was lower than that in patients without CMV viremia, which also reflects the fact that an inflammatory reaction caused by CMV intensified the decrease in serum albumin. A variety of opportunistic infections occur at the same time, increasing the disease burden in patients. Excessive wasting of the body also leads to a decrease in serum albumin. Therefore, improved nutrition should be encouraged for patients with more complications. CMV viremia did not cause significant differences in other laboratory results. However, other reasons for the low level of serum albumin such as impaired liver synthesis and nephropathy were not noted, and this flaw may undermine the aforementioned explanation.

In this study, patients with AIDS and disseminated NTM disease who had a CD8 cell count of more than 250 cells/uL had a better prognosis. A study has reported that the CD8 counts may predict prognosis independent of the CD4 counts (*28*). In most cases, the end stage of HIV infection can cause both CD4 and CD8 depletion (*29*). The CD8 cell count may also indicate poor outcomes for AIDS patients with severe opportunistic infections since the low CD4 cell count in people infected with HIV is one of the main reasons for co-infection of NTM and CMV. The incidence of new cases of CMV end-organ disease has declined by ≥ 95% with the advent of ART (*30,31*). Therefore, early diagnosis of HIV infection and timely ART are crucial.

There are several limitations to this study. First, this study was a retrospective one, so many patients with HIV and a disseminated NTM infection were tested for CMV DNA. This may have reduced the sample size and produced bias in analysis. Second, some laboratory results are missing, reducing the statistical power. Third, data on HIV RNA were not collected in this study, so the impact of the HIV burden on prognosis could not be evaluated in more detail. Last, this was a single-center study, and caution should be exercised when extrapolating the current findings to the whole population in areas with a high incidence of NTM disease.

In conclusion, CMV viremia increases the risk of a poor prognosis in patients with AIDS and a disseminated NTM infection. A routine CMV DNA test should be performed on patients with AIDS and disseminated NTM disease in order to reduce the risk of death.

Authors' contributions HZL, JJS, BT, and YZS conceived and designed this study; BT, JJS, LL, RFZ, YZS, JC, and TKQ collected the data. JJS, BT, CXL, JSB, and JL analyzed the data; JJS, BT, YZS, and HZL interpreted the results. BT, JJS, CXL, JSB, and JL wrote the first draft; JJS, BT, YZS, and HZL contributed to the final version. All authors have read and approved the manuscript.

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Commentary

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Clinical guidelines for the diagnosis and treatment of HIV/AIDS in China: Their potential benefits and impact on public health

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SUMMARY Since the first edition of the guidelines for the diagnosis and treatment of AIDS was published in 2005, the AIDS Specialists Group of the Society of Infectious Diseases has updated the guidelines three more times to include more thorough, practical, standardized, and specific content. The latest edition (the 2021 version) has recently been updated in China in accordance with clinical practice nationwide and results of the latest research. Compared to the four previous editions, the 2021 edition references the latest information on the epidemiology of HIV, the prevention of HIV transmission, standardized lab diagnosis, and clinical management. First, the guidelines highlight the concept of "enhancing the combination of early intervention, prevention, and treatment". The guidelines specify more detailed clinical phases (three clinical stages), the clinical staging and progression of AIDS, and patient prognosis. The guidelines also specify diagnostic criteria – HIV antibodies, HIV RNA tests, CD4 cell counts, and the patient's epidemiological history – to use in conjunction with symptoms to confirm an HIV infection. In addition, the guidelines summarize more advanced HIV/AIDS research in China by describing the different circulating recombinant forms (CRFs) and unique recombinant forms (URFs) in Chinese patients, by summarizing the most prevalent strains in the Chinese population, and by comparing disease progression by route of transmission and by the CD4+T cell count. Lastly, this edition describes ways to optimize programs to prevent mother-to-child transmission, strategies for diagnosis and treatment of opportunistic infections, the aging patient population, and specialized ART treatment programs for different populations living with HIV. The guidelines should not only help to prolong the life of people living with HIV and improve their quality of life but also encourage successful collaboration between scientific researchers and physicians in the area of HIV.

Keywords clinical guidelines, diagnosis, treatment, HIV/AIDS, public health

1. Introduction

As of 2020, 38 million people were living with HIV/AIDS (PLWH) and 1.5 million were newly infected worldwide. Twenty-six million patients, or 71% of all patients with AIDS, received antiretroviral therapy (ART), and 62% of new infections came from high-risk populations and their sexual partners (*1*). If the 188 countries worldwide were divided into 10 levels of prevalence based on the survival rate of AIDS, the rate of new infections per year, and the mortality rate per year, China would rank in the eighth level. Moreover, 75% of PLWH live in 15 countries, including China. By the end of October 2021, China (excluding Hong Kong, Macao, and Taiwan) had reported 1.14 million HIV infections. From January to October 2021, 111,000 AIDS cases were reported nationwide, 97% of which were sexually transmitted (*2*). Although Chinese AIDS prevention and control efforts have made considerable progress over the past nearly 30 years, AIDS prevention and control in China is clearly still facing challenges comparing to the prevalence of HIV elsewhere in the world and infection status elsewhere in the world (*3*).

To optimize HIV/AIDS diagnosis, treatment, and management, the AIDS Specialists Group of the Society of Infectious Diseases, Chinese Medical Association has continuously updated Chinese guidelines for the diagnosis and treatment of HIV/AIDS (Guidelines for China) (*4*). The guidelines are now in their fifth edition since the first edition was published in 2005. Chinese guidelines for the diagnosis and treatment of HIV/AIDS (2021 edition) were published In December 2021; the latest version is more comprehensive, practical, standardized, and advanced.

2. Background of the update

Due to the aging population with HIV/AIDS, the longer duration of treatment, changes in the types of ART drugs, and complex management of non-AIDS-related diseases (NAD), the clinical management of patients with AIDS poses considerable challenges. The guidelines need to be updated continuously in accordance with new findings from clinical trials, real-world studies, and clinical practice and new thinking about HIV prevention and comprehensive disease management.

3. Advantages of the update

3.1. Drafted in less time

In 2005, Chinese Medical Association organized experts to draft Chinese guidelines for the diagnosis and treatment of HIV/AIDS. China had just launched its national program for free ART and partially subsidized treatment of opportunistic infections at that time, and clinical practitioners experience and clinical data were lacked. Thus, the first edition of the guidelines was published in 2006 over the course of a year, mainly based on guidelines and literature from other countries. Since then, the guidelines were updated in 2011, 2015, and 2018 (*5-7*). As clinical and real-world studies have been conducted in China and a new generation of ART drugs has become more available and affordable, the AIDS and Hepatitis C Specialists Group of the Society of Infectious Diseases, Chinese Medical Association recently initiated steps to update the guidelines in April 2021 to include concepts like promoting pre-exposure prophylaxis (PrEP) and comprehensive disease management. Top experts and clinical practitioners in HIV/AIDS gathered and discussed those topics. In October 2021, a first draft was completed , and the final version of the guidelines was published. The update and revision process illustrates China's advances in combating HIV/AIDS and the continued and diligent efforts by researchers and clinical practitioners over the past few decades (*8*).

3.2. Drafted more thoroughly

The 2021 edition of the guidelines was drafted bytop domestic experts in epidemiology, lab management, perinatal transmission, and basic research. Therefore, information about the epidemiology of HIV, prevention of its transmission, standardized lab diagnosis, free ART and other treatments available nationally has been incorporated in the guidelines, making them more comprehensive.

3.3 More scientific evidence involved

The 2021 edition of the guidelines refers to results of relevant domestic research and evidence from clinical practice, including the Expert Consensus on HIV Pre-exposure Prophylaxis (PrEP) Drugs and the Expert Consensus on Diagnosis and Treatment of Patients with AIDS and Pneumocystis Pneumonia in China. The guidelines also comprehensively cite and refer to results of foreign research. Moreover, the guidelines also highlight HIV/AIDS prevention, clinical diagnosis, patient follow-up and management, ART, HIV, and management of opportunistic infections in light of clinical practice in China and views of personnel at different levels of the healthcare system.

4. Highlights

4.1. Source control and early intervention

(*i*) The guidelines put forward the concept of "enhancing the combination of early intervention, prevention, and treatment."
The chapter on epidemiology describes UNAIDS' 6 95% targets to end AIDS (*e.g.*, 95% of PLWH know their HIV status and 95% of PLWH who know their status initiate treatment) for the first time. For the first time, the chapter also mentions PrEP and post-exposure prophylaxis (PEP) for high-risk groups to reduce HIV transmission.

The guidelines further encourage and ask clinical practitioners to pay attention not only to the diagnosis and treatment of diseases but also to the actions and goals promoted by epidemiological and public policies. There are no leakage in prevention and treatment.

(*ii*) In the chapter on PrEP and PEP, treatment of occupational exposure, follow-up of non-occupational exposure, and treatment options are highlighted in light of actual needs in China. Moreover, the cohorts in which the safety and efficacy of innovative ART drugs for PEP in China are being tested are described for the first time. Further data shall be added once available.

The guidelines also describe the eligible population, initiation process, treatment regimens, precautions, and follow-up survey of PrEP in detail. This indicates that PrEP is being implemented and promoted as a public health policy in China. In addition, the significance of HIV RNA tests before and after taking drugs for PrEP is also highlighted for the first time. Patients must take drugs for PrEP for 7 more days after the last high-risk sexual behavior. These aspects align with the current public health policy to "enhance prevention and prophylaxis and enhance regular follow-up" in China in order to provide clinical support of reducing new HIV infections (*9*).

4.2. Standardized diagnostic criteria

In the chapter on clinical staging and diagnosis, the concept of and diagnostic criteria for HIV/AIDS are

clearly described. The stage of disease includes acute HIV infection (stage I), chronic HIV infection (stage II), and AIDS (stage III). The importance of a nucleic acid test (NAT) for diagnosis is highlighted throughout the guidelines.

The 2021 edition of the guidelines illustrates that HIV antibody and HIV RNA tests are used to confirm an HIV infection, the epidemiological history for the diagnosis of acute HIV infection and HIV in infants, CD4 cell counts and symptoms for disease staging, and AIDS-related symptoms for diagnosis and treatment of AIDS.

4.3. Advances in research on HIV/AIDS in China

In the chapter on pathogenesis, results from pathology and epidemiology studies conducted by Chinese researchers are widely cited. The different circulating recombinant forms (CRFs) and unique recombinant forms (URFs) in Chinese patients are described for the first time.

Molecular epidemiology data from China has also been published. According to the fourth National HIV Molecular Epidemiology Study in 2015, the most prevalent strain in China is CRF07_BC, CRF01_AE, CRF08_BC and subtype B.

Data indicate that the disease progresses faster in men who have sex with men (MSM) who are infected with HIV, with an average of 4.8 years' development before AIDS stage.

For the first time, the guidelines mention that adequate immune reconstitution is not achieved in 10-40% of patients with HIV/AIDS despite long-term virological suppression. These patients are referred to as "inadequate immunological responders" or "immunological non-responders." Compared to patients in whom adequate immune reconstitution has been achieved, the inadequate immunological responders have a higher risk of progression to AIDS and non-AIDS events and a higher mortality rate (*10*).

4.4. Optimized programs to prevent mother-to-child transmission based on circumstances in China and clinical studies Prevention of mother-to-child transmission and single-positive family fertility based on clinical study of China.

ART regimens for pregnant women have been updated based on findings from recent research. Dolutegravir (DTG) is included in the first-line regimen for ART in pregnant women and tenofovir alafenamide (TAF)/ emtricitabine (FTC) is included in the second-line regimen.

Neonatal risk assessment indicators are clearer and more readily assessed. Different ART regimens will be selected based on the level of risk of perinatal HIV transmission. A newborn with a high risk of perinatal HIV transmission will receive standard triple therapy.

Another update is specification of the timing forearly HIV tests in newborns with perinatal HIV exposure. For accurate diagnosis, the newborn must undergo an HIV NAT test within 48 hours and at 6 weeks and 3 months after birth. HIV antibody tests will be performed at 12 and 18 months after birth. Based on clinical practice and results of research in China, babies with HIV exposure who have negative NAT results and positive antibody test results will need to undergo another HIV antibody test 24 months after birth.

For the first time, the guidelines mention that when an HIV-positive man without virological suppression attempts to have sex without a condom to conceivean HIV-negative woman, should take tenofovir (TDF)/ FTC (or TDF + lamivudine [3TC]) continuously 20 days prior to the date of sexual behavior and for 1 month after for prophylaxis.

4.5. Significance of lab testing to guide effective ART

The 2021 edition of the guidelines highlights the benefits of viral load and HIV drug resistance testing in HIV diagnosis and ART initiation. The guidelines now highly recommend increasing the frequency of HIV viral load tests, they cite the reason for increasing the test frequency and for use of ultrasensitive HIV viral load testing, and they recommend reducing the frequency of CD4 tests. For the first time, the guidelines indicate that HIV drug resistance testing should be done before ART, regardless of the treatment regimen used.

4.6. Diagnosis and treatment of opportunistic infections and when to start ART

(*i*) The Xpert MTB/RIF and Xpert MTB/RIF Ultra molecular diagnosis tests are clearly recommended as initial diagnostic tests for diagnosis of HIV/tuberculosis (HIV/TB). The updated guidelines emphasize that ART must be started within 2 weeks of tuberculosis treatment, but does not include tuberculous meningitis and drug-resistant tuberculosis

RAL is no longer recommended as a preferred protocol for HIV/TB.

The most important update is the description of an HIV/TB diagnosis that does not rely on LTBI test results, consistent with the WHO guidelines for a prophylactic drug strategy for TB.

(*ii*) Primary prophylaxis for nontuberculous mycobacteria (NTM) is not recommended for patients with rapid initiation of ART.

(*iii*) A treatment for cryptococcal pneumonia was specifically updated, and a treatment for cryptococcal antisepsis has been recommended for the first time. In the consolidation stage for treatment of cryptococcal meningitis, the recommended dose of fluconazole was increased to 600-800mg/d, which was based on clinical

studies by and evidence from Chinese researchers.

4.7. A solution for China: ART

(*i*) In alignment with international guidelines, the Chinese guidelines now propose that rapid initiation of ART or same-day initiation of ART can be considered for eligible patients.

(*ii*) A regimen including integrase inhibitors (INSTI) is one of the main recommended regimens for ART;

(*iii*) Based on clinical practice and an expert consensus in China, dual therapy is cited as a first-line regimen;

(*iv*) The single-tablet regimen is listed as the preferred treatment option. At the same time, generic drugs developed in China, such as azvudine and ainuovirine, are included in the guidelines for the first time.

(*v*) The dose of efavirenz (EFV) is specified as 400 mg, while EFV should not be used for patients with a viral load greater than 500,000 copies/mL (*11*).

(*vi*) Suitable INSTIs should be used in first-line ART regimens for children.

(*vii*) Albuvirtide is recommended for certain patients under special circumstances, including those with drug resistance.

After the publication of the 2021 guidelines, experts on the Editorial Committee have recently start training other medical personnel around the country on those guidelines. Experts will teach, combine practice with theory, and travel to places with a high incidence of HIV/AIDS and patients receiving long-term treatment. Information in the guidelines will be emphasized, highlights of the update will be cited, and actual cases from local clinical practice will be discussed. This should provide ample opportunities for training of and discussions with primary physicians across the country.

In conclusion, the 2021 edition of the guidelines covers results of the latest domestic and foreign research, it focuses on the clinical benefits of standardized treatment in clinical practice, it proposes ART regimens more suitable for PLWH in China, and it promotes whole process management of HIV/AIDS. The guidelines include findings from research and clinical studies by Chinese researchers, and the guidelines are more suitable for Chinese patients. During the period of the 14th Five-Year Plan, research on multidisciplinary treatment of HIV/AIDS and on metabolic syndrome will be conducted as the guidelines are promoted and implemented. This research will combine clinical practice and clinical research to further explore advanced medical technologies such as functional cures and gene therapies. This will help to lower the incidence and mortality rate, prolong the life of PLWH, increase their survival rate, and improve patients' quality of life.

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Commentary

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From SARS to the Omicron variant of COVID-19: China's policy adjustments and changes to prevent and control infectious diseases

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SUMMARY The COVID-19 pandemic has been the biggest public health crisis in a century. Since it was initially reported in 2019, the duration and intensity of its impacts are still in serious question around the world, and it is about to enter its third year. The first public health revolution failed to achieve its ultimate targets, as previously contained infectious diseases seem to have returned, and new infectious diseases continue to emerge. The prevention and control of infectious diseases is still a public health priority worldwide. After SARS, China adjusted a series of its infectious disease policies. In order to ensure the effectiveness and implementation of prevention and control interventions, the government should integrate the concept of public health. Perhaps we need a global public health system at the government level to fight the potential threat of infectious disease. This system could include multifaceted strategies, not just specific prevention and control interventions, and it could also be a comprehensive system to ensure unimpeded communication and cooperation as well as sustainable development.

Keywords COVID-19, infectious disease, physical containment strategies, vaccination, global health, public health

The COVID-19 pandemic has been the biggest public health crisis in a century (Figure 1). According to the World Economic Outlook in April 2020, the International Monetary Fund ranked this crisis as the Great Lockdown. In addition to the public health crisis, this health emergency had triggered a global financial crisis, and containment measures have made stimulating aggregate activity more challenging (*1*). Worldwide, severe uncertainty about the duration and intensity of the pandemic's impacts remains in 2021, and a WHO Emergency Committee concluded that the pandemic is far from over. The Delta variant has become one of the most infectious viruses (R0:5-9·5) (*2*).

New strains pop up continuously. on Nov 26, 2021, WHO designated a new variant, Omicron. After its was first identified in southern Africa, Omicron has been found in 76 countries across the world as of Dec 14, 2021 (*3*). In the United States, 43 individuals infected with Omicron were identified from Dec. 1 to Dec. 8 (*4*); one individual was hospitalized but no deaths were reported. China has also identified 11 cases infected with Omicron until Dec. 15, 2021 (*5.6*). There is substantial uncertainty regarding Omicron's transmissibility and severity (*7*). The researchers from The University of Hong Kong found that Omicron SARS-CoV-2 infects and multiplies 70 times faster than the Delta variant and original SARS-CoV-2 in human bronchus, but the infection in the lung is significantly lower than the original SARS-CoV-2 (*8*). Given the persistent mutation of the virus, if the SARS-CoV-2 virus becomes more transmissible and it continues to exist with human beings over the long time, then presumably the disease's severity will decrease and it will become "another type of influenza."

Omicron's immune escape potential is also uncertain. Breakthrough infections are likely to occur, which means relying more on physical containment strategies. The WHO also recommends that individuals socially distance and wear masks (*7*).

1. China's experience and physical containment strategies.

During the global fight against COVID-19, China has reported only 0.05% of the total number of global cases (more than 265 million) despite its huge population base. Unlike some Western countries, China mobilized quickly and coordinated a national campaign to contain outbreaks in Hubei and related epidemics (*9.10*). In about three months, China normalized prevention and control with a focus on imported cases and related outbreaks. Since

Aug 2021, China has attempted to enter a new phase through a "dynamic zero tolerance" approach, which aims to formulate precise and differentiated strategies to prevent and control COVID-19 (Figure 2).

China's experience shows that physical containment strategies are not magic. Physical containment strategies that seek to interrupt the transmission of pathogens are effective at preventing and controlling COVID-19 and other infectious diseases (*11*). What is special is the implementation of these strategies. Traditional contact tracing and quarantine are always effective at identifying secondary cases and avoiding continued transmission. During outbreaks in Dalian (July 2020) and Guangzhou (May 2021), 74% and 65% of cases, respectively, were identified among close contacts (*9*). Implementation of social distancing and movement restrictions effectively

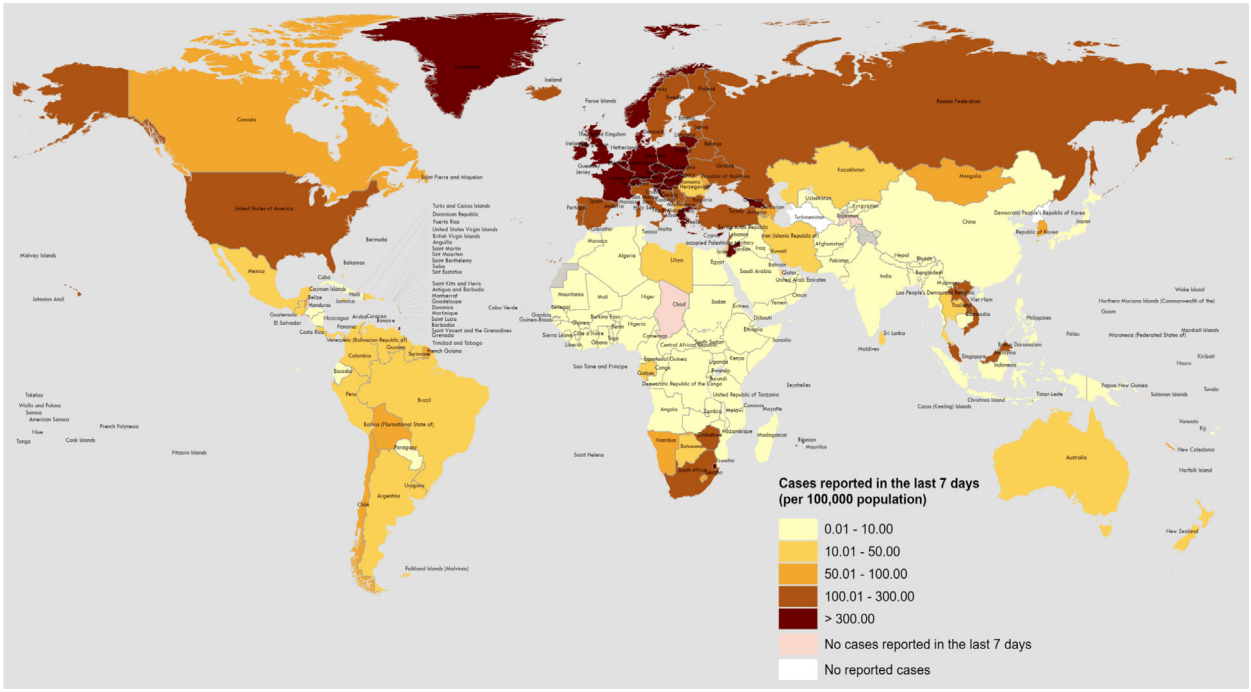

Figure 1. COVID-19 cases per 100,000 population reported by countries, territories, and areas, Dec-14 2021. Figures are from the COVID-19 Weekly Epidemiological Update. Data presented are based on official laboratory-confirmed COVID-19 cases and deaths reported to the WHO by country/territories/areas, largely based upon WHO case definitions and surveillance guidance.

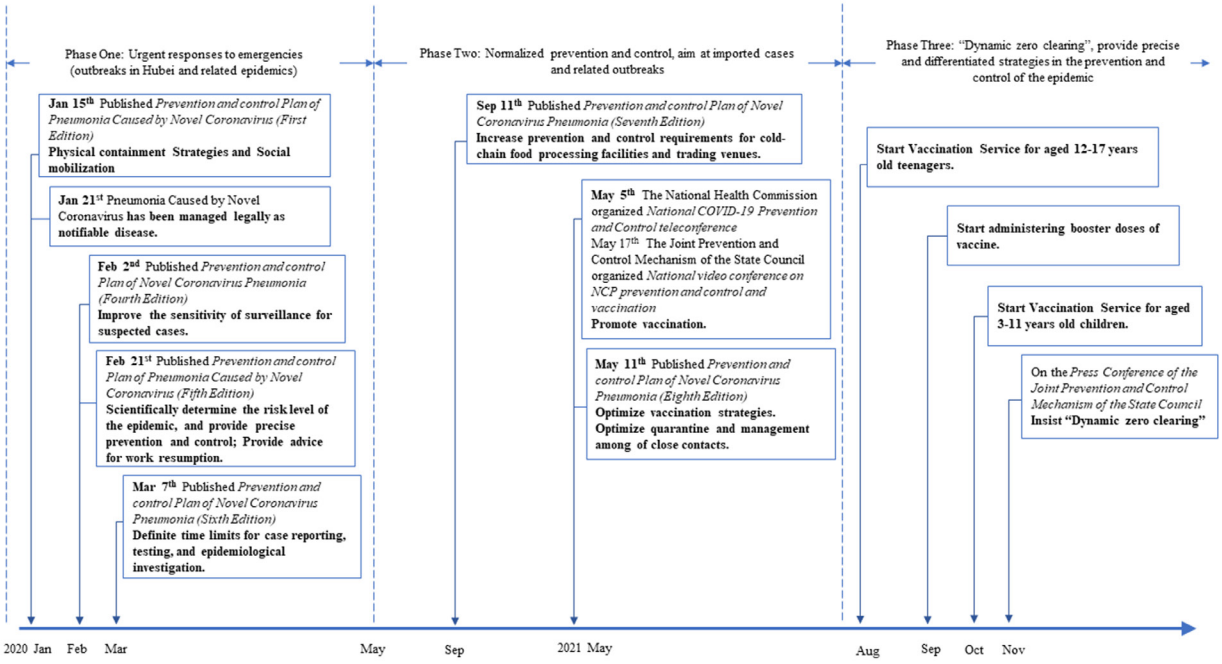

Figure 2 Prevention and control processes and strategies to combat COVID-19 in China since 2020.

reduced the secondary attack rate among household contacts. During the Wuhan outbreak (from Dec 2019 to April 2020), the household reproduction number declined by 52% among primary cases and by 63% among secondary cases (*12*).

Containment interventions also mean a certain degree of infringement upon liberty. Policy-making needs to be based on scientific evidence to assure the legality of an intervention and to balance personal privacy and public health. Government also needs to support effective public mobilization and management. Thanks to rapid and sustained containment strategies, such as the lockdown in Wuhan, China avoided a 67-fold increase in cases (interquartile range: 44-94-fold) within one month (*13*).

2. Over the long term, prevention and control of infectious diseases is the basis of public health.

The first public health revolution failed to achieve its ultimate targets, as previously contained infectious diseases seem to have returned, and new infectious diseases continue to emerge (emerging infectious diseases, or EIDs). In the late 20th century, most experts concurrently reached the conclusion that ideas on fitness and medical models should change and evolve. What causes the highest proportion of deaths has changed from acute infectious diseases to chronic diseases, and this is especially true in developed countries. However, outbreaks or pandemics of infectious diseases have presented a significant worldwide threat since 2000. These diseases include reemerging diseases, such as HIV/AIDS, malaria, tuberculosis, and West Nile virus, as well as emerging ones, such as severe acute respiratory syndrome (SARS) and COVID-19. (Table 1) There are two types of infectious diseases that represent the greatest threat to public health worldwide. One is respiratory infectious disease; the other is vector-borne diseases.

3. The concept of a global healthcare community should be integrated into all policies and regulations.

Each epidemic or pandemic is the result of a complex interplay of natural evolution and human advancement. Many EIDs appear to be caused by zoonotic pathogens and involve interaction between humans and wildlife (*14,15*). Paralleling natural evolution and human advancement, human activity seems to have expanded since mankind entered the 21st century, increasing the probability of pandemics (*14*). In addition, climate change brought about by human activity may profoundly affect the transmission of pathogens and vectors (*16,17*). Governments should once again acknowledge the actual and potential burden of infectious diseases.

Regrettably, global prevention and control efforts and awareness among the healthcare community worldwide have been far outpaced by the global spread of EIDs. Intervention strategies for infectious diseases and public health systems worldwide are not prepared for future challenges from EIDs. The WHO should expand its implementation and range of responsibilities by enhancing the "One Health" concept. A collaborative system should be created by health authorities and authorities related to wildlife and the environment. The relationship among human beings, other creatures, and diverse natural ecosystems should be fundamentally reconsidered, and the concept of global healthcare community should be devised.

Public health should be emphasized globally. In addition to development of effective vaccines in the near future, new International Health Regulations (IHR) (2022) may be modified and adopted, and a treaty or agreement with international support to fight infectious disease pandemics should also be concluded. Over the long term, political leadership at the global level is needed. The National Provider Identifier Standard (NPI) should be emphasized. Public health should be integrated into the United Nations' Sustainable Development Goals to be implemented by every member country and to facilitate global health initiatives.

Vaccines are the most crucial intervention to achieve herd immunity and prevent the spread of infectious disease. As a result of massive resources worldwide, COVID-19 vaccines have been rapidly developed in comparison to conventional vaccines. As the virus continues to mutate, research on new and highly effective vaccines should be accelerated. In the future, increased emphasis should be placed on biological research, and vaccine research and stockpiles should be improved to respond to the long-term challenges of EIDs.

To fight against potential infectious disease pandemics, training of public health personnel should be accelerated. In addition, leaders in global public health should be trained instead of relying on the CDC. There are many examples of exceptional teamwork in fighting pandemics: 1) The Epidemic Intelligence Service (EIS) of the US CDC has greatly contributed to the eradication of smallpox and many other immunization programs (*18*). 2) In 2003, Trainees from China's Field Epidemiology Training Programs helped provide scientific evidence for identification of and intervention in the SARS pandemic (*19*).

The COVID-19 pandemic has focused our attention on infectious diseases once again. We need to face the fact that the SARS-CoV-2 virus will coexist with human beings over the long term. As globalization continues, governments need to realize that the threat of infectious diseases is ever-present. We need to learn from past experience to build a global public health system to fight the potential threat of infectious diseases. This system should have multifaceted strategies and not just specific prevention and control interventions. It should also be a comprehensive system to ensure unimpeded

Table 1. Outbreaks of Emerging Infectious Diseases (EIDs) since 2000

Time of Occurrence /Duration	Sites of initial outbreaks or large outbreaks	Name of disease or virus	Events and Burden	Response of WHO	Declared a Public Health Emergency of International Concern (PHEIC)?
1999-	Perak, Malaysia	Nipah virus	1. Nipah virus was first recognized in 1999 during an outbreak in Perak, Malaysia. 2. Then in Bangladesh in 2001. 3. The disease has also been identified periodically in eastern India and other areas.	Support technical guidance 1. Use a One-Health approach. Cooperate with the agricultural sector, detect cases, and establish an animal health or wildlife surveillance system to provide an early warning to veterinary and human public health authorities. 2. Control infection in health-care settings. Implement standard infection control precautions among health-care workers.	
2002.11 -2003.07	Guangdong, China	SARS	The total number of SARS cases worldwide reached 8,437, with cases in 29 counties. Mortality from SARS is estimated to be 10-12% (*20*). SARS (R0:2.2-3.6) is more transmissible than MERS and Ebola (*2,21*).	Take effective control measures including international collaboration supported at the highest political level. Seek to apply the spirit of several regional and international efforts in fighting the SARS epidemic, including the ASEAN +31 Ministers of Health Special Meeting on Severe Acute Respiratory Syndrome (SARS) (Kuala Lumpur, 26 April 2003), the Special ASEAN-China Leaders Meeting on the Severe Acute Respiratory Syndrome (SARS) (Bangkok, 29 April 2003), and other high-level meetings (*22*).	No, WHO issued a global warning on Mar 12, 2003.
2003	From Asia to Europe and Africa	H5N1 virus	H5N1 virus has spread from Asia to Europe and Africa, and outbreaks have resulted in millions of poultry infections, several hundred human cases, and many human deaths.	1. Continuously monitor avian and other zoonotic influenza viruses closely through its Global Influenza Surveillance and Response System (GISRS). 2. Collaborate with the World Organisation for Animal Health (OIE) and the Food and Agriculture Organization (FAO), conduct surveillance at the human-animal interface, assess the associated risks and coordinate the response to zoonotic influenza outbreaks. 3. Provide guidance and develop surveillance, preparedness, and response strategies and intervention recommendations.	
2007	The Island of Yap (Federated States of Micronesia)	Zika virus disease	1. Since 2015, outbreaks and evidence of transmission soon appeared throughout the Americas, Africa, and other regions of the world. 2. As of July 2019, a total of 87 countries and territories have reported evidence of mosquito-transmitted Zika infection (*23*).	1. Advance research in prevention, surveillance, and control of infection and associated complications. 2. Develop, strengthen, and implement integrated surveillance systems for infection and associated complications. 3. Strengthen the capacity of laboratories to test for infection worldwide. 4. Support global efforts to implement and monitor vector control strategies aimed at reducing Aedes mosquito populations. 5. Strengthen care and support of affected children and families.	Yes, on Feb 2, 2016
2009		H1N1	As of 1 August 2010, more than 214 countries and overseas territories or communities have reported laboratory-confirmed cases of pandemic influenza H1N1 2009, including over 18,449 deaths.	Raise the level of influenza pandemic alert to phase 6 in late April. Strengthen national, regional and global influenza response capacities including diagnostics, antiviral susceptibility monitoring, disease surveillance, and outbreak responses. Increase vaccine coverage among high-risk groups. In collaboration with other partners, monitor influenza activity globally through the WHO GISRS system and recommend vaccine formulations.	Yes, on April 24, 2009

Table 1. Outbreaks of Emerging Infectious Diseases (EIDs) since 2000 (Table continued)

Time of Occurrence /Duration	Sites of initial outbreaks or large outbreaks	Name of disease or virus	Events and Burden	Response of WHO	Declared a Public Health Emergency of International Concern (PHEIC)?
2010-	Endemic in more than 100 countries	Dengue	1. As of October 27, 2014, a total of 41,155 dengue cases and 6 deaths were reported in Guangdong Province, China. 2. Large dengue outbreaks, with the Region of the Americas reporting more than 2.38 million cases in 2016 (*24*). 3. The largest number of dengue cases ever reported globally was in 2019. All WHO Regions were affected, and dengue transmission was recorded in Afghanistan for the first time. 4. About 129 countries have been at risk. The America, South-East Asia, and Western Pacific regions are the most seriously affected, with Asia representing 70% of the global burden of disease (*25*).	1. Support countries in the confirmation of outbreaks through its collaborating network of laboratories. 2. Provide technical support and guidance to countries for the effective management of dengue outbreaks. 3. Support countries to improve their reporting systems and capture the true burden of the disease. 4. Provide training on clinical management, diagnosis, and vector control at the country and regional level with some of its collaborating centers. 5. Support countries in the development of dengue prevention and control strategies and adopting the Global Vector Control Response (2017-2030) 6. Review the development of new tools and publish guidelines and handbooks for surveillance, case management, diagnosis, dengue prevention, and control for Member States.	
2012.09	Saudi Arabia	MERS	1. The average MERS case fatality rate is around 35%. 2. Largest outbreaks seen in Saudi Arabia, United Arab Emirates, and the Republic of Korea.	1. Provide updated information on the situation. 2. Conduct risk assessments and joint investigations with national authorities. 3. Convene scientific meetings and develop guidance and training for health authorities and technical health agencies on interim surveillance recommendations, laboratory testing of cases, infection prevention and control, and clinical management.	
2013	China	Avian influenza A (H7N9) virus	In 2013, 139 confirmed cases were identified in 12 areas of China (*26*). As of Sep 5, 2018, a total of 1,567 laboratory-confirmed human cases, including at least 615 deaths, have been reported to the WHO.	1. Continuously monitor avian and other zoonotic influenza viruses closely through its Global Influenza Surveillance and Response System (GISRS). 2. Collaborate with the World Organisation for Animal Health (OIE) and the Food and Agriculture Organization (FAO), conduct surveillance at the human-animal interface, assess the associated risks and coordinate the response to zoonotic influenza outbreaks. 3. Provide guidance and develop surveillance, preparedness and response strategies and intervention recommendations.	
2014-	West Africa	Ebola	The average case fatality rate is around 50%.	Prevent Ebola outbreaks by maintaining surveillance for Ebola virus disease and supporting at-risk countries to develop preparedness plans. Publish guidance and advice to prevent and control potential outbreaks. In 2015, the WHO published a list of top emerging diseases likely to cause major epidemics. The initial list of disease priorities needing urgent R&D attention comprises: Crimean Congo hemorrhagic fever, Ebola virus disease and Marburg, Lassa fever, MERS and SARS coronavirus diseases, Nipah, and Rift Valley fever. The list will be reviewed annually or when new diseases emerge.	Yes, on Aug 8, 2014 and on Jul 17, 2019.
2019	Wuhan, China	COVID-19	1. As of November 28, over 260 million confirmed cases and nearly 5.2 million deaths have been reported globally. The International Monetary Fund ranked this crisis as The Great Lockdown. 2. New strains pop up continuously. The Delta variant has even become one of the most infectious viruses (R0:5-9·5) (*2*). 3. The new Omicron variant has spread to 38 countries.	Provide support around the world to fight against this pandemic. Issue the COVID-19 Strategic Preparedness and Response Plan (SPRP) for 2021 and accompanying documents as a package aimed at guiding the coordinated action that we must take at national, regional, and global levels to overcome the ongoing challenges in the response to COVID-19.	Yes, on Jan 30, 2020.

communication and cooperation as well as sustainable development.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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BST
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