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Editorial

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More effective vaccines and oral antivirals: Keys for the battle against Omicron

Hongzhou Lu*

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SUMMARY With the rapid roll out of vaccination programs and extraordinary non-pharmaceutical interventions (NPIs) by the government, China has maintained a "dynamic zero-COVID-19" policy over the last two years. However, the global pandemic and immune evasion of Omicron variant poses a huge challenge to China. Currently, about 87.69% of the Chinese population has been vaccinated, most with inactivated vaccines. Although seroepidemiological data on the vaccinated are lacking, published data suggested that even a homologous booster of an inactivated vaccine displayed very limited neutralizing activity against the Omicron variant and that neutralizing activity was significantly lower than that of a heterologous booster or mRNA vaccine alone. A great concern is whether the neutralizing antibodies induced by inactivated vaccines can provide sufficient protection against the Omicron variant since local transmission of the Omicron variant is now occurring in China. The era of extraordinary NPIs by governments and countries to control the transmission of SARS-CoV-2 is going to change. Omicron's immune evasion of neutralizing antibodies induced by current vaccines and the majority of existing therapeutic SARS-CoV-2 monoclonal antibodies (mAbs) suggest an urgent need for more effective vaccines and highly effective oral antivirals, which will be the keys for the battle against Omicron in the future.

Keywords Omicron, vaccine, oral antivirals

Due to its strong tropism in the upper respiratory tract and its considerable evasion of antibody neutralization, the Omicron variant has spread rapidly and efficiently around the world (*1,2*). Currently, more than 2 million newly confirmed COVID-19 cases are reported to the WHO around the world every day (*https://covid19.who.int/*), and over 90% of the SAS-CoV-2 sequences recently uploaded to the GISAID database (*https://www.gisaid.org/*) were the Omicron variant. Despite the obvious increase in transmissibility, the disease burden of the Omicron variant has been found to be lower than that of the Delta variant. Real-world studies conducted in South Africa revealed a decrease in severity and mortality for the Omicron variant in comparison to other variants (*3-5*). For children under the age of 5 with an initial SARS-CoV-2 infection, the risk of a visit to the emergency department (ED), hospitalization, intensive care unit (ICU) admission, or placement on mechanical ventilation within 3 days of infection is significantly lower in the Omicron cohort than in the matching Delta cohort (*6*). Based on the high transmissibility and low pathogenicity of the Omicron variant, several countries have rescinded policies to control the spread of Omicron variant and instead placed their hopes on infection-acquired immunity, though this obviously ignores the increase in fatalities among the huge infected population, the long-term health consequences of COVID-19, and the accompanying social issues related to the commitment of medical resources (*7,8*). In addition, naive infection with the Omicron variant induces limited cross-variant immunity (*9*), and an mRNA-Omicron vaccine boost may not provide greater immunity or protection compared to a boost with the current mRNA-1273 vaccine (*10*).

With the rapid roll out of vaccination programs and extraordinary non-pharmaceutical interventions (NPIs) by the government, China has maintained a "dynamic zero-COVID-19" policy over the last two years (*11*). However, the "dynamic zero-COVID-19" policy is now facing huge challenges due to the global pandemic caused by the Omicron variant. Currently, about 87.69% of the Chinese population has been vaccinated, mostly with inactivated vaccines (*https://ourworldindata.org/coronavirus*). Although seroepidemiological data on the vaccinated are lacking, published data suggested that even a homologous booster of an inactivated vaccine

displayed very limited neutralizing activity against the Omicron variant (*12-14*) and that neutralizing activity was significantly lower than that of a heterologous booster or mRNA vaccine alone (*15-20*). Moreover, breakthrough infections with the Omicron variant have also been found in individuals who received a homologous booster with an mRNA vaccine (*21*). A great concern is whether the neutralizing antibodies induced by inactivated vaccines can provide sufficient protection against the Omicron variant since local transmission of the Omicron variant is now occurring in China. Several oral antivirals have been authorized for emergency use in the treatment of mild-to-moderate COVID-19 by the US Food and Drug Administration (FDA), including Molnupiravir and Paxlovid. In clinical trials, these oral antivirals significantly reduced hospital admissions and deaths among people with COVID-19 who are at high risk of severe illness in comparison to a placebo (*22,23*). Until recently, there were no such oral antivirals in China, although several remdesivir derivatives that were designed and modified by Chinese researchers were found to be safe and highly effective in preclinical studies (*24,25*).

Currently, there are three theories on the origins of the Omicron variant (*26*). Although determining which of the three is true is difficult, more variants are sure to appear with unpredictable mutations. The era of extraordinary NPIs by governments and countries to control the transmission of SARS-CoV-2 is going to change. Omicron's immune evasion of neutralizing antibodies induced by current vaccines and the majority of existing therapeutic SARS-CoV-2 monoclonal antibodies (mAbs) suggest an urgent need for more effective vaccines and highly effective oral antivirals, which will be the keys for the battle against Omicron in the future.

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Editorial

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Increasing demand for point-of-care testing and the potential to incorporate the Internet of medical things in an integrated health management system

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SUMMARY As the number of people with COVID-19 increases daily around the world, point-of-care testing (POCT) is gaining attention as a tool that can provide immediate test results and greatly help to deter infection and determine what to do next. POCT has several drawbacks such as a low sensitivity and specificity, but according to studies POCT has increased sensitivity on par with that of polymerase chain reaction testing. The advantage of POCT is that the results can be obtained quickly, regardless of the location. To further enhance its benefits, POCT is being developed and researched in conjunction with the Internet of medical things (IoMT), which allows POCT results to be collected, recorded, and managed over a network. IoMT will be beneficial not only for the use of POCT simply as a testing tool but also for its integration into diagnostic and health management systems. IoMT will enable people to regularly receive their test results in their daily lives and to provide personalized diagnosis and treatment of individual conditions, which will be beneficial in terms of disease prevention and maintenance of health.

Keywords point-of-care testing, Internet of medical things, COVID-19, Japan

Point-of-care testing (POCT) is a concept that has been gaining attention due to the global outbreak of COVID-19. POCT is a simple test that can be performed at the patient's side by a healthcare professional or the patient himself. Information on the results of a test for a suspected disease is essential to making a proper diagnosis or deciding a treatment in a medical setting. With POCT, tests can be performed quickly in the required time, and results obtained in real time eliminate the time lag between diagnosis and care and enable a rapid response. In addition, quick testing and immediate results will improve the quality of medical care provided to patients and their quality of life. POCT is a concept that refers not only to testing equipment and reagents, but to the entire system for quick testing and immediate results (*1,2*). In the past, clinical tests were performed at specialized facilities such as testing centers using large specialized analyzers, but testing methods, kits, and systems are being researched and developed each year in response to the needs of the medical field for quick testing and immediate results.

POCT does not necessarily have to be done in a hospital or other medical facility. If necessary, it can be done at the patient's home or workplace, as long as the test results can be obtained immediately and can be used for testing and diagnosis in a location near the patient. The location-independent advantage of POCT was evident during the global outbreak of COVID-19. In the early stages of COVID-19 testing, PCR testing was the main method used, but PCR testing requires a special machine to be used after specimens are collected, and test results can take more than a day to be obtained (*3*). With the number of infected people increasing every day, the PCR test alone cannot keep up with the required number of tests. Antigen test kits were later introduced as POCT, and results can be obtained in about 30 minutes after specimen collection (*3*). In addition to antigen testing, nucleic acid testing using the Loop-mediated isothermal amplification method and biosensor testing using new sensor detection technologies such as surface plasmon resonance and electrochemical methods are also being studied, but immunochromatography is currently the main method in commercial use because of its stability and cost (*4*). According to a survey by the Tokyo Metropolitan Government, when the number of tests performed peaked in the sixth wave of COVID-19 on

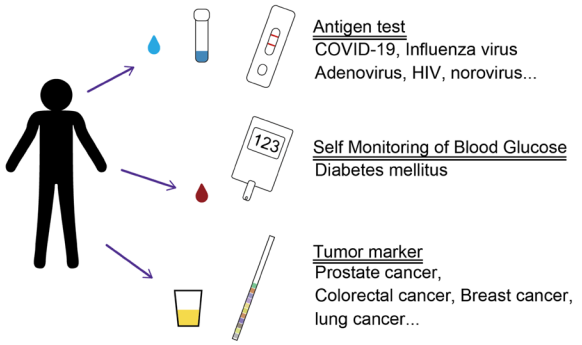

Figure 1. Test methods available for point-of-care testing.

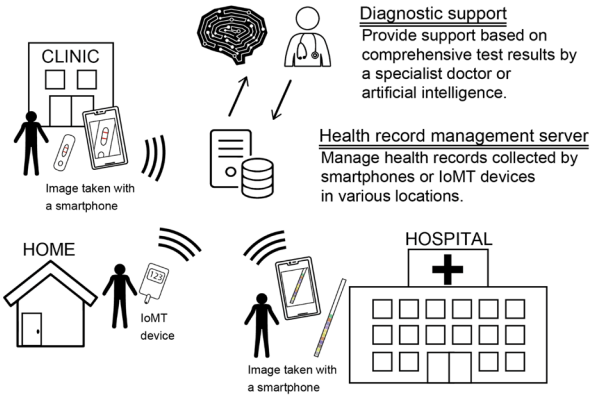

Figure 2. A location-independent health management system with point-of-care testing using the Internet of medical things and immediate support from expert doctors or artificial intelligence.

January 24, 2022, 35,182 people were tested in Tokyo, of which 11,154 underwent antigen testing (*5*). Although there are concerns about the availability of sufficient numbers of antigen test kits and their diagnostic accuracy, POCT, which can provide test results quickly and in the necessary time, will greatly help to decide diagnosis and treatment.

Current status of POCT

The area of POCT has been attracting attention as a test for COVID-19, but POCT itself has been actively developed and researched, and test kits have been developed and used for a variety of subjects. Figure 1 summarizes the testing methods that can be used in POCT and the conditions that can be tested for, including antigen tests and tumor markers (*1,2,6*). Antigen tests are used to test for antigens against various viruses such as SARS-CoV-2, which causes COVID-19, and reagents and test kits have been developed to test for influenza virus antigens, adenovirus antigens, HIV antigens, norovirus antigens, and antigens of many other infectious microorganisms. Immunochromatography is the main method used for antigen testing and POCT for tumor markers. The advantage of immunochromatography is that the results can be confirmed visually on the spot. However, there may be differences in negative and positive results depending on the lot and reagent.

In addition to the development of the tests for various infectious diseases and cancers as described above, the testing methods used in POCT are also being improved. Although POCT enables rapid testing, high accuracy is also required because POCT plays a major role in determining diagnosis and treatments. However, POCT has the disadvantage of having a lower sensitivity and specificity than an enzyme-linked immunosorbent assay or a polymerase chain reaction, which are the test methods commonly used in laboratories. In contrast, immunochromatography, which is often used to test for antigens of infectious microorganisms, is a test method involving the use of a paper test strip; it is low cost and easy to perform but tends to be less accurate. In recent years, research has been conducted to improve assays and enrich samples via preamplification to solve the problem of low sensitivity. Moreover, a low specificity can be increased by assay optimization and the identification and use of highly specific affinity molecules (*7*). Research has progressed to the point where POCT has performance on par with PCR testing.

Future development of POCT

POCT is an area where significant market growth is expected in the future. The global market for POCT reached $24.8 billion in 2021 and is expected to grow to over $43.5 billion by 2026 (*8*). Increasing demand for kits to test for infectious diseases, the incidence of cancer and chronic diseases, and a large elderly population are the major factors driving that market growth. In addition to the increase in the number of people who are candidates for testing, patient needs for quality medical care and convenience in obtaining results remotely and in a familiar place (home, office, or nearby clinic) are also factors that are expected to contribute to the growth of the market worldwide. Medical professionals have needs as well due to the shortage of medical personnel as a result of the aging population, a decrease in the size of the workforce, and the uneven distribution of medical personnel in Japan (*9*). Therefore, tests and systems that can be used remotely or by patients themselves, such as POCT, are expected to be developed.

POCT using the Internet of medical things (IoMT) is being researched in order to allow remote POCT systems to collect, record, and manage test results (*10,11*). Early detection and real-time treatment of infectious diseases is important to reducing the spread of infection, but it is a difficult task for limited medical personnel. As shown in Figure 2, systems that communicate via the IoMT and that collect, manage, and analyze information obtained via POCT, smartphones, and small testing devices will be beneficial. Diagnosis of infectious diseases using machine learning is also becoming a possibility (*12,13*). In addition, smart applications that screen for conditions based on physical information such as blood

glucose levels, blood pressure, and weight are being brought to market (*14*). In the future, POCT could share information *via* the IoMT, applications could analyze that information, and medical facilities could collaborate to create a system that immediately provides people with useful diagnostic information. POCT that can be easily and regularly performed in daily life would allow health to be gauged on a regular basis; this will lead not only to the early detection of lifestyle-related diseases but also to the immediate detection of their signs. Based on those signs, an analysis by an application or a doctor's remarks in real time would provide information on immediate lifestyle modifications. POCT using the IoMT will be beneficial in terms of public health and disease prevention.

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Review

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What does oral care mean to society?

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SUMMARY Oral care is defined in a narrow sense as cleaning of the teeth, oral cavity, and dentures, and in a broad sense as the maintenance of oral functions (feeding, swallowing, chewing, speech, aesthetics, *etc*.), dental treatment, feeding and swallowing training, and articulation training. In the past, it was recognized as simply cleaning the mouth, but the concept of oral care has gradually expanded, and many studies and surveys have been conducted in cooperation with various other professions. As a result, oral health care is involved not only in the prevention of pneumonia, but also in the onset and suppression for severity of diabetes, cardiovascular diseases, some malignant tumors, cerebrovascular diseases, rheumatoid arthritis, dementia, *etc*. It is also a powerful supportive therapy in cancer treatment. In the terminal stages of life, oral health care can help people to maintain their dignity by continuing to consume food orally until the end of their lives, and in times of disaster, oral health care has been found to be as important as attention to deep vein thrombosis. It has also been found to be effective in preventing severe diseases such as COVID-19. And, although it has not been discussed much, it has been found to have medical and economic benefits such as reducing the duration of hospitalization and treatment costs. This article reviews the results of research to date.

Keywords oral care, supportive therapy, perioperative oral management, economic benefit

1. Introduction

Dentistry was originally focused on the treatment of dental caries and periodontal disease. Subsequently, emphasis gradually shifted to prevention, such as the pursuit of effective methods of brushing to remove dental plaque as a method of prevention and treatment of dental caries and periodontal disease, and the development of drugs. However, these efforts were mostly limited to patients visiting dental clinics and did not encompass inpatients, patients whose general condition was deteriorating, or elderly patients with weakened immunity.

That said, cleaning of the oral cavity of inpatients has been performed by nurses for a long time as a part of physical care. At home, too, mouth cleaning called "mouth care" have been used since around the 1980s with the spread of at-home care. However, this was probably based on the same concept as that of cleaning the body of patients who could not take a bath. In addition, the act of cleaning another person's mouth was time-consuming and difficult to perform safely and reliably and was often postponed due to busy schedules and lack of personnel.

The relationship between oral bacteria and other diseases has been discussed in terms of focal infections such as palmoplantar pustulosis. Knowledge was limited: infective endocarditis was known to be associated with oral infection, and periodontitis in diabetic patients tended to become severe. A breakthrough in oral care in hospitals was made in 1999 when Yoneyama *et al*. reported that thorough oral care was effective in preventing aspiration pneumonia (*1*). Since then, the importance of oral care has been highlighted, and oral care has been recognized as an evidence-based practice (*2*).

Oral care is defined in a narrow sense as cleaning of the teeth, mouth, and dentures, and in a broader sense as the maintenance of oral functions (eating, swallowing,

chewing, speech, aesthetics, *etc*.), dental care, eating and swallowing training, and articulation training. The concept of oral care has gradually expanded, and many studies and surveys have been conducted in collaboration with various professions.

The next section will review these studies, and the latter half of this section will look at the health economics of oral care, which have seldom been discussed until now.

2. What are the benefits of oral care?

2.1. Prevention of aspiration pneumonia and ventilator-associated pneumonia

The number of deaths from pneumonia decreased with the introduction of penicillin and other antimicrobials.

However, the number of deaths began to increase in the 1980s with the aging of the Japanese population, and according to the Vital Statistics of the Ministry of Health, Labor, and Welfare (MHLW), pneumonia, including aspiration pneumonia, surpassed stroke as the third leading cause of death in 2011 (*3*).

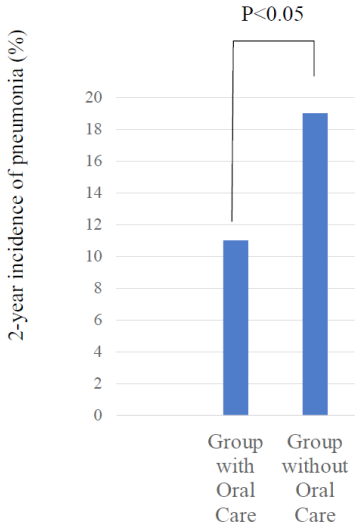

Figure 1. Comparison of the 2-year incidence of pneumonia between the oral care group and the control group. One group (oral care group) was visited once a week by a dentist or dental hygienist for professional oral cleaning, while the other group (control group) received conventional care. Results indicated that the incidence of pneumonia decreased by about 40%.

Oral care is known to facilitate the prevention of aspiration pneumonia in the elderly. In 1999, Yoneyama *et al*. reported on the potential of oral care to prevent aspiration pneumonia in a randomized controlled trial (RCT) in which residents of a special nursing home were randomly assigned to two groups (*1,4*). One group (oral care group) was visited once a week by a dentist or dental hygienist for professional oral cleaning, while the other group (control group) received conventional care. Results indicated that the incidence of pneumonia decreased by about 40% (Figure 1, Table 1). However, the problem is that no meta-analysis yielding reliable evidence has been conducted since that study.

There is a large body of literature suggesting that oral care is effective in the prevention of ventilator-associated pneumonia (VAP). In patients undergoing cardiac surgery, oral rinsing with chlorhexidine has been reported to reduce the incidence of VAP by a factor of 0.56 (95% confidence interval 0.41-0.77) compared to no rinsing (*5*). A study of 12 RCTs by Labeau *et al*. found a similar reduction in the incidence of VAP of 0.72-fold (95% CI 0.55-0.94) (*6*). That said, the effectiveness of rinsing with povidone-iodine and the effectiveness of oral cleaning with a toothbrush at preventing VAP have not been sufficiently proven. The problem is that a chlorhexidine concentration of 0.12% to 0.2% is used for oral rinsing, which is not a concentration that can be used in Japan. The cases of anaphylactic shock caused by chlorhexidine gluconate have been reported (*7*), and thus the undiluted concentration of chlorhexidine gluconate in mouthwashes is limited to 0.05%.

The current authors have been teaching about oral care in the ICU of a hospital in Vietnam, and the incidence of VAP has been successfully reduced. In addition, the hospital is also actively engaged in nosocomial infection control, teaching basic countermeasures, providing VAP care bundles, and conducting training in VAP countermeasures. Therefore, the results of these measures may have also contributed to the decrease in the incidence of VAP, and the results of oral care alone cannot be adequately evaluated (Data not shown)

As described above, oral care is thought to play a major role in the prevention of respiratory diseases, but dentistry cannot be said to play an active role in this area. The creation of a social system in which dentistry

Table 1. Comparison of the number of fever cases, pneumonia cases, and pneumonia deaths between the oral care group and the control group

Group	Number of patients	Age Mean ± SD	Male/Female	Baseline ADLs Mean ± SD	Baseline MMSE Mean ± SD	Number of fever cases (%)	Number of pneumonia cases (%)	Deaths due to pneumonia (%)
Oral Care	184	82.0 ± 7.8	36/148	16.3 ± 6.5	13.6 ± 6.9	27** (15)	21* (11)	14** (7)
No Oral Care	182	82.1 ± 7.5	37/145	16.2 ± 6.7	13.9 ± 6.9	54 (29)	34 (19)	30 (16)

MMSE: Mini-Mental State Examination. There were significantly fewer cases of fever and pneumonia in the oral care group than in the control group. There were also significantly fewer deaths from pneumonia. *$p < 0.05$ and **$p < 0.01$ in a test of significant differences between the oral care group and control group.

can play an active role will lead to improvement in the effectiveness of oral care and societal recognition.

2.2. Relationship between diabetes and periodontal disease

Many diabetics have long been known to suffer from periodontal disease, but the mutual causal relationship between periodontal disease and diabetes has become increasingly apparent in recent years.

Watanabe *et al*. reported that oral administration of *Porphyromonas gingivalis* for 22 weeks induced prediabetes in mice with chronic periodontitis (*8*) and that *P. gingivalis* was detected in pancreatic islets (*9*).

An excellent observational study, the Hisayama Study, had all examinees undergo a fasting 75 g oral glucose tolerance test (OGTT). A report on the relationship between the severity of periodontal disease and glucose intolerance using probing pocket depth (PPD) as an index to analyze the relationship between the severity of periodontal disease and the occurrence of glucose intolerance 10 years later (*10*). Results indicated that the odds ratio (OR) for the development of glucose intolerance 10 years later was two to three times higher in the group with severe periodontal disease than in the group with mild periodontal disease. This suggested that the presence of severe periodontal disease may affect the development of glucose intolerance and diabetes itself, possibly because the inflammatory response caused by long-term periodontal disease may affect insulin resistance and insulin secretion itself. However, these studies were all observational studies, and the effect of periodontal disease treatment on the risk of developing diabetes awaits further verification through interventional studies.

Another topic to consider is the relationship between periodontal disease and the treatment of diabetes mellitus. In the Hiroshima Study, high levels of sensitive C-reactive protein (hsCRP) decreased from > 0.25 mg/dL to <0.15 mg/dL and HbA1c improved from > 8.5% to about 6.5% in the high hsCRP group (> 0.05 mg/dL: severe periodontitis group) after 6 months of periodontal treatment (*11*). In contrast, the hsCRP level did not decrease in the low hsCRP group (less than 0.05 mg/dL: group of patients with mild periodontitis) even after 3 months of periodontal treatment, and HbA1c did not change significantly from less than 7% at the time of initial treatment. The same trend was also observed in the high hsCRP group and the low hsCRP group, which received only basic treatment without antimicrobials to treat periodontal disease.

Although meta-analyses have noted improvement in HbA1c, the following problems have been cited: few studies were analyzed, the sample size was too small, comparisons are difficult because of the wide range of periodontal disease and diabetes treatments when the study was conducted, and the alleviation of periodontal disease by periodontal treatment is unclear in some studies. In addition, studies have pointed out that the effects of infections other than those in the oral cavity should be considered when antimicrobials are administered systemically. However, there is a group of patients whose diabetes was alleviated by periodontal treatment according to a systematic review and the results of interventional studies (*12,13*) in Chinese patients, who are similar in size to Japanese patients. In the future, interventional studies should be conducted with a standardized protocol.

In 2018, the American Academy of Periodontology (AAP) and European Federation of Periodontology (EFP) periodontal classifications were revised for the first time in 19 years. The classification aimed to provide a "current assessment (severity, extent, complexity)" of periodontal disease through stage classification and to estimate "future projections (future risk, systemic involvement)" through grading. In addition to the degree of alveolar bone resorption and the depth of periodontal pockets as the main indices, the grading system included HbA1c as a grade modifier and hsCRP as a reference finding. The background for the inclusion of diabetes in the grading system is a US retrospective observational cohort study of more than 300,000 people, which indicated that the medical costs and hospitalizations for type 2 diabetes were significantly lower by about 40% in the group that visited the dentist four or more times a year than in the group that visited the dentist one to three times a year (*14*).

Although periodontal disease was once regarded as an inflammatory disease confined to the periodontium, it is now attracting attention as an "oral systemic link," that is, as a disease associated with systemic diseases.

2.3. Cancer treatment and oral care

During cancer treatment, various adverse events originating in the oral cavity occur at a high frequency, not only increasing patient suffering but also adversely affecting cancer treatment itself.

When a patient is immunosuppressed due to the use of anticancer drugs, dental infections can become severe and progress to systemic infections, which can have a significant impact on prognosis, including death. In addition, a study has reported that the risk of oral infections is increased not only by common bacteria but also by fungi and herpes viruses (*15-18*).

In radiotherapy, oral mucositis is inevitable when the oral cavity is included in the radiation field, and the degree of oral mucositis tends to be more severe and prolonged than mucositis caused by drug therapy. Oral mucositis not only causes pain and distress to the patient, but also, in severe cases, can prevent oral intake, leading to malnutrition and dehydration, and can cause systemic infections such as sepsis from secondary infections at the ulcer site (*19-22*). In addition to adversely affecting

the cancer treatment itself, it also adversely affects the patient's everyday life, which involves eating by mouth. The long-term use of bone-modifying drugs such as bisphosphonates and anti-RANKL antibodies, which are used to prevent fractures and alleviate symptoms in patients with bone metastases from cancer, as well as molecularly targeted drugs that inhibit angiogenesis, has been reported to cause medication-related osteonecrosis of the jaw (MRONJ). In a systematic review, the incidence of bisphosphonate osteonecrosis (BON) was 6.1% in all studies, 13.3% in studies with a follow-up, 0.7% in studies without a documented follow-up, and 1.2% in epidemiological studies (*23*).

Osteoradionecrosis (ORN) can easily occur within the radiation field due to invasive surgery such as tooth extraction, tooth-derived infection, or mucosal damage from ill-fitting dentures. In a systematic review of the prevalence of ORN in patients with head and neck cancers, the weighted prevalence of ORN was 7.4% for conventional radiation therapy, 5.1% for intensity-modulated radiation therapy (IMRT), 6.8% for chemoradiation therapy, and 5.3% for intra-tissue radiation therapy (*24*). Moreover, the oral cavity after radiation therapy is associated with decreased saliva. Furthermore, the oral cavity after radiotherapy is more susceptible to caries and more prone tooth loss due to the effects of treatment, such as decreased saliva production and qualitative changes in the oral microbiota (*25*).

Oral management of dental infections should consider the expected degree and duration of myelosuppression, and patients should be examined and treated for the presence of a focus of infection in the oral cavity to the extent that the situation permits before the start of cancer treatment. Moreover, the risk of infection should be controlled during the period of myelosuppression by oral cleaning with a focus on brushing. Periodontal disease has a high risk of causing acute infection during cancer treatment and must be evaluated and managed before treatment begins. These oral management practices have been found to be effective in preventing systemic complications during cancer treatment (*26-29*).

The total incidence of ORN after tooth extraction in the irradiated field was 7%, and the highest risk was observed in the extraction of mandibular molars in the irradiated field in patients with radiation doses exceeding 60 Gy (*30*). This is not only because the act of tooth extraction is bad, but because the patient has chronic inflammation that requires tooth extraction. In other words, since the risk of ORN remains the same even many years after irradiation, preventive measures are important. Teeth with a poor prognosis in the irradiation field should be extracted or treated appropriately at least 2 weeks before the start of treatment. Continuous dental and oral management is also important to avoid tooth extraction as much as possible (*31-33*).

Similarly, MRONJ is not only caused by tooth extraction, but by chronic inflammation that is left untreated. MRONJ is not incurable, and early conservative treatment can alleviate symptoms and inhibit disease progression. A study has proposed that the prodromal stage of MRONJ, when the mucosa is destroyed and the sequestrum is exposed, be called "stage 0," and the study contended that treatment in this stage may improve prognosis (*34*).

For oral management of oral mucositis, there are no preventive medicines or treatments that can completely suppress the onset of the disease. Symptomatic treatment is mainly focused on alleviating pain and other symptoms, reducing the risk of secondary infection, and promoting healing. During cancer treatment, the secretion of saliva decreases, causing dryness in the mouth, and nausea and fatigue make good oral hygiene difficult. However, , the symptoms of mucositis can be alleviated, the severity of the disease can be reduced, and the duration of the disease can be shortened by providing appropriate oral hygiene guidance tailored to the situation and making efforts to keep the oral cavity clean and moist (*35-38*).

2.4. Relationship between oral microbiota and cancer

Research on oral microflora and carcinogenesis is also progressing. In gastrointestinal cancers, *Helicobacter pylori* eradication has been found to reduce the risk of developing gastric cancer, and in colorectal cancers (CRC), many studies have reported on the relationship between *Fusobacterium nucleatum*, a type of periodontal bacterium, and colon cancer. However, the pathway of *F. nucleatum* infection in colon cancer tissues was unknown. Fourteen patients (10 males and 4 females, mean age: 69.4 years) with no history of antimicrobial use within one month were randomly selected from among 84 patients diagnosed with CRC according to colonoscopy. CRC tissues and saliva samples were collected via endoscopy and cultured using Fusobacterium selective medium. A total of 1,351 colonies were isolated and 361 isolates of *F. nucleatum* were detected by a specific primer polymerase chain reaction (PCR). As a result, *F. nucleatum* was detected in both colon cancer tissues and saliva in eight cases. When *F. nucleatum* isolated from these eight cases was analyzed at the strain level using arbitrarily primed PCR (AP-PCR), the same strain was detected in both colon cancer tissue and saliva in six cases (*39*).

A study performed a genetic analysis and comparison of the bacterial flora in 206 saliva and stool samples (103 saliva samples and 103 stool samples) collected from 52 CRC patients and 51 healthy controls. Results indicated that the oral and intestinal microflora contained oral indigenous bacteria (*Peptostreptococcus stomatis*, *Streptococcus anginosus*, *S. koreensis*, and *Solobacterium moorei*) that were suggested to be involved in carcinogenesis and cancer progression. These bacterial species were more prevalent in both

saliva and stool samples from the CRC group than from the control group. *S. moorei* and *P. stomatis* were present in relatively high amounts not only in stool but also in saliva, suggesting that they may originate from the oral cavity. *S. moorei* was detected in significantly higher amounts in both saliva and stool samples from patients with advanced stage CRC than from patients with early-stage CRC (*40*).

High concentrations of periodontal bacteria as well as *S. anginosus* were detected in cancerous tissues from patients with esophageal cancer, and the characteristics of oral bacterial flora and periodontal disease status were investigated for the relationship between oral infectious bacteria and esophageal cancer (*41*). Samples of subgingival dental plaque and unstimulated saliva were collected to evaluate the prevalence and abundance of oral bacteria. In the esophageal cancer group, the prevalence of all bacteria except *F. nucleatum* in dental plaque, the prevalence of *Aggregatibacter actinomycetemcomitans* in saliva, and the prevalence of all bacteria except *F. nucleatum* and the prevalence of all bacteria except *F. nucleatum* and *Prevotella intermedia* in dental plaque, the prevalence of *A. actinomycetemcomitans* in saliva, and the prevalence of *A. actinomycetemcomitans* and *S. anginosus* in dental plaque were significantly higher. *P. gingivalis* and *Aggregatibacter* have also been reported to be associated with a higher risk of pancreatic cancer (*42*).

In the future, examining oral flora through saliva tests and combining those results with responses to questionnaires on lifestyle may lead to the detection, risk detection, and prevention of cancer.

2.5. Cardiovascular disease and oral care

Since Mattila *et al*. reported that oral health was associated with the occurrence of myocardial infarction (*43*) in 1989, the relationship between periodontal disease and ischemic heart disease has attracted attention, but epidemiological studies to date have not necessarily reached a consensus on the existence of a causal relationship. Senba *et al*. conducted a large-scale study in Japan and found that the proportion of patients with periodontal disease who had coronary heart disease was significantly higher than that of those without periodontal disease (male: OR = 1.51, 95% CI: 0.90-2.52, female: OR = 1.48, 95% CI: 0.95 to 2.32) (*44*).

Periodontal disease has been associated with an increased prevalence of ischemic heart disease and associated mortality. In addition, periodontal disease affects systemic inflammation and vascular endothelial cell function. However, designing a study that excludes all confounding factors that may affect both is extremely difficult, and there is insufficient evidence to indicate its association with the onset or progression of ischemic heart disease.

However, recent studies have found that the relative risk of cardiovascular disease increases in patients with periodontal disease when the target population is limited to those under 65 years of age and that periodontal disease associated with systemic bacterial infection increases the risk of coronary artery disease (*45,46*). These findings suggest the need for well-designed follow-up and intervention studies that take these factors into account in the future.

2.6. Cerebrovascular disease and oral care

Cerebrovascular disease is the second leading cause of Japanese people requiring nursing care. Although the percentage of cerebrovascular disease has been declining in past Basic National Life Surveys, it still accounts for 16.6% in the latest figures for 2019 (*47*). Cerebrovascular disease causes motor impairment not only in the limbs but also in the orofacial region, which in turn causes deterioration in oral health. In addition, lifestyle habits that may cause cerebrovascular disease may also worsen oral health, suggesting the existence of common risk factors that affect both. Thus, oral health status and cerebrovascular disease are closely related to each other, and many studies have examined both.

In recent years, research has been focused on *S. mutans*. Cnm-positive *S. mutans*, which express the highly collagen-binding protein Cnm, is known to be involved in intracerebral hemorrhage, and about 20% of the entire population is said to have Cnm-positive *S. mutans*. When *S. mutans* was administered to stroke-prone spontaneously hypertensive rats, intracerebral hemorrhage was extensive, and streptococci infiltrated the extravascular lumen of blood vessels (*48*). Among the four disease types (hypertensive cerebral hemorrhage, lacunar infarction, cardiogenic cerebral embolism, and atherothrombotic cerebral infarction), the rate of detection was highest in cases of hypertensive cerebral hemorrhage, reaching 26%, and the OR for the other disease types was 5.66 (95% CI: 1.34 -23.9) (*49*). In addition, Cnm-positive *S. mutans* carriers had a higher incidence of intracerebral microhemorrhage, including microhemorrhages deep in the brain, suggesting that microhemorrhage has a negative impact on the brain (*50*).

However, studies have not sufficiently demonstrated that treatment of periodontal disease reduces cerebrovascular disease, and the causal relationship is not clear. As was mentioned earlier, designing a trial that excludes all possible confounding factors that may affect both the oral environment and stroke is difficult, making evaluation very difficult as well.

2.7. Rheumatoid arthritis and oral care

A study has suggested that rheumatoid arthritis and periodontitis exist in a mutually causal relationship (*51*). Rheumatoid arthritis (RA) predisposes patients to periodontitis. The aforementioned study investigated

the relationship between rheumatoid arthritis and periodontitis. In addition, Sjogren's syndrome and poor dental behavior can lead to tooth loss.

In contrast, other studies have suggested that periodontitis itself can be an aggravating factor for RA through periodontopathogenic bacteria. *P. gingivalis*, a commensal oral bacterium and one of the causes of periodontal disease, secretes an enzyme (PPAD) that functions similarly to peptidylarginine deiminase (PAD) in the body. Since periodontal patients routinely suffer from bacteremia, not only does *P. gingivalis* invade the synovial fluid, but citrullinated PPAD also invades the synovial fluid. It is thought to cause an autoimmune reaction in the joints, resulting in exacerbation of rheumatoid arthritis (*51*).

Interventional studies have indicated the effects of periodontal treatment in patients with rheumatoid arthritis (*53-55*), and case-control studies using data from the NHANESIII, a large US health survey, have also noted a relationship between periodontal disease and rheumatoid arthritis (*56-58*).

The results of intervention studies, case-control studies, cross-sectional studies, and basic research have indicated that periodontal disease and rheumatoid arthritis are related and that prevention and treatment of periodontal disease alleviates some of the symptoms of rheumatoid arthritis. However, improvement is only one aspect of evaluation, and some studies have not described discrete improvement, so further research is needed.

2.8. Dementia, nursing care, and oral care

As the world's population ages, dementia is becoming a major problem because of the need for nursing care. Many cross-sectional studies have indicated that people with dementia have poor oral health (*59,60*). These cross-sectional studies suggested that patients had dementia, which resulted in poor oral health due to inadequate oral care. In recent years, however, a study has reported that oral health affects the subsequent development of dementia and cognitive decline (*61*).

In a study by Dominy *et al*, gingipains, which are a proteolytic enzyme produced by *P. gingivalis*, were found in the hippocampus of patients with Alzheimer's disease (AD), and inhibition of gingipains suppressed amyloid β protein (Aβ) production, reduced neuroinflammation, and inhibited hippocampal neuron loss (*62*). In addition, the study found that (*i*) *P. gingivalis* is present in saliva and cerebrospinal fluid of patients with AD, (*ii*) local injection of gingipains into the hippocampus of mice causes neurodegeneration, which is inhibited by administration of gingipain inhibitors, (*iii*) Aβ42 increases in the brains of mice infected with *P. gingivalis*, and this increase is inhibited by administration of gingipain inhibitors, (*iv*) *P. gingivalis* proliferation in the brains of mice is inhibited by administration of gingipain inhibitors, and (*v*) gingipain inhibitors do not cause drug resistance. Therefore, there are possible pathways by which these periodontal diseases lead to the onset of dementia and decline in cognitive function.

In addition, tooth loss may cause degenerative changes in cognitive areas of the brain due to decreased chewing ability and decreased stimulation of the brain by chewing (*63*). In addition, decreased chewing ability may decrease the number of raw vegetables consumed, resulting in deficiencies in nutrients such as vitamins (*64*). These vitamin and other nutritional deficiencies are risk factors for the development of dementia.

Dental treatment for the increasing number of patients with dementia requires the detection of dementia in an early stage, a response to that condition in cooperation with the family physician, and provision of appropriate oral function management depending on the patient's condition. Therefore, dentists need to improve their ability to deal with dementia.

2.9. End-stage medical care and oral care

In patients with so-called terminal cancer, the condition of the oral cavity often deteriorates due to decreased saliva secretion, resulting in damage to the oral mucosa and taste disorders, as well as dental caries, periodontal disease, and denture incompatibility (*65*). In addition to medical treatment of systemic and psychological symptoms, efforts also need to be made to maintain the basic function of eating and to prevent systemic diseases such as pneumonia, and oral care can play a role in supporting patients with cancer so that they retain their human dignity until the end.

2.10. Disaster medical care and oral care

After the Great East Japan Earthquake, the number of suspected cases of "disaster-related deaths," in which symptoms developed or worsened in the wake of a disaster leading to death, increased (*66*). Of the 615 additional recipients of disaster condolence money compiled by the city of Kobe by January 1996, one year after the Great Hanshin-Awaji Earthquake, 89.6% were age 60 or older. By cause of death, 37.9% had cardiovascular disease (28.8% had heart disease and 9.1% had brain disease), 35.0% had respiratory disease (26.2% had pneumonia and 8.8% had some other respiratory disease), 3.6% had gastrointestinal disease, 2.0% had hematopoietic disease, 0.7% committed suicide, and 21.0% had exacerbation of a pre-existing condition (*67*).

The reasons for the prominence of cardiovascular and respiratory diseases are the poor diet and living conditions in shelters after an earthquake, the dust, and the lowered immunity due to the prolonged stay in shelters, as well as the inability to brush one's teeth and maintain good oral hygiene due to the extreme lack of water. In addition, extreme lack of water, poor oral hygiene due to the inability to brush one's teeth,

and difficulty removing dentures at night due to lack of privacy are said to be related to the increase in oral bacteria and subclinical aspiration, in which oral bacteria are swallowed along with saliva during sleep. In addition, many patients die from aspiration pneumonia as a result of worsening of chronic diseases such as hypertension, diabetes, and cerebrovascular disease due to the difficulty in taking prescriptions and regular medications and the inability to exercise and control one's diet.

Unlike deep vein thrombosis (DVT), the recognition that aspiration pneumonia caused by inadequate oral care can directly affect life or death has not yet fully entered the consciousness of doctors, nurses, public health nurses, and other medical professionals, as well as the government. Even if it is recognized, medical personnel are busy immediately after a disaster and tend to overlook or postpone the importance of aspiration pneumonia, just like brushing one's teeth. While there are many measures to prevent pneumonia, such as pneumonia and influenza vaccinations, management of chronic diseases, and early improvement of diet and living conditions, the importance of oral care must also be recognized.

2.11. COVID-19 and oral care

The novel coronavirus (SARS-CoV-2) infects people by binding to the angiotensin-converting enzyme (ACE) 2 receptor (*68*). ACE2 is expressed predominantly in the gastrointestinal tract, kidneys, and heart. Although pneumonia is a problem with COVID-19, ACE2 is expressed less in the lungs than in the gastrointestinal tract and is more abundant in salivary glands than in the lungs (*69*).

A comparison of the sensitivity and specificity of RT-PCR at detecting the nucleic acids of SARS-CoV-2 in saliva and nasopharyngeal/nasal swabs noted no significant differences between the two, and saliva was comparable to nasopharyngeal/nasal swabs (*70,71*). Saliva is equivalent to nasopharyngeal swabs, the gold standard for virus detection, suggesting the presence of infectious viruses in saliva.

In addition, SARS-CoV-2 damages the epithelial cells and weakens the defense mechanisms of the lungs, causing secondary bacterial pneumonia due to aspiration of oral bacteria. Thorough oral hygiene is important to prevent the salivary glands from becoming reservoirs of SARS-CoV-2 and to prevent secondary bacterial pneumonia.

3. Health economics of oral care

3.1. Perioperative oral function management (POM) in Japan

In Japan, POM was added to dental insurance in the April 2012 revision of medical fees (*72*). Although the practice had been in place for some time, this was the start of a full-scale effort to provide oral care at medical facilities throughout Japan.

POM includes oral hygiene instruction, oral care (moisturizing and cleaning), removal of the tongue coating, professional oral care by a dental hygienist, treatment by a dentist to remove sources of infection (periodontal disease and tooth decay) in the teeth and mouth, and adjustment of dentures. As a result of the widespread recognition of the importance of POM, the April 2014 revision of points for coverage by medical insurance includes the following. When surgery is performed to remove a malignant tumor from the face, oral cavity, neck, chest, or abdomen or cardiac or vascular surgery (excluding that on arteries or veins) is performed under general anesthesia within one month after POM by medical doctors, additional points were assigned to surgery after POM, which led to enhanced medical and dental cooperation. As of April 2018, the scope of coverage has been expanded to include the prevention of postoperative complications such as complications caused by oral bacteria in patients with dental diseases or poor oral hygiene (surgical site infection and local infection), local infection caused by decreased immunity due to invasive surgery or drug administration, aspiration pneumonia caused by endotracheal intubation during artificial respiration, aspiration pneumonia caused by feeding dysfunction due to stroke, and infections related to postoperative nutritional disorders. The scope of coverage has been expanded to include the prevention of complications. Covered surgeries have also been expanded to include malignant tumors of the head and neck, respiratory, and digestive systems, cardiovascular surgery, orthopedic surgery such as hip replacement, organ transplantation, hematopoietic stem cell transplantation, and surgery for stroke (*73*).

3.2. Validation of POM using big data

How has including POM in the Japanese social insurance system, as was mentioned in the previous section, affected the prevention of postoperative complications? There is an interesting analysis using big data.

Kurasawa *et al*. conducted a multicenter study to investigate the risk factors for the development of pneumonia in postoperative patients and the effectiveness of POM at preventing pneumonia (*74*). Eight hospitals in the region were surveyed over a 4-year period from April 2010 to March 2014. Of 346,563 patients without pneumonia at the time of admission (sample population), 25,554 patients with cancer who underwent surgery (the target population) were selected and evaluated. The incidence of pneumonia in these patients was determined and significant predictors of pneumonia were identified using multiple logistic regression analysis. When the incidence of pneumonia before and after the introduction of POM was compared, the incidence of pneumonia

after cancer surgery decreased from 2.0% to 0.8% in the target population. Results indicated that the OR for the development of pneumonia after the introduction of POM was 0.44, indicating that the risk of pneumonia decreased.

Ishimaru *et al*. conducted a retrospective cohort study on the effectiveness with which oral care prevented the development of pneumonia in patients after surgery for a malignant tumor based on an analysis of the National Database of Health Insurance Claims and Specified Health Examinations (NDB) (*75*). They conducted a retrospective cohort study on the effectiveness with which oral care prevented the incidence of head and neck cancer, esophageal cancer, gastric cancer, CRC, lung cancer, and liver cancer from May 2012 to December 2015. Patients who underwent resection surgery for head and neck, esophageal, gastric, colorectal, lung, and liver cancers between May 2012 and December 2015 were examined. The primary outcomes were pneumonia and all-cause mortality within 30 days after surgery. Patient background served as a propensity score (PS), and background items including comorbidities that affected the intervention effect were included as covariates in the analytical model to adjust for effects in the intervention group.

In that study, the oral hygiene group was assumed to include a mixture of patients with good oral hygiene, who are thought to have a low incidence of postoperative pneumonia, and patients with poor oral hygiene, who are thought to have undergone surgery without adequate oral hygiene because of the brief period before surgery. Therefore, the influence of these factors on results needed to be examined. Of 509,179 patients studied, they reported that 81,632 (16.0%) received preoperative oral care by dentists, 15,724 (3%) developed postoperative pneumonia, and 1,734 (0.34%) died within 30 days after surgery. Preoperative oral care by dentists was significantly associated with a reduction in postoperative pneumonia (3.28 *vs*. 3.76%, risk difference: -0.48%, 95% CI: -0.64 to 0.32) and a reduction in all-cause mortality within 30 days after surgery (0.30 *vs*. 0.42%, risk difference: -0.12%, 95% CI: -0.17 to 0.07).

In other words, analyses based on big data, have demonstrated the effectiveness of preoperative oral management in cancer surgery in terms of preventing postoperative pneumonia. However, POM does not cover patients on dialysis, patients taking steroids, non-surgical heart failure, stroke, an examination for a fever of unknown origin, and confirmation before bisphosphonate administration. Therefore, evidence that these conditions can also be alleviated by POM needs to be collected.

3.3. POM systems

Although POM is practiced in general practitioners' clinics, according to data on social medical practice from June 2020, it is mostly practiced in dental departments of hospitals (*76*). A hospital would have to perform POM for a huge number of patients, and POM for all of them would take up human resources, putting pressure on other practices such as dental care.

Therefore, Sekiya *et al*. attempted to institute the practice at a hospital's perioperative medical center (*77*). The center is a one-stop department for the management of patients admitted to the hospital and who will be undergoing surgery involving general anesthesia. Such departments have now been created in many hospitals in Japan. In 2012, the year following the establishment of the department, an "oral triage" system was instituted in which dental hygienists screen the oral cavity and select patients who require preoperative oral hygiene and functional management. In other words, only those patients who need dental intervention after screening receive oral hygiene treatment under this system. The rate of dental intervention was stable at around 20%. Moreover, the system has the advantage that oral hygiene procedures can be performed by similarly trained staff in the same department at a standardized frequency and in a standardized manner. A total of 37,557 patients who underwent surgery at that hospital from April 2010 to March 2019 (2 years before and 7 years after the system implementation) were evaluated, and the sustainability and effectiveness of the system implementation were examined in 7,715 patients undergoing cancer surgery. Oral management was performed at a rate of 20% and the incidence of postoperative pneumonia was significantly reduced (adjusted OR = 0.50, $p = 0.03$), so the system successfully eliminated the reservoir of oral infections within a sufficient treatment period of two weeks. Instituted at a perioperative medical center, the system proved to be a sustainable and evolving method of perioperative oral management. This system may be a useful strategy with which to manage surgical patients with minimal human resources.

3.4. Benefits of POM in terms of reducing health care costs

Few studies have examined POM in terms of medical costs. The Japan Dental Association presented data on an interventional study at Chiba University Hospital at the 84th meeting of the Medical Insurance Committee of the Social Security Council in November 2014 as "a case study of the effects of oral function management and medical and dental collaboration functioning effectively (*78*).

Patients undergoing surgery by the Department of Oral and Maxillofacial Surgery, the Department of Gastroenterological Surgery, and the Department of Cardiovascular Surgery, patients receiving radiotherapy in the Department of Oral and Maxillofacial Surgery, and patients receiving chemotherapy for malignant tumors in the Department of Pediatrics and the Department of Hematology during the 9 years and 10 months from

January 2004 to October 2013 were included. The term "management of oral functions" here refers not only to cleaning, but also to keeping oral functions as normal as possible through specialized treatment of periodontal pockets, caries, root canals, the root apex, the jaw, the salivary glands, and other specialized areas. The "unmanaged group" is the group that received general oral care such as mouth rinsing, which was mainly performed by nurses in the past, while the "managed group" refers to the group that was examined by dentists and that received specialized oral function management performed by dentists and dental hygienists.

First, the length of hospitalization for surgical patients was as follows: 77.9 days for the managed group (n = 210) undergoing dental surgery compared to 102.4 days for the unmanaged group (n = 271), 42 days for the unmanaged group (n = 52) undergoing gastrointestinal surgery compared to 29 days for the managed group (n = 108), and 38 days for the unmanaged group (n = 53) undergoing cardiovascular surgery compared 29 days for the managed group (n = 110). Hospitalization was significantly shorter for the managed group (84.2 days, n = 55) receiving chemotherapy for a malignancy in Pediatrics compared to the unmanaged group (135.3 days, n = 64) and for the managed group (96 days, n = 103) in Hematology compared to the unmanaged group (108 days, n = 60). A significant reduction in the duration of hospitalization was observed in all departments. The effect of oral function management on the length of hospitalization for patients undergoing radiotherapy for oral malignancies was also found to be shorter in the managed group (75.2 days, n = 54) compared to the unmanaged group (84 days, n = 33), and fewer days were required for recovery in the managed group (25.6 days, n = 54) compared to the unmanaged group (31.5 days, n = 33). Fewer days were required for recovery in the managed group (25.6 days, n = 54) compared to the unmanaged group (31.5 days, n = 33). These results suggest that a significant reduction in the recovery period after the completion of radiotherapy contributes to a shorter hospitalization. The duration of postoperative antimicrobial therapy for patients with oral malignancies was significantly shorter in the managed group (n = 210, 5.6 days) than in the unmanaged group (n = 271, 9.9 days). Cardiovascular surgery is considered to have the fewest external factors such as infections affecting postoperative recovery. In In cardiovascular surgery, the duration of postoperative antimicrobial therapy was significantly shorter in the managed group (n = 110) than in the unmanaged group (n = 53) 1, 7, 14, and 21 days after surgery.

The same study also presents the medical costs of Asahi General Hospital. For a laparoscopic gastrectomy, the average hospitalization was 6.6 ± 0.94 days in the managed group (n = 20) and 11.1 ± 11.2 days in the unmanaged group (n = 58), and the average medical costs were 1,483,901 yen in the managed group (n = 20) and 1,678,465 yen in the unmanaged group (n = 58). For an open gastrectomy, the average hospitalization was 11.7 ± 10.5 days in the group with oral health care (n = 57) and 16.0 ± 16.1 days in the group without oral health care (n = 57). The average medical costs were 1,627,496 yen in the managed group (n = 57) and 1,758,704 yen in the unmanaged group (n = 57). Thus, POM significantly shortened the duration of required hospitalization and it reduced medical costs.

Sekiya *et al*. confirmed that the average treatment costs for patients who did not develop pneumonia after hospitalization were 1,295,762 (± 1,007,162) (yen/person), and the average treatment costs for patients who developed pneumonia after hospitalization were 2,962,771 (± 1,964,419) (yen/person) (*79*). Therefore, the treatment costs for patients who developed pneumonia after hospitalization were calculated to differ by 1,667,009 (yen/person). POM was estimated to have prevented pneumonia in 161 patients. The total costs would have been 268,388,449 yen (1,667,009 × 161). In contrast, POM cost 18,020,320 yen, so medical costs for 13,668 patients would have differed by an estimated 250,368,129 yen (approximately 250 million yen). These results suggest that oral function management leads to early postoperative recovery and that the effects of oral function management are not limited to mere prevention but also include therapeutic benefits and reduced medical costs.

3.5. Increased revenue to dentistry from POM

Of the 2,082 facilities belonging to the Japan Hospital Association, only 739 have dental and oral surgery. In many hospitals, dentistry has been eliminated or reduced for financial reasons. However, changes have occurred since 2012, when insurance points were assigned for POM.

One change is that hospitals are recognizing the importance of POM as a supportive care, and another is that POM is a powerful tool for dentistry to increase patients and revenue.

The current authors' department was asked to perform POM in about 5 cases per month in 2012, just after the introduction of POM. However, the department is now receiving as many as 300 requests per month as a result of the increase in the number of target diseases and awareness of POM, the establishment of the Admission and Discharge Support Center in 2017, and the simplification of procedures to request POM (Figure 2). The department receives a fee for preparation and a preoperative and postoperative reimbursement for each request, which has resulted in an increase in the number of patients seen and an increase in revenue. In addition, dentists and dental hygienists with fewer years of experience can be actively involved in the practice and task shifting.

4. The future of POM

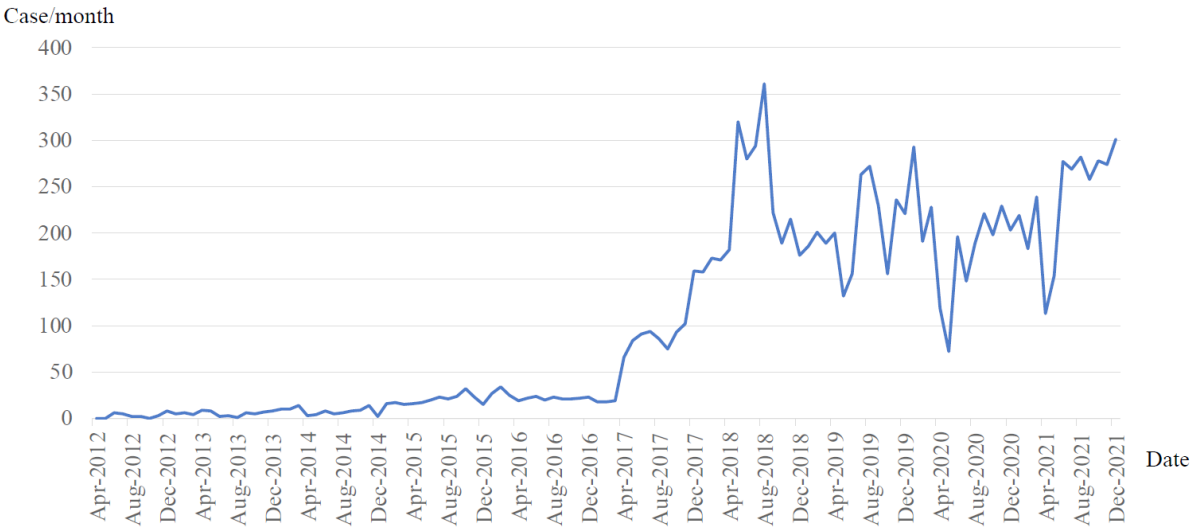

Figure 2. POM performed monthly by the Department of Oral and Maxillofacial Surgery, Center Hospital, National Center for Global Health and Medicine. The Department of Oral Surgery, National Center for Global Health and Medicine was requested to perform POM about 5 times per month in 2012, just after the introduction of POM. However, due to an increase in the number of target diseases and increased awareness of POM, as well as the creation of the Admission and Discharge Support Center in 2017 to simplify the procedures for requesting POM, as many as 300 requests are now being receiving each month (that said, the number decreased temporarily due to the COVID-19 pandemic).

As indicated in the previous section, POM has been found to have had a significant impact on health, far beyond the role it was initially envisioned to play. From a health economics perspective, POM has also been linked to reduced medical costs and increased dental revenue. Therefore, there are increasing attempts to set up oral care teams in hospitals with medical and dental facilities and to implement POM in cooperation with general practicing dentists as well as dentists in hospitals. In the future, medical reimbursement may be added to "team medicine" like palliative care, respiratory care, and pressure ulcer management.

Japan already has a superannuated population, and the number of elderly people is estimated to peak at 39 million in 2042. Therefore, the MHLW is promoting the establishment of a community-based comprehensive support system by 2025 in order to help the elderly maintain their dignity and live independently so that they can continue to live their own lives in their communities as much as possible (*80*).

What can dentistry offer? In addition to so-called dental treatment, such as the treatment of decayed teeth and periodontal disease, dentistry has a role to play in combating dementia, at-home care, delaying the need for nursing care, and nursing care facilities, but the main focus of care is to facilitate eating by mouth.

Oral functions such as chewing and swallowing, eating conditions, and eating posture can be properly evaluated by a multidisciplinary team observing care recipients at mealtime. Additional perspectives can be incorporated professionals in multiple disciplines exchanging opinions. This should enhance eating support, contribute to the intake of required nutrients, lead to weight gain, and prevent aspiration pneumonia. These attempts can be considered as part of oral care in a broader sense.

There is a movement to promote oral care as an academic discipline. The Japanese Society for Oral Care consists of professionals from various disciplines, including dentists, doctors, pharmacists, nurses, dental hygienists, speech pathologists, midwives, care workers, nutritionists, public health nurses, physical therapists, teachers, childcare workers, and home helpers, and it conducts academic research on oral health care. It also aims to promote health maintenance and publicize the results of that research. Moreover, the Society aims to make a significant contribution to improving the QOL of people by facilitating communication and by preventing aspiration pneumonia in the elderly, as well as promoting drinking, eating, and talking.

The Society devices practices, it conducts training, surveys, and research, and it facilitates the exchange of information among health, medical, and welfare professionals from many academic organizations to provide oral health care to the healthy, sick, disabled, homebound, hospitalized, infants, and elderly people.

The International Society of Oral Care was established in 2021 with the aim of contributing to oral health-related science on a global scale.

In conclusion, the field of oral health care has evolved greatly from the days of "cleaning the mouth" to the present, and new and surprising findings are successively being announced. The hope is that new findings will continue to be obtained and that practices will continue to be refined in the future.

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Review

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The clinical management of hepatocellular carcinoma worldwide: A concise review and comparison of current guidelines: 2022 update

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SUMMARY Hepatocellular carcinoma (HCC) is the fifth most common malignancy and the second leading cause of cancer-related mortality worldwide. This review is an updated version that summarizes comprehensive guidelines published from January 2001 to January 2022 worldwide with a focus on the clinical management of HCC. The electronic databases MEDLINE, the Chinese SinoMed, and the Japanese CiNii were systematically searched. A total of 22 characteristic guidelines for HCC management were ultimately included, including 1 international guideline, 11 guidelines from Asia, 5 from Europe, 4 from the America, and 1 from Australia. If guidelines were published in multiple versions, the most recent update was included, and surveillance, diagnosis, and treatment were compared. The composition of and recommendations in current guidelines on HCC varied, so these guidelines were regrouped and diagnostic and treatment algorithms were summarized graphically to provide the latest information to clinicians. The diagnostic criteria were grouped into 2 categories: a "Size-based pathway" and a "Non-size-based pathway". The treatment criteria were summarized according to different treatment algorithms, and mainstream treatment options were reviewed. Findings from comparison of current guidelines might help target and concentrate efforts to improve the clinical management of HCC. However, further studies are needed to improve the management and outcomes of HCC. More straightforward or refined guidelines would help guide doctors to make better decisions in the treatment of HCC in the future.

Keywords hepatocellular carcinoma, clinical guideline, surveillance, diagnosis, treatment

1. Introduction

As the most common primary malignancy of the liver, hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide, with an increasing incidence of approximately > 500,000 new cases per year (*1,2*). Over the past 2 decades, various studies have examined the clinical management of HCC, markedly improving treatment options, which include new drug combinations. Despite the considerable advancement that has occurred, the overall outcomes of HCC are still far from satisfactory. For better treatment of HCC, various types of guidelines and expert consensus opinions have been issued in multiple regions and subspecialties. If adequate guidelines are devised, they could serve as roadmaps for clinicians to develop individualized decision-making algorithms, improve the quality of care and patients' outcomes, and assist regional and national authorities in allocating resources (*3*).

Since 2001, when the European Association for the Study of the Liver (EASL) issued their HCC guideline, at least 20 comprehensive clinical guidelines for HCC have been published or updated, and each has its own advantages. That said, gaps in knowledge and areas of controversy regarding certain aspects of HCC management are still evident and cannot be ignored.

In a previous review, we summarized 18 comprehensive guidelines published worldwide from 2001 to 2017 with a focus on the clinical treatment of HCC; those guidelines have been significantly revised since (*4*). To provide the latest information for clinicians, the current review has summarized the current editions of those guidelines up to 2022. Twenty-two characteristic guidelines were selected, including 1 international

guideline, 11 from Asia, 5 from Europe, 4 from the America, and 1 from Australia. These characteristic guidelines have been compared and summarized in order to describe new aspects of the surveillance, diagnosis, and treatment of HCC.

2. An update to characteristic guidelines for the clinical management of HCC

Like the previous review, the current review involved a systematic search of mainstream databases in English, Chinese, and Japanese, including MEDLINE, the Chinese SinoMed (*http://www.sinomed.ac.cn/zh/*), and the Japanese CiNii (*http://ci.nii.ac.jp/*), for applicable results from January 2001 to January 2022. No language restriction was applied. Search terms (medical subject headings or keywords) included: "hepatocellular carcinoma", "guidelines/practice guidelines", "consensus", "strategy", "liver cancer", and "liver carcinoma".

Inclusion criteria were as follows: *i*) credibility, as measured by whether the guidelines were widely cited by subsequent guidelines or other publications regarding the management of HCC after the original guidelines were published; *ii*) influence, an indication that the guidelines were created with the support of government or academic/medical societies and that the guidelines attracted nationwide attention with respect to their implementation and the standard care for HCC; and *iii*) multifaceted, meaning that the guidelines included aspects of the diagnosis and treatment of HCC at a minimum. Hence, many specialized guidelines, though credible and influential, did not make the list of 22 guidelines but they are still discussed in specific subsections. If the guidelines were published in multiple versions, the most recent update was analyzed. Moreover, references listed in guidelines were manually searched for other potential sources. The title and abstract of retrieved studies were evaluated for relevance and compliance. If compliance was not clearly defined by the abstract, the full text was reviewed for further assessment.

In line with the criteria above, 22 comprehensive guidelines published between 2001 and 2022 were identified for analysis, including 1 international guideline, 11 guidelines from Asia, 5 from Europe, 4 from the America, and 1 from Australia (Table 1) (*1,5-25*). Among the 18 guidelines included in our previous review, 10 of them have been updated within the last 5 years. Besides, 5 new guidelines were included for the first time. These 22 characteristic guidelines were examined with a focus on the clinical management of HCC, and surveillance, diagnosis, and treatment in those guidelines were compared.

3. High-risk population and surveillance of HCC

Identification of the risk factors for HCC and devising of appropriate methods for surveillance of the high-risk population are crucial to early diagnosis and a better outcome. This process is usually divided into 3 parts: *i*) determining risk factors, *ii*) screening the population with risk factors for individuals who need to be monitored, and *iii*) devising the form of surveillance that yields the most benefit.

The current review found that 17 of the 22 guidelines clearly described risk factors and surveillance. The guidelines contained a lot of similar information on those topics, but there were discrepancies among guidelines due to regional differences in disease and other variables. HCC has been proven to be linked to liver disease independently, and its major risk factors can be divided into those that are cirrhosis-related and those that are non-cirrhosis-related. The former includes hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, alcoholic cirrhosis, genetic causes (hemochromatosis and tyrosinosis), nonalcoholic fatty liver disease (NAFLD), stage IV primary biliary cholangitis, alpha one antitrypsin deficiency, and other causes of cirrhosis; the latter includes being an HBV carrier with a family history of HCC, being Asian and elderly (males ≥ 40 years and females ≥ 50 years), and being an African/North American black infected with hepatitis B (*1,21*). Among these risk factors, cirrhosis caused by various etiologies is the strongest predictor of HCC, with an associated annual incidence of HCC of 1-8% (*26,27*). Hepatitis B is the leading cause of HCC in East Asia and Africa while hepatitis C is the leading cause in Western countries (*28*). In recent years, NAFLD-related HCC has attracted more attention since a growing population worldwide is estimated to have NAFLD (*29,30*).

HCC surveillance is cost-effective, especially for high-risk groups. Ultrasound (US) is the most widely recommended method of HCC detection (*1,7,22,31,32*). However, whether alpha-fetoprotein (AFP) should serve as a routine screening test for HCC is still being debated. The NCCN/AASLD recommendations suggested US surveillance with or without AFP (*22,31*). The EASL guideline described AFP as "suboptimal" as a serological test for surveillance since its levels were interfered with by viral replication and underlying liver disease, so they often do not appear abnormal in the early stages of HCC (*1*). Several studies indicated that AFP alone has limited and inconsistent sensitivity and specificity as a screening biomarker and that elevated levels of AFP may be found in < 20% of patients with early-stage HCC (*33-35*).

In contrast, some expert panels consider AFP to be a good surveillance marker due to its wide utility in diagnostic settings, where it has been studied extensively (*36*), and its role in combination with US, which can significantly maximize early detection of HCC, despite the lack of evidence concerning improvement in survival (*37,38*). In the 22 guidelines that were reviewed here, 6 recommended US for screening with AFP, 6 suggested US alone, and the others considered AFP to be optional.

Table 1. Twenty-two characteristic guidelines for the clinical management of HCC

No.	Area	Year (latest update)	Guidelines	Drafted by	Aspects covered	*Ref.*
	International					
1		2010	WGO Guideline	• World Gastroenterology Organization	D&T+E+P+S	(*5*)
	Asia					
2		2020	Pan-Asian adapted ESMO Guideline	• ESMO Asia Meeting	D&T+E+P+S+F	(*6*)
3		2017	APASL Guideline	• Asian-Pacific Association for the Study of the Liver	D&T+E+P+S	(*7*)
4		2009	AOS Guideline	• Asian Oncology Summit 2009	D&T+P+S	(*8*)
5	China	2020	CSCO Guideline	• Chinese Society of Clinical Oncology	D&T+S+F	(*9*)
6	Japan	2021	JSH Consensus Statements and Recommendations	• Japan Society of Hepatology	D&T+S	(*10*)
7		2021	J-HCC Guideline	• Group formed to establish "Guidelines for evidence-based clinical practice for the treatment of liver cancer"		
8		2019	JSH Guideline	• Japan Society of Hepatology	D&T+S	(*11*)
9	Korea	2014	Korean Guideline	• Korean Liver Cancer Study Group and National Cancer Center	D&T+E+P	(*12*)
10	Saudi Arabia	2020	Saudi Guideline	• Saudi Association for the Study of Liver diseases and Transplantation	D&T+E+P+S	(*13*)
11	India	2019	INASL Guideline	• The Indian National Association for Study of the Liver	D&T+E+P+S	(*14*)
12		2019	ICMR Consensus	• Indian Council of Medical Research	D&T+E+P+S+F	(*15*) (*16*)
	Europe					
13		2021	ESMO Guideline	• European Society for Medical Oncology	D&T+E+P+S+F	(*17*)
14		2018	EASL Guideline	• European Association for Study of the Liver, European Organization for Research and Treatment of Cancer	D&T+E+P+S	(*1*)
15	Belgium	2004	BASL Guideline	• Belgian Association for the Study of the Liver	D&T+E+P+S	(*18*)
16	Britain	2003	BSG Guideline	• British Society of Gastroenterology	D&T+E+S	(*19*)
17	Italy	2009	GOIM Guideline	• Italian Southern Oncological Group	D&T+E	(*20*)
	America					
18		2021	NCCN	• National Comprehensive Cancer Network	D&T+E+S	(*21*)
19		2018	AASLD	• American Association for the Study of Liver Disease	D&T+S	(*22*)
20		2010	NCI Guideline	• United States National Cancer Institute	D&T+E	(*23*)
21		2007	ACS Guideline	• American College of Surgeons*	D&T	(*24*)
	Oceania					
22	Australia	2020	GESA Guideline	• Gastroenterological Society of Australia	D&T+E+P+S+F	(*25*)

Abbreviations: D&T: diagnosis and treatment, E: epidemiology, P: prevention, S: surveillance, F: follow-up. *A review article published on J Am Coll Surg by the American College of Surgeons.

The usefulness of other biomarkers, including the lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) and des-gamma-carboxy prothrombin (DCP), has been studied (*39,40*). Concomitant use of these biomarkers is recommended as a regular screening method by the 2019 updated JSH Guideline (*12*). In contrast, the 2018 EASL guideline described AFP, AFP-L3 and DCP as "suboptimal in terms of cost-effectiveness for routine surveillance of early HCC". The debate goes on.

The ideal surveillance interval should be evaluated from the perspective of cost-effectiveness by considering the clinical status and available resources. Generally, the surveillance interval is 6 to 12 months for the high-risk population according to guidelines. A prospective cohort study found that patients with HBV had a better survival with a surveillance interval of 6 months than with 12 months (*41*). However, other studies have found no significant differences in survival or the rate of HCC detection with intervals of 6 and 12 months (*42,43*). Of the 22 guidelines that were reviewed here, 8 tended to recommend a surveillance interval of 6 months and 2 recommended an interval of 6 to 12 months.

The definition and description of the high-risk population varied according to the guidelines. According to the 2019 update of the JSH Guideline, individuals with a high risk of developing HCC who need to be surveilled are classified as the high-risk population and the very-high-risk population (*12*). The high-risk population includes: *i*) individuals with chronic hepatitis B, *ii*) individuals with chronic hepatitis C, and *iii*) individuals with liver cirrhosis (due to causes other than HBV or HCV). The recommended form of surveillance is US and tumor marker measurement (AFP/DCP/AFP-L3) every 6 months. The very-high-risk population includes: *i*) individuals with hepatitis B-related liver cirrhosis and *ii*) individuals with hepatitis C-related liver cirrhosis. The surveillance protocol for those individuals is US and tumor marker measurement (AFP/DCP/AFP-L3) every 3-4 months, with alternative dynamic CT/MRI especially for those who cannot readily undergo US due to liver atrophy, severe obesity, or post-operative deformity.

The NCCN Guideline, INASL Guideline, and EASL Guideline classified patients who are at risk of developing HCC into a group with cirrhosis and a group without cirrhosis (*1,15,31*). The EASL/INASL/Saudi Guideline also took liver function (Child-Pugh) into consideration for the group with cirrhosis. Those 2 guidelines stressed that patients on the waiting list for liver transplantation (LT), regardless of their liver function status, should be screened for HCC in order to detect tumor progression (whether it exceeds conventional criteria) and to help prioritize transplantation. The NCCN Guideline did not recommend surveilling the group without cirrhosis for chronic HCV with advanced fibrosis, but the INASL Guideline and EASL Guideline do recommend surveilling that group. Similarly, the Saudi Guideline suggests surveillance of all cirrhotic patients, but it also stated that there was insufficient evidence to advise surveillance for patients with chronic hepatitis C but without cirrhosis. The WGO Guideline divided the criteria for HCC screening into 3 parts: hepatitis B carriers, cirrhosis not due to hepatitis B, and general patients. General patients referred to patients who were previously eligible for HCC screening and included cirrhotic patients who were successfully treated for chronic viral hepatitis. The AASLD guideline grouped together patients who would benefit from surveillance and patients in whom there was no evidence of a benefit from surveillance. The remaining guidelines did not divide the population who needed to be surveilled into smaller groups.

Obviously, there are regional differences in epidemiology that might change with time. For example, the importance of HBV as a cause of HCC is declining, but the importance of NAFLD and nonalcoholic steatohepatitis (NASH) as risk factors for HCC is on the rise (*29,30*). Future guidelines should pay close attention to these changes, and each country could devise its own method of HCC surveillance depending on local epidemiology. The current comparison of guidelines could help organizations devise a meaningful and easily understood form of surveillance.

4. Diagnostic criteria for HCC according to characteristic guidelines worldwide

The diagnosis of HCC is generally based on a combination of clinical and laboratory features as well as radiographic and histopathologic presentation. The diagnostic algorithms in the 22 guidelines that were reviewed here have been summarized in a flowchart (Figure 1). Although there were differences among these guidelines, the final diagnosis of HCC was based on imaging techniques or biopsy. With the recent advancement of various types of imaging techniques even for "indeterminate lesions" as described by the AASLD guideline, biopsy is only suggested in selected cases.

In general, if US reveals a nodule or mass in an at-risk individual, there are mainly 2 pathways for diagnosis of HCC according to current guidelines. For simplicity, these 2 categories have been designated as the "Size-based pathway" and the "Non-size-based pathway".

4.1. Size-based pathway for HCC diagnosis

The "Size-based pathway" for diagnosis of HCC starts with tumor size (generally larger or smaller than 1 cm. In the latest CSCO guideline, this was subdivided into < 1 cm, 1-2 cm, and > 2 cm). HCC nodules with a small diameter are difficult to distinguish from cirrhotic nodules, and previous studies found that small nodules, and especially those with a diameter < 1 cm, were unlikely to be HCC nodules (*44,45*). This is the main

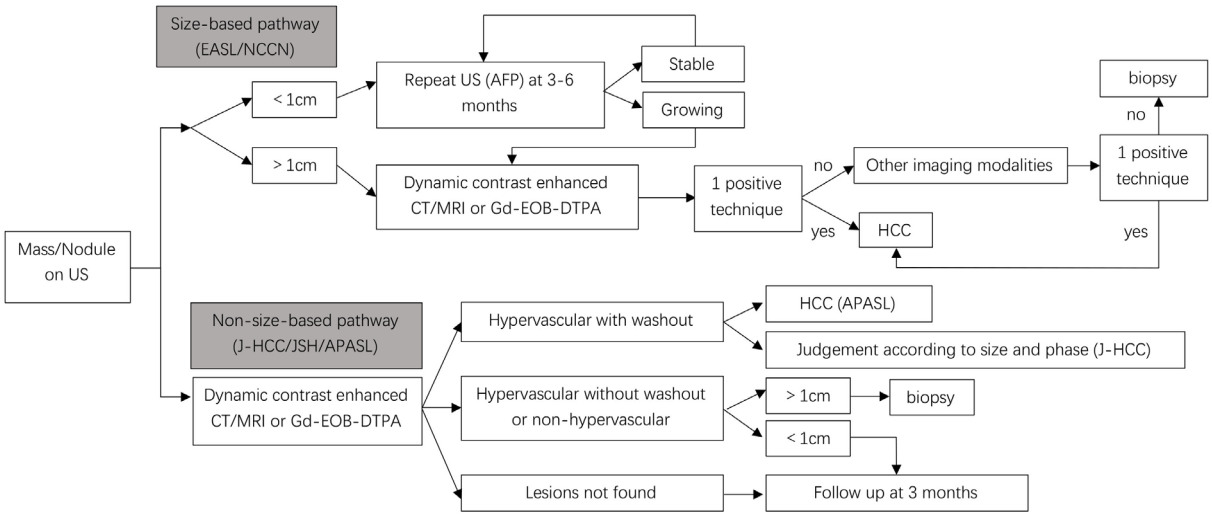

Figure 1. The diagnostic algorithm for hepatocellular carcinoma in current guidelines. The diagnostic criteria were grouped into 2 categories of a "Size-based pathway" and a "Non-size-based pathway". *Abbreviations*: EASL: European Association for the Study of the Liver; NCCN: National Comprehensive Cancer Network; JSH: Japan Society of Hepatology; APASL: Asian-Pacific Association for the Study of the Liver; US: ultrasonography; AFP: alpha-fetoprotein; Gd-EOB-DTPA: gadolinium ethoxybenzyl-diethylenetriaminepentaacetic acid.

reason why the AASLD/EASL Guideline recommend that those patients be closely followed-up with US every 3 or 4 months. The NCCN Guideline recommends repeat US plus AFP every 3 to 6 months. Kim *et al*. argued that hyper-intensity on both T2 and diffusion-weighted images is helpful in the diagnosis of hypervascular HCC nodules smaller than 1 cm in diameter (*46*). The Korean Guideline established stricter criteria for diagnosis of HCC nodules < 1 cm. Nodule size according to 2 or more imaging modalities is a typical hallmark of HCC in combination with elevated serum AFP and absence of hepatitis activity (*13*). The technique that first detected nodules should be performed again 3 to 6 months later. If the nodules remain the same size, a close follow-up should be performed. Otherwise, special attention should be paid to the growing nodule size.

Liver nodules larger than 1 cm in size should be evaluated with dynamic contrast-enhanced CT/MRI or Gd-EOB-DTPA MRI. Evidence of one or more radiological hallmarks of HCC, arterial hypervascularity, and venous/late-phase washout is considered indicative of HCC. A non-biopsy diagnosis based on a nodule size > 1 cm has been updated several times. According to the 2002 version of the EASL Guideline, a positive imaging finding plus AFP levels > 400 ng/mL can lead to a diagnosis of HCC when nodules > 2 cm (*47*). In 2005, the AASLD Guideline excluded AFP from the diagnostic algorithm and recommended radiological hallmarks according to 2 imaging techniques to diagnose HCC nodules between 1 and 2 cm in size. For nodules > 2 cm, a hallmark detected by 1 imaging technique would be sufficient. The 2010 version of the AASLD Guideline updated the criterion: an imaging technique revealing a radiological hallmark of HCC is sufficient for diagnosis of tumors 1-2 cm in diameter. The 2018 EASL guideline also stated that non-invasive criteria can apply to nodules over 1 cm in diameter. This indicates that, as imaging techniques such as gadolinium-based MRI advance, smaller nodules are diagnosed more accurately through non-invasive approaches.

Needle biopsy of a suspicious liver lesion could guide management for patients who do not exhibit a classic imaging presentation and serology, although it is not recommended generally because of the possibility of tumor dissemination outside the liver. The overall incidence of needle-tract tumor seeding following biopsy of HCC is 0.9-2.7% per year (*48*). Moreover, the NCCN Guideline stresses that a negative biopsy result does not rule out HCC if a nodule or mass has increased in size.

4.2. Non-size-based pathway for HCC diagnosis

In the "Non-size-based pathway", patients will be scheduled for dynamic imaging regardless of tumor size. All of the guidelines indicate that HCC can be definitively diagnosed when dynamic CT/MRI reveals intense arterial uptake followed by a "washout" of contrast. Moreover, ever since the 2014 JSH Guideline included Gd-EOB-DTPA MRI (gadoxetic acid disodium, a liver-specific contrast agent) as a tool for first-line surveillance and diagnosis of HCC, multiple guidelines have cited gadoxetic acid-enhanced MRI as a first-line imaging technique. In principle, this contrast agent is specifically absorbed by normal hepatocytes, resulting in contrast enhancement. Therefore, HCC nodules lacking normal hepatocytes are hypo-intense, and this difference can help distinguish tumors from non-tumorous ("normal") nodules (*49*).

When an advanced imaging technique reveals only hypervascularity with no washout, the diagnostic algorithms differ among the guidelines that were reviewed here. Recommendations in the J-HCC Guideline depend on tumor size. If the tumor diameter is larger than 1 cm, other optional examinations should be performed, including Gd-EOB-DTPA-MRI, SPIO-MRI, CEUS, CTA, and biopsy. A 3-month follow-up is recommended for patients with a tumor < 1 cm in diameter and elevated levels of tumor markers while dynamic CT/MRI is recommended for a larger tumor. In the JSH Guideline, a tumor that is hypo-intense during the hepatobiliary phase of GD-EOB-DTPA-MRI can be diagnosed as HCC provided that cavernous hemangioma is first ruled out by other modalities. A biopsy is necessary if the tumor is iso-intense or hyper-intense in the hepatobiliary phase. According to the APASL Guideline, a lesion can be diagnosed as HCC when high SPIO-enhanced MRI signals or a defect in the Kupffer phase of Sonazoid-enhanced US is evident (*7*). However, the APASL Guideline only recommends a close follow-up instead of a biopsy for patients with intense uptake in SPIO-MRI or CEUS.

There is still a lack of a broad consensus on the most appropriate diagnostic algorithm to use when initial dynamic CT/MRI reveals a hypo-vascular mass in the arterial phase. The updated J-HCC Guideline suggested that an optional examination should be undergone by patients with a tumor larger than 1.5 cm and it suggested a follow-up of 3 months for those with a tumor smaller than 1.5 cm. The JSH Guideline stresses presentation in the hepatobiliary phase of GD- EBO-DTPA-MRI. If hypo-intensity is present, Sonazoid CEUS is recommended; otherwise, follow-up should be continued. The APASL Guideline tended to recommend SPIO-enhanced MRI or Sonazoid CEUS for those patients. A close follow-up was recommended in the event of a negative imaging finding.

5. Treatment criteria for HCC according to characteristic guidelines worldwide

The treatment algorithm for HCC is constantly changing as the criteria for hepatic resection expand, locoregional therapies advance, novel targeted systemic therapies are introduced, techniques for internal and external radiation therapy improve, and the possibility of receiving a transplant increases. However, long-term outcomes of HCC depend on both the medical complexity of HCC (involving multiple confounding factors: tumor heterogeneity, liver function and performance status) as well as the choice of an appropriate treatment, posing a challenge for both patients and clinicians.

An important aim of clinical guidelines is to feature up-to-date, specific, quality evidence to help clinicians select the most appropriate treatment. Compared to our previous review (*4*), the updated guidelines include those by the NCCN (2021), AASLD (2018), CSCO (2020), JSH (2019), INASL (2019), ESMO (2021), EASL (2018), and Saudi Arabia (2020). New guidelines published between 2017 and 2022 added to the current review are the Pan-Asian adapted ESMO (2020), ICMR (2019), and GESA (2020) guidelines. The treatment algorithms in these updated guidelines and in other guidelines were reviewed here and are discussed in terms of surgical and non-surgical approaches.

Different staging systems are used to select the best treatment option for patients, which is the main difference between the guidelines. Typically, Japanese guidelines (J-HCC/JSH guidelines) use the Child-Pugh score for the very first evaluation for treatment options, while AASLD, ESMO, EASL, Saudi, and INASL guidelines involve an initial evaluation based on BCLC staging system. A flowchart has been included here to provide an overview of the diverse staging systems (Figure 2).

5.1. Surgical approaches

Basically, all of the staging systems focus on the determination of tumor resectability, since surgery is still recommended as the best treatment option for selected patients, with a 5-year survival rate as high as 80% (*1*). Initially, tumor resectability should be evaluated based on parameters like liver function, the presence of portal hypertension, tumor location, and the presence of extrahepatic metastases. If a tumor is resectable, resection or radiofrequency ablation (RFA) (for a tumor with a small diameter) is recommended. LT should also be considered for patients with cancer that is Child-Pugh class C. LT has become the first-line treatment for patients with unresectable tumors that nonetheless meet the Milan or United Network for Organ Sharing (UNOS) criteria. If those patients are not optimal candidates for transplantation, the choice of locoregional therapy, sorafenib, or supportive care depends on individual circumstances (including tumor location, liver function, and institutional capabilities). Moreover, the NCCN Guideline added that transplantation can be considered or recommended for those patients who initially failed to meet the Milan criterion but who received successful downstaging therapy.

The BCLC staging system takes tumor stage, liver function, and physical status into account, and this system had been widely adopted for HCC staging and treatment (*50*). Moreover, the BCLC staging system is the only staging system that assigns treatment strategies based on specific prognostic subclasses, an approach that has proven effective (*51*). The spectrum of treatment options with curative intent may be a subject of some controversy, but it generally consists of liver resection, LT, and ablation. Patients with stage 0 or stage A liver cancer may have a 5-year survival rate of 40-70% after treatment with curative intent. Liver resection still

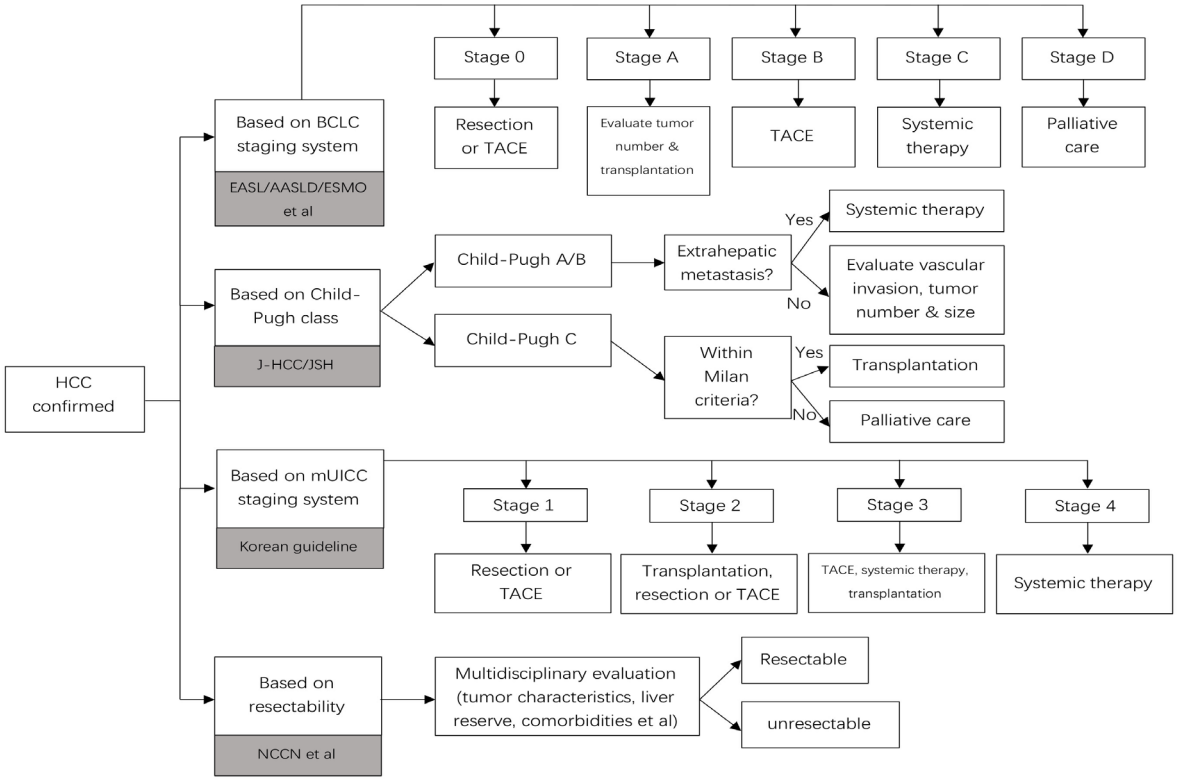

Figure 2. The treatment algorithm for hepatocellular carcinoma in current guidelines. Four clinical pathways based on different staging systems are shown. *Abbreviations*: BCLC staging system: Barcelona Clinic Liver Cancer staging system; mUICC staging system: modified Union of International Cancer Control staging system; EASL: European Association for the Study of the Liver; AASLD: American Association for the Study of Liver Disease; ESMO: European Society for Medical Oncology; JSH: Japan Society of Hepatology; NCCN: National Comprehensive Cancer Network; TACE: transarterial chemoembolization.

remains the mainstay of HCC treatment in non-cirrhotic patients or in selected cirrhotic patients with a single lesion. The AASLD Guideline repeatedly stresses the usefulness of measuring portal pressure in predicting patient outcomes and optimizing patient selection for liver resection; the usefulness of this index has also been verified in Japan (*52*). The AASLD Guideline also indicated that patients with portal hypertension or multiple lesions could receive a survival benefit from resection. The algorithm in the ESMO Guideline excluded hypertension and it expanded the criteria for clinical decision-making with regard to resection (*17*).

LT is indicated for patients with BCLC stage A cancer meeting the Milan criterion (solitary HCC nodule < 5 cm in size or fewer than 3 nodules, none larger than 3 cm in diameter). Patients with cancer meeting the Milan criterion had a 5-year overall survival rate of 65-78% after LT, which is why this criterion was integrated into the BCLC staging system (*53*). This strict criterion also has certain limitations. According to the ESMO Guideline, LT is ruled out for patients with cancer meeting the Milan criterion and poor liver function (Child-Pugh class C), who would be classed as BCLC stage D. The University of California San Francisco (UCSF) criterion extends beyond the Milan criterion, and the UCSF criterion results in comparable outcomes according to the INASL Guideline (*54*). On the whole, primary recommendations for LT have remained the same.

The 2014 Korean Guideline adopted the mUICC as its primary staging system. Its recommendations for first-line treatment are based on mUICC staging system, but its algorithm only applied to patients with Child-Pugh class A HCC, no portal hypertension, and an Eastern Cooperative Oncology Group (ECOG) performance status of 0-1. The basic criteria of the mUICC staging system include: *i*) the number of tumors, *ii*) the diameter of the largest tumor, and *iii*) vascular or bile duct invasion. The best treatment option for a stage I tumor (single/≤ 2 cm/VI-) is resection or RFA. There are 3 options for Stage II cancer: *i*) resection or RFA (tumor size ≤ 3 cm) is recommended for treatment of stage IIa cancer (single/> 2 cm/VI-); *ii*) LT (for cancer meeting the Milan criterion) is the first option for treatment of stage IIb cancer (multiple/≤ 2 cm/VI-) and transarterial chemoembolization (TACE) or RFA is an alternative when there are more than 3 nodules; and *iii*) stage IIc cancer (single/≤ 2 cm/VI+) is amenable to TACE. The mainstay for treatment of stage III cancer is TACE or sorafenib. However, LT must be taken into account when cancer meets the Milan criterion. Sorafenib is better suited to treatment of a stage IV tumor. The Korean

Guideline also added that external beam radiation therapy could be useful in alleviating symptoms caused by primary HCC or metastases.

An algorithm based on the Child-Pugh class of liver function is utilized in Japan. The class is based on 3 factors: liver function, the number of tumors, and tumor size. Before a Child-Pugh class is assigned, whether extrahepatic spread is present is first determined. If extrahepatic spread is present, chemotherapy is the treatment of choice for Child-Pugh class A cancer. Palliative care is recommended for patients with decreased liver function. Undoubtedly, liver resection has been the first option for a solitary tumor that is Child-Pugh class A/B. According to the 2021 updated version of the J-HCC guideline, RFA is also recommended for a tumor < 3 cm. For patients with 2 to 3 tumor nodules, resection or RFA/TACE is recommended depending on their size (*12*). For patients with more than 4 tumor nodules, TACE is first recommended, but the JSH Guideline contends that resection can sometimes be performed, and ablation is sometimes performed in combination with TACE.

LT is recommended for patients younger than 65 with cancer meeting the Milan criterion, even if they have class C liver function according to the Child-Pugh score.

5.2. Non-surgical approaches

5.2.1. Ablation

RFA and percutaneous ethanol injection (PEI) are the most widely used forms of ablative treatment. They are considered the standard treatment for HCC that is BCLC 0-A stages and that is not amenable to surgery. Previous studies have found that RFA or PEI, as first-line treatment, can yield similar outcomes to surgical resection when tumors are smaller than 2 cm in size and BCLC stage 0 (*55,56*). A study in 2019, the SURF trial, recommended RFA for patients with 1-3 tumors smaller than 3 cm (*57*). In contrast, the INASL Guideline only recommends that patients with stage 0 undergo ablation when they are not potential candidates for LT (*15*). Substantial evidence is required to verify the effectiveness of ablation as a first-line treatment for very early HCC.

Patients in the terminal stage (BCLC stage D) should receive the best supportive care. External beam radiation therapy has only been tested in non-controlled studies. The INASL Guideline contends that radiation therapy cannot be recommended for management of HCC until its effectiveness is verified in clinical trials.

5.2.2. Embolization

In recent years, HCC interventional therapy has made huge advances, such that it has become an independent subspecialty. TACE was listed as the primary treatment option for BCLC stage B HCC in guidelines such as those from the EASL, and that procedure is described as being supported by strong evidence (*58,59*). The current guidelines reviewed here recommend TACE at about the same level as they did previously. Recent studies have found transarterial radioembolization (TARE), also called selective internal radiation therapy (SIRT), might outperform TACE in terms of tumor downstaging, and its combined use with Yttrium-90 microspheres may result in an encouraging outcome in terms of survival (*60,61*). Thus, TARE with Yttrium 90 could be considered as an alternative to TACE, particularly in cases of HCC with portal vein thrombosis.

5.2.3. Systemic therapy

Molecularly targeted therapy has made vast progress over the past few years. Traditionally, sorafenib is indicated when BCLC stage C HCC or BCLC stage B HCC progresses after TACE. Two widely cited RCTs have revealed that sorafenib can serve as a first-line treatment in patients with HCC who still have liver function but who can no longer be treated with other more effective therapies (*62,63*). Previous studies on sorafenib have reported its safety data and its efficacy in prolonging survival (*64-66*). Another first-line drug recommended by recently updated guidelines is lenvatinib. In a randomized phase 3 trial (about 2/3 of the included patients were from the Asia-Pacific region), the efficacy of lenvatinib was not inferior to that of sorafenib (*67*). In the study in question, lenvatinib displayed superior efficacy in the Chinese subgroup, and the overall survival time was prolonged by 4.8 months. Lenvatinib has a survival benefit for HBV-related HCC. According to the AASLD guideline, there is no evidence to support whether second-line treatment options such as regofinib or nivolumab can be used for patients with tumor progression receiving lenvatinib, but sequential use of tyrosine kinase inhibitors with a similar mechanism of action can be considered.

In the latest NCCN guideline, however, the recommended dose of sorafenib was reduced and the preferred regimen was changed to atzumab + bevacizumab (referred to as the T + A regimen, Child-Pugh class A only). Data presented at the 2019 ESMO Asia Congress indicated that the T+A regimen was superior to sorafenib in patients with unresectable HCC (*68*). Nevertheless, the cost-effectiveness of the T+A regimen still needs to be optimized (*69*).

6. Conclusion

This work has reviewed updated information from current comprehensive guidelines for HCC management published worldwide between 2001 and 2022. Twenty-two characteristic guidelines were identified, including 1 international guideline, 11 guidelines from Asia, 5 from

Europe, 4 from the US, and 1 from Australia. Those guidelines were compared in terms of surveillance, diagnosis, and treatment with a focus on the clinical management of HCC. The composition of and recommendations in current guidelines on HCC varied, so these guidelines were regrouped and diagnostic and treatment algorithms were summarized graphically to provide the latest information for clinicians.

Over the past few decades, HCC has changed from an almost universal death sentence to a cancer that can be prevented, detected in an early stage, and effectively treated, but HCC is still the second leading cause of cancer-related mortality worldwide, and the leading cause of death among patients with chronic liver disease (*2*). Findings from this comparison of current guidelines may help target and concentrate efforts to improve the clinical management of HCC. However, further studies are needed to improve the management and outcomes of HCC. More straightforward or refined guidelines would help guide doctors to make better decisions in the treatment of HCC in the future.

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Review

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Use of chemotherapy to treat hepatocellular carcinoma

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SUMMARY Hepatic malignancies remain a global challenge. Hepatocellular carcinoma (HCC) accounts for around 90% of patients with liver cancer and is the sixth most common neoplasm worldwide and the fourth leading cause of cancer-related death. However, the long-term prognosis for HCC remains far from satisfactory, with a late diagnosis and limited treatment. DOX has served as conventional chemotherapy with the longest history of use. Although conventional chemotherapy is being challenged by molecular therapy and immune therapy, there is renewed optimism and interest in both systematic and locoregional therapy. Combined chemotherapy is widely used in clinical practice. In specific terms, FOLFOX can serve as a first-line (category 2B) option as recommended by the 2021 NCCN guidelines, while the efficacy of LTLD plus RFA has been confirmed in the phase III HEAT study. These approaches have challenged the dominant status of molecular therapy in terms of health economics and they have potential benefits in Asia, where HBV-related hepatocellular carcinoma is prevalent. Moreover, locoregional chemotherapy can be achieved with TACE and HAIC (possibly involving FOLFOX, DOX, mitomycin C, cisplatin, epirubicin, *etc*.). TACE was officially recommended by the 2021 NCCN guidelines for patients with Child-Pugh class B liver disease. In addition, HAIC has demonstrated a potential advantage in preliminary clinical practice, although it hasn't been included in any guidelines. Hence, this review summarizes large-scale trials and studies examining the development and innovative use of chemotherapeutic agents. Mounting clinical evidence warrants an exploration of the efficacy of chemotherapy.

Keywords chemotherapy, hepatocellular carcinoma, immune therapy, molecular therapy

1. Introduction

Primary hepatic malignancies include hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma originating from the parenchyma. In addition, rare primary lesions originating from the mesenchyme develop into liver sarcoma. HCC is the sixth most common neoplasm worldwide and the fourth leading cause of cancer-related death, and it accounts for around 90% of patients with hepatic malignancies with an unfavorable prognosis due to its largely asymptomatic natural history (*1*), high recurrence, and ineffective therapeutic strategies for advanced HCC (*2-5*) (Figure 1). Hepatitis B virus (HBV) infection is an independent high risk factor for HCC among unvaccinated persons, mostly in Asia and sub-Saran Africa (*6*). In addition, hepatitis C virus (HCV) infection, dietary exposure to aflatoxin B1, and alcoholic liver disease (ALD) and nonalcoholic fatty liver disease (NAFLD) have become the leading causes of HCC in developed countries (*7,8*).

Currently, there are various controversies regarding the therapeutic options for HCC. Since localized liver cancer is asymptomatic for much of its natural history, the major obstacles are a late diagnosis and a subsequently low resection rate, limiting treatment alternatives. Thus, a significant fraction of patients will eventually become eligible for chemotherapy. Chemotherapy has become a conventional option for HCC as a result of drug research and development. These drugs play an indispensable role in systematic therapy and are also being developed to act on locoregional targets through approaches such as transarterial chemoembolization (TACE) and hepatic artery infusion chemotherapy (HAIC). Here, chemotherapy is outlined and its corresponding role in treating HCC has been described.

The stage of HCC is identified using various staging systems, namely Barcelona Clinic Liver Cancer (BCLC) tumor staging, the Hong Kong Liver Cancer staging system (*3,9*), and the Cancer of the Liver Italian Program (*10*). The BCLC is most widely used and was introduced

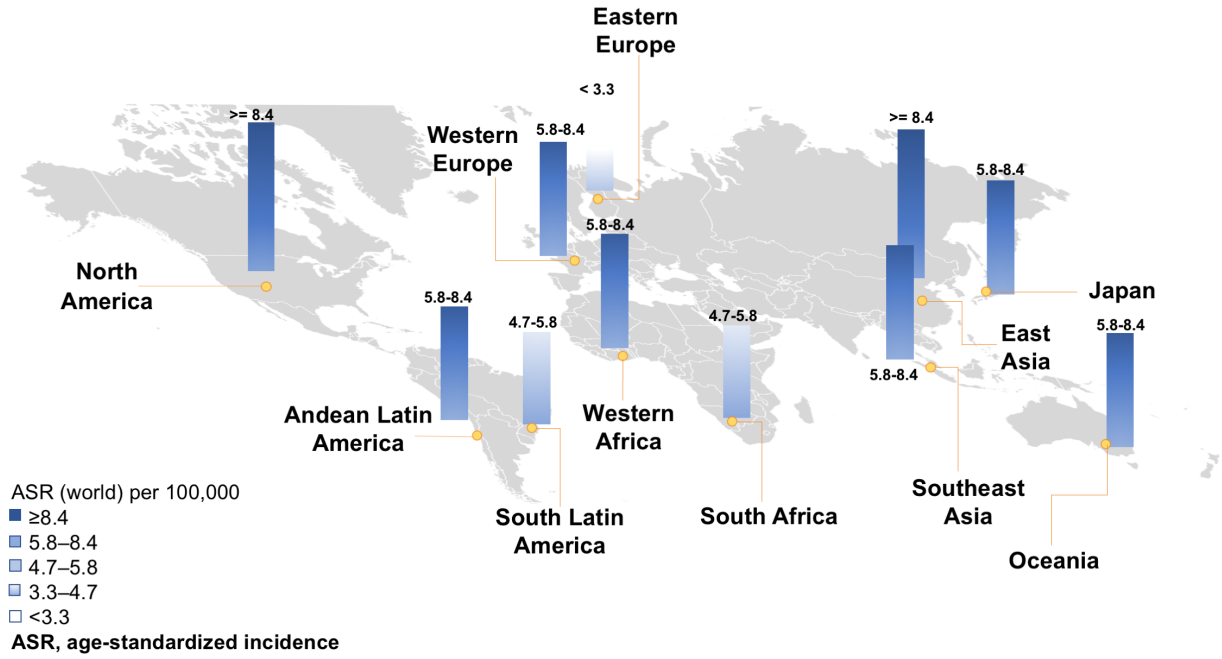

Figure 1. The incidence of HCC by geographical area. HCC is a worldwide problem and it is concentrated in East Asia and North America, as reflected by the age-standardized incidence.

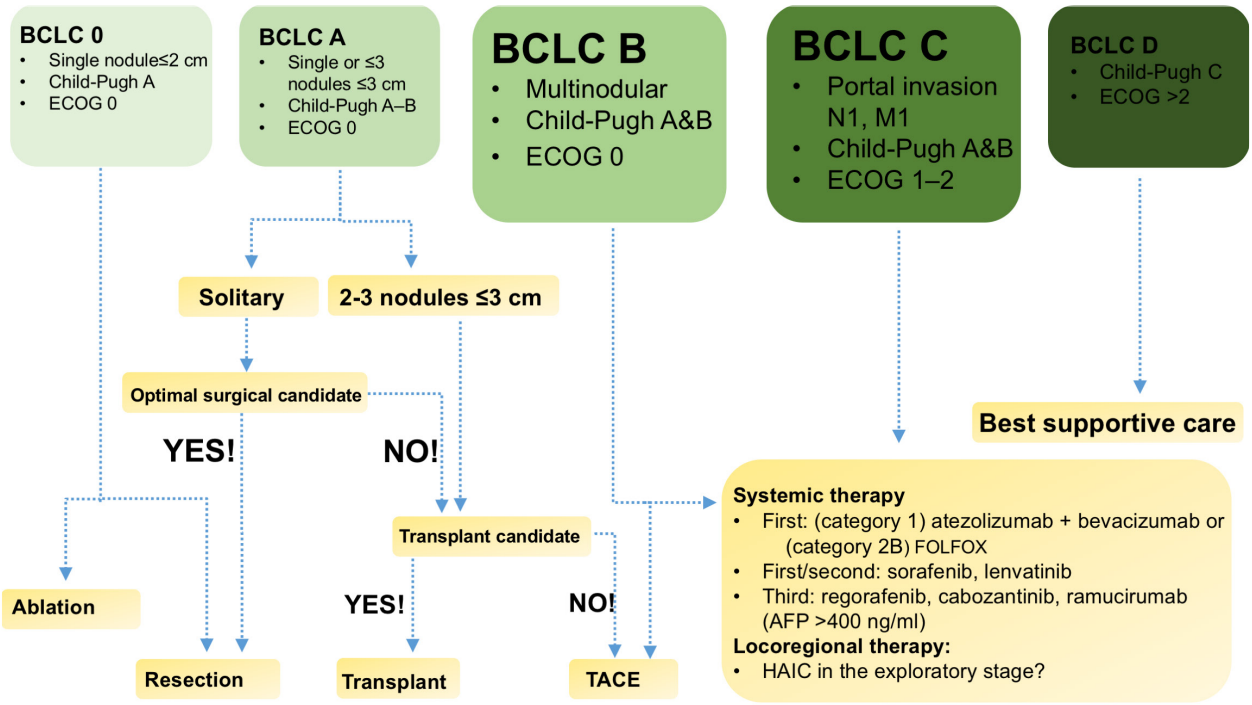

Figure 2. Clinical algorithm for the management of HCC. This algorithm, based on the Barcelona Clinic Liver Cancer algorithm, classifies patients into one of five stages, corresponding to graded therapies recommend by the 2021 NCCN guidelines.

in 1999 (*3,11*). Since it provides a comprehensive evaluation based on liver function, performance status, and tumor burden, the BCLC system has been approved by the European Association for the Study of the Liver (*4,10-12*). The algorithm classifies patients into one of five stages and it provides treatment recommendations for each stage (*3*). (Figure 2) Based on this classification system, the use of chemotherapeutic agents for different stages of HCC has been described here (Figure 3).

2. Systemic chemotherapy

Systemic therapies are, along with transarterial therapies, recommended for patients with BCLC stage B or C HCC

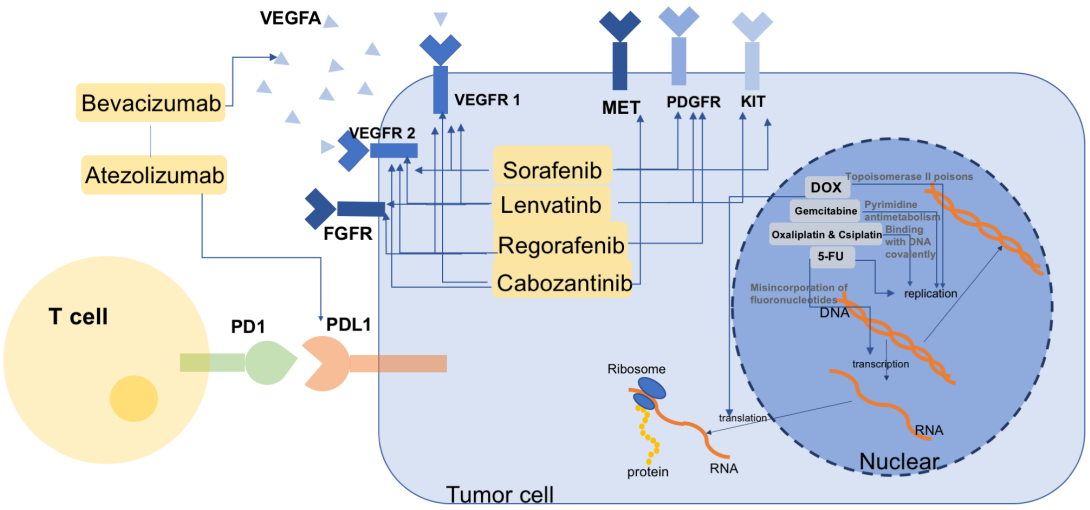

Figure 3. Mechanism of action of chemotherapy, molecular therapy, and immune therapy. The mechanism of action of chemotherapeutic agents: DOX inhibits the replication and translation of DNA via various approaches including as topoisomerase II poisons. Gemcitabine specifically kills cells in the process of DNA synthesis by disrupting pyrimidine metabolism. Platinum covalently binds directly with DNA. 5-FU induces the misincorporation of fluoronucleotides by replacing dUMP to form a ternary complex with a higher binding affinity and greater stabilization. **The mechanism of molecularly targeted agents:** They suppress multi-kinase, which is the key substance that activates tyrosine kinases such as VEGFRs, PDGFR, MET, and KIT. These tyrosine kinases play a key role in cell proliferation and angiogenesis in the tumor microenvironment (TME). **The mechanism of immune therapy:** Atezolizumab is targeted PDL1 expressed in HCC tumor cells that inhibits PD1 expressed by effector lymphocytes. Bevacizumab (a VEGF inhibitor) synergistically enhances the effectiveness of PD1 inhibition.

with progression (*3*). In some developing countries, 50-70% of patients are initially diagnosed with BCLC Stage C HCC because of limited systematic screening. Liver transplantation is currently with the most effective treatment, it is available for only select patients with early-stage HCC. In addition, surgical resection and ablative techniques have demonstrated advantages in terms of long-term survival, but less than 25% of patients are fortunate enough to have access to these treatments even in the West (*13,14*). Despite the widespread use of TACE in patients with unresectable tumor lesions, systemic therapy with doxorubicin has been the standard for many years (*15*).

2.1. Doxorubicin (DOX)

DOX was used as a first-line chemotherapeutic drug for many years until the appearance of sorafenib in 2008 (*13*), albeit without evidence of a significant survival benefit (*16*). It was first introduced in the early 1960s (*17*) and was widely used to treat solid tumors. A bold attempt has been made to use DOX to treat HCC. Nonetheless, controversies surrounding DOX's mechanism of action abound. In a nutshell, DOX inhibits the replication and translation of DNA in various way including as a topoisomerase II poison (*17*) and targeting p53 (*17*). Like other antitumor agents, DOX was soon approved, but its drawbacks have been serious cardiac adverse events, namely chronic cardiomyopathy and congestive heart failure (CHF), and progressive drug resistance after completion therapy within a year (*18*). These faults have limited the maximum recommended volume of DOX to 40 to 75 mg/m^2 according to successive clinical studies conducted from 1977 to 2007 (*16*). (Table 1) As a monotherapy for HCC, DOX only conferred a survival benefit of 3.0 to 4.1 months and an ORR of 19% (16,19). As diagnosis and technology have advanced, fortunate patients are more likely to receive systemic treatment in earlier stages (*16*), but DOX's marginal survival benefit has not changed.

2.2. DOX derivatives

After DOX was introduced, DOX derivatives were examined as chemotherapeutic agents. Pegylated liposomal doxorubicin (PLD) has long-acting pegylated ‘stealth' liposomes encapsuling a doxorubicin hydrochloride inner core for intravenous administration to target HCC lesions. PLD has a better permeability and liposolubility that delay its clearance from the circulation *via* leaky capillaries, resulting in an attenuated circulation time and a superior cardiac safety profile (*20*). However, a phase II study found that PLD has almost no effect in advanced HCC (*20,21*), with a response rate of 10-17% at best (*20,22*).

When liposomal doxorubicin (LD) is pegylated, it does not have significant systemic efficacy, but lyso-thermosensitive LD (LTLD) locally releases a high concentration of doxorubicin and quickly diffuses into local tissues when heated to ≥ 40°C (*23,24*). The tumor concentration of doxorubicin increases 25-fold (*23*). Tumor microvasculature is more permeable than normal

Table 1. Summary of outcomes of systemic use of DOX according to clinical trials from 1977 to 2007

Years	Clinical Trials	Demographic Characteristics	Doses of DOX	Median OS	Response	Treatment-related Adverse Events	
						Most Common Grade 3-4	Leading to Death
1978	—	1. Patients were ineligible for surgery 2. Patients with grade A or B liver function	**60 mg/m^2**; **30 mg/m^2**: WBC < 2,000 μL or PLT < 100,000 μL; **15mg/m^2**: WBC < 1,000 μL or PLT < 50,000 μL, Calculated maximum: **550 mg/m^2**	2-5 months	Objective response: 32%	NR	NR
1984	NCZ vs. DOX	1. Patients from South Africa 2. Contraindications for patient eligibility included previous treatment and poor liver function	**60 mg/m^2** for anicteric patients **40 mg/m^2** for icteric patients and AST > 2 times normal	22 weeks	NR	NR	NR
1984	Eastern Cooperative Oncology Group Trial	1. Patients from the US, Europe, and South Africa 2. Contraindications for patient eligibility included previous treatment and poor liver function	**60 mg/m^2** for anicteric patients; **40 mg/m^2** for icteric patients	17 weeks	Objective response: 12%	Common adverse events: Leukopenia (leukocyte count: 2,000/mm^3) and/or neutropenia (neutrophil count: 1,000/mm^3): 21%	NR
1985	Clinical trials of DOX in Italy	1. Patients from Italy 2. Grade A or B liver function 3. Unresectable HCC	**60 mg/m^2**; **30 mg/m^2**: WBC < 3,500 μL or PLT < 80,000 μL, DB > 2 mg/mL Calculated maximum: **550 mg/m^2**	4.1 months	Objective response: 24%	Common adverse events: Alopecia: 100%	NR
1988	DOX vs. no antitumor therapy	1. Patients from China 2. Inoperable tumor 3. Liver function with acceptable therapy 4. Without detectable cardiac diseases	Initial dose **60 mg/m^2** with subsequently increased dose of **75 mg/m^2**, NPC < 3.5 × 10^9, **50 mg/m^2**; NPC < 1 × 10^9, **37.5 mg/ m^2**	10.6 weeks	Partial response: 3.3%	Cardiotoxicity; Neutropenia	25%
2005	DOX *vs*. PIAF	1. Unresectable HCC 2. Liver function with acceptable therapy	**60 mg/m^2**	6.83 months	Overall response: 10.5%	Neutropenia: 63% Anemia: 28%; Thrombocytopenia: 24%	NR

Abbreviation: NCZ, neocarzinostatin; ORR, objective response rate; NR, not report; OS, overall survival.

blood vessels, so LTLD is better able to reach tumors and reduce systemic toxicity (*24-26*). Given the significant correlation between LTLD and heat, the best approach might be to increase the local temperature using radiofrequency ablation (RFA). Recent studies involving a combination of intravenous administration of LD and RFA suggested that RF-induced thermal energy at 42°C in particular might yield better efficacy, improving the release of DOX from the long-circulating drug/liposome complex and resulted in accumulation of a higher concentration at the target lesion (*27*). In the recent phase III HEAT study, the initial complete response of multinodular intermediate-sized lesions (3-7 cm) to RFA + LTLD was > 94% and the therapeutic failure was < 5% (*23*) according to a sub-analysis of relatively large tumors (5-7 cm), and median PFS was 13.9 months and the median OS was 53 months (*23,24*). That said, there were no statistically significant differences between the RFA + LTLD arm and the RFA alone arm. However, a subsequent post hoc study suggested that prolonging the RF ablation of larger tumors would be more likely to increase efficacy and have a survival benefit (*28*).

2.3. Other cytotoxic agents

Nevertheless, DOX continues to be used as conventional chemotherapy. Continued innovation in chemotherapeutic agents is expected to result in the replacement of DOX, but studies have yielded conflicting results. For instance, fluoropyrimidine 5-fluo-rouracil (5-FU) has been widely used in a vast number of regimens and it has been used as an essential component of transarterial treatment as well (*29-31*). Like DOX, 5-FU suppresses DNA and RNA synthesis *via* the misincorporation of fluoronucleotides, and it inhibits the nucleotide-synthesizing enzyme thymidylate synthase (TS) as well. Tegafur-uracil is an oral prodrug metabolized to 5-FU mostly in the liver, and it has higher efficacy and is better tolerated (*32*). Nolatrexed (NOL) is a novel anticancer agent that also inhibits TS. NOL is taken up into cells without active transport, and it acts without polyglutamation (*16*). However, a large-scale randomized controlled trial (RCT) has compared the efficacy of DOX and NOL and found that NOL resulted in a negligible improvement in survival, with an OS of 20.7 weeks, compared to DOX (*16*). Thus, numerous novel cytotoxic agents have been examined, including gemcitabine, capecitabine, and oxaliplatin (*33*). These agents have demonstrated modest efficacy alone but considerable efficacy when used in combination (Table 2) (*29,34*). For instance, gemcitabine kills cells in the progress of DNA synthesis by inhibiting pyrimidine metabolism, so it is specific to certain cell phases. Moreover, it is attractive as a component of a combined strategies due to its favorable nonhematologic toxicity spectrum and mild and reversible hematological toxicity profile (*35,36*). Like gemcitabine, capecitabine targets fluoropyrimidine but *via* an oral protocol (*37*). Capecitabine resulted in an ORR of 11%, (including complete remission in 1 patient), and a disease control rate of 22% (*29,38*). The antitumor role of platinum was subsequently discovered by accident, and platinum-based drugs were approved in 1978. Platinum's mechanism of action, as confirmed by a number of highly reliable studies, differs from the mechanisms mentioned thus far since it interferes with DNA synthesis by covalently binding directly with DNA (*39,40*). Typical platinum-based chemotherapeutic agents include cisplatin, carboplatin, and oxaliplatin. Cisplatin was initially found to play a significant role in treating reproductive system tumors, namely testicular and ovarian cancers, with notable toxicity to the kidneys and gastrointestinal tract (*39*). Cisplatin resulted in a response rate of 16-27% when used in combination to treat advanced HCC (*29,31*), but it did provide a marginal survival benefit when used alone. Cisplatin is also used in intra-arterial strategies. Indeed, cisplatin has a marginal survival benefit because its interference with DNA binding and repair diminishes as HCC becomes resistant to the drug. Carboplatin, a second-generation platinum-based drug, has greatly reduced nephrotoxicity but it has an efficacy similar to that of cisplatin. Oxaliplatin (1R,2R-diaminocyclohexane oxalatoplatinum (II)) is actively antagonistic to tumors with acquired resistance to cisplatin (*39,41,42*), and it even overturned the previously accepted view that platinum-based drugs are insensitive to colorectal cancer (*39*). Interferons (IFNs) are a group of signaling cytokines secreted by immune cells. They display antitumor action by provoking antitumor immune responses and regulating the expression of proliferation-related genes (*43*).

2.4. Combination chemotherapy

Since single agents had limited efficacy against HCC, the question is whether those drugs would be efficacious when used in combination. A phase II study indicated that a gemcitabine plus PLD regimen resulted in a response rate of 24% and it increased opportunities for surgery, including resection and transplantation, for eligible patients with unresectable HCC (*21,35,44,45*). Due to its different mechanisms of action, it resulted in an acceptable toxicity and it prevented cross-resistance (*35*). In specific terms, gemcitabine promotes topoisomerase II expression, which is a process that PLD targets (*35,46*). Thus, better results are achieved when gemcitabine is administered before PLD. In addition, a gemcitabine plus oxaliplatin (GEMOX) regimen is likely to be well-tolerated when treating primary NAFLD according to a phase II study (*47*). The regimen results in an ORR of 18% and a disease control rate of 76%. Although these figures seem to indicate a marginal benefit, the regiment resulted in durable stabilization of HCC characterized by chemo-resistance (*47*). Combination chemotherapy with PIAF (cisplatin, interferon, doxorubicin, and

Table 2. Examination of combination chemotherapies

Combination Regimens	Pharmacological Mechanism	Administration	Median PFS	Median OS	ORR	Treatment-related Adverse Events		
						Grade 3-4	Most Common Grade 3-4	Leading to Death
Gemcitabine + PLD	**Gemcitabine**: pyrimidine antimetabolite	Gemcitabine 1,000 mg/m^2 on Days 1 and 8, followed by pegylated liposomal doxorubicin 30 mg/m2 on Day 1.	5.8 months	22.5 months	25.00%	31.70%	**Neutropenia**: 17% **Anemia**: 7%	NR
Gemcitabine + Oxaliplatin (GEMOX)	**Oxaliplatin**: covalently binds directly with DNA to interfere with DNA synthesis	Gemcitabine 1,000 mg/m^2 on Day 1 and oxaliplatin 100 mg/m2 on Day 2	6.3 months	11.5 months	18.00%	42.00%	**Thrombocytopenia**: 26% **Neutropenia**: 21% **Neurotoxicity**: 9%	No toxic deaths occurred
PIAF (cisplatin, interferon, doxorubicin, and 5-fluorouracil)	**Cisplatin**: covalently binds directly with DNA; **5-FU**: misincorporation of fluoronucleotides into RNA & DNA, inhibits thymidylate synthase **IFN**: increases immune response	Cisplatin (20 mg/m^2) on Days 1 through 4), interferon α-2b (5 MU/m^2) on Days 1 through 4, doxorubicin (40 mg/m^2) on Day 1, and 5-fluorouracil (400 mg/m2) on Days 1 through 4 every 3 weeks for up to six cycles.	NR	8.67 months	20.90%	NR	Most common toxicities: **Neutropenia**: 82% **Thrombocytopenia**: 57%	NR
FOLFOX4 (fluorouracil, leucovorin, and oxaliplatin)	**Leucovorin**: increases the binding affinity of a ternary complex mainly consisting of 5-fluorodeoxyuridine monophosphate (FdUMP) converted by 5-FU	OXA 85 mg/m^2 intravenously on Day 1; LV 200mg/m^2 IV from hour 0 to 2 on Days 1 and 2; and FU 400 mg/m2 IV bolus at hour 2, then 600 mg/m^2 over 22 hours on Days 1 and 2, once every 2 weeks	2.93 months	6.40 months	8.15%	55.74%	**Neutropenia**: 30.6%; **AST**: 11.96%	6.01%

Abbreviation: PLD, pegylated liposomal doxorubicin; ORR, overall response rate; OXA, oxaliplatin; LV, leucovorin; IV, intravenously; NR, not report.

5-fluorouracil) has yielded positive outcomes in terms of a pathologic complete response (*15*) in a small but marked proportion of patients, and it resulted in a marginally prolonged median survival of 8.67 months (range = 6.36 to 12.00) versus DOX (6.83 months (range = 4.80 to 9.56)) (*31*). Attention has continued to focus one combination chemotherapy. The drug 5-FU induces misincorporation of fluoronucleotides by replacing dUMP to form a ternary complex with a higher binding affinity and increased stabilization. The presence of leucovorin can boost ternary complex formation, and oxaliplatin can further increase efficacy. Combination chemotherapy with the FOLFOX (fluorouracil, leucovorin, and oxaliplatin) regimen has been widely used in metastatic colorectal cancer (mCRC) with demonstrable efficacy (*48*), and it has also been examined in advanced HCC. Although FOLFOX4 conferred a slim advantage as indicated by a median PFS of 2.93 months (2.43 to 3.53) versus DOX (1.63 to 2.30 ; $P < 0.001$), it was not effective at prolonging OS (6.4 months versus 4.97 months, $P > 0.05$) according to a phase III study (*49*). That said, FOLFOX4 offered a statistically significant benefit in terms of OS when used to treat metastatic HCC (*49*). Given its limited superiority in terms of a statistically significant survival benefit, the 2021 clinical practice guidelines of the NCCN recommend that FOLFOX serve as a first-line (category 2B) option (*1*).

Numerous studies have examined chemotherapy with various combinations of drugs, such as capecitabine plus cisplatin (XP) (*29*) and gemcitabine plus carboplatin (*50*). Nevertheless, these trials noted only a modest and inconsistent efficacy.

In summary, HCC is a multidrug-resistant tumor caused by a high level of MDR1 expression (*51*). Randomized trials of novel therapeutics have failed to note a significantly improved survival until recently, with a median OS of 6 to 8 months (*35*). Systematic chemotherapy is modestly effective in treating HCC (*49*).

2.5. Chemotherapy and molecular therapy

Examination of subsequent approaches to treating advanced HCC has ushered in a new era of molecularly targeted agents (MTAs) (Table 3) and immune therapy (*52*). Sorafenib was the first systemic molecular agent approved by the FDA, and it is rapidly replacing DOX as the drug of choice for frontline therapy (*3*). Sorafenib targets multi-kinases, including the serine-threonine kinases Raf-1 and B-Raf, and tyrosine kinases, which are key substances that activate vascular endothelial growth factor receptors (VEGFRs) and platelet-derived growth factor receptors (PDGFR-β). These receptors play a key role in cell proliferation and angiogenesis in the tumor microenvironment (TME) (*53-55*). The SHARP trial (a phase III RCT) initially noted an improved prognosis in cases of advanced HCC, with a median survival benefit of nearly 3 months and a median OS of 10.7 months versus the placebo group (*52,53*). The National Institute for Health and Clinical Excellence argued that sorafenib had limited cost-effectiveness as a first-line treatment for advanced HCC (*35*). Therefore, studies examined MTAs, including erlotinib (56), brivanib (*57*), sunitinib (*58*), linifanib (*59*), and everolimus (*60*), as a way to achieve greater efficacy at a lower cost. However, a global phase III trial found that these agents had efficacy no better than or on par with what of sorafenib (*3*). Studies on MTAs appeared to have reached an impasse, but lenvatinib subsequently appeared as an alternative for advanced HCC with a broader pharmacological mechanism profile against VEGFR, FGFR, PDGFR α, RET, and KIT (*61*). According to a phase III trial, lenvatinib resulted in an OS of 13.6 months (95% CI 12.1-14.9 months) similar to that of sorafenib (12.3 months, 95%CI 0.79-1.06 months) (*61*). However, all secondary efficacy endpoints were statistically superior, namely PFS, TTP, and OR (*61*). Moreover, a recent cost-utility analysis found that lenvatinib was superior in cost (*62*). Lenvatinib is reasonably given priority (*62*). Recently, the 2021 clinical practice guidelines of the NCCN recommended sorafenib and lenvatinib as a category 1 option for patients with Child-Pugh class A liver function (*1*). In addition, the later phase III RESORCE trial (*63*) and CELESTIAL trial confirmed the role of regorafenib and cabozantinib, both of which are oral multikinase inhibitors, as subsequent-line therapy in the event of disease progression after sorafenib administration (*1*).

Although clinical trials have demonstrated the benefits of sorafenib and lenvatinib, a retrospective study in South Korea reached the opposite view. In that study, the efficacy of conventional chemotherapy (fluorouracil plus doxorubicin and platinum) was not inferior to that of sorafenib (*34*); this finding is presumably due to the fact that trials included patients with Child-Pugh class B or C liver disease. Moreover, sorafenib alone had a limited benefit in select patients with extrahepatic disease (*49*). In addition, a pivotal phase III study in Asia demonstrated that sorafenib has modest efficacy, with an OS of 6.5 months (95% CI 5.56-7.56 months) versus 4.2 months in the placebo arm (95% CI, 3.75-5.46 months). Although the HRs were comparable between that study and the SHARP trial, the OS in Asia was inferior to that in the West (*64*). This is presumably due to the higher proportion of patients infected with HBV or poor screening in developing countries (*64,65)*. A subsequent systemic review confirmed that sorafenib had superior efficacy in cases of non-metastatic HCC caused by HCV (*5*). The rapid emergence of sorafenib resistance in the majority of patients and the conflict between high costs and low incomes has limited its use in Asia (*34*). Use of MTAs and alternative chemotherapies is hotly contested in abound in certain countries.

Would the combination of sorafenib and chemotherapeutic agents result in a considerable

Table 3. Examination of molecular therapy to treat advanced HCC

Clinical Trial	Molecular Strategies	Median OS (months)	Median PFS (months)	ORR	Treatment-related adverse events				Comments
					Grade 3-4	Most Common Grade 3-4	Leading to Death	Leading to Discontinuation	
SHARP	Sorafenib	10.7	Symptomatic progression: 4.1	PR: 2% DCR: 71%	45%	Diarrhea: 8% HFS: 8% Fatigue: 4%	NA	NA	Patients are from Europe, North America, South America, and Australasia.
Sorafenib *vs*. placebo	Sorafenib	6.5	2.8	DCR 35.3%	NR	HFS: 10.7% Diarrhea: 6.0% Fatigue: 3.4%	NA	rarely	Patients are from the Asia-Pacific region
SEARCH	Sorafenib + Erlotinib *vs*. Sorafenib + placebo	9.5 *vs*. 8.5 $p = 0.408$	3.2 *vs*. 4.0, $p = 0.18$	6.6% *vs*. 3.9%, $p = 0.102$	87%	Diarrhea: 18.5% Fatigue: 17.1%	NA	NA	—
BRISK-FL Study	Brivanib	9.5	4.2	12%	11.70%	Hyponatremia: 4% Fatigue: 2.5%	NA	43.00%	—
Sunitinib *vs*. Sorafenib	Sunitinib	7.9	3.6	6.20%	NR	Thrombocytopenia: 29.7% Neutropenia: 25.7%	NA	13.30%	—
Linifanib *vs*. Sorafenib	Linifanib	9.1	5.4	13%	85.30%	Hypertension: 20.8%	NA	36.30%	—
EVOLVE-1	Everolimus	7.6	3	DCR 56.1%	Grade 3: 52.1% Grade 4: 18.8%	Anemia:7.8% Asthenia: 7.8% Decreased appetite: 6.1%	NR	NR	—
REFLECT	Lenvatinib	13.6	7.4	24.1	75%	Hypertension: 23% Decreased weight: 8%	NR	NR	—
RESORCE	Regorafenib	10.6	3.1	7%	50%	Hypertension: 13% HFS: 13% Fatigue: 9%	2%	10%	—
CELESTIAL	Cabozantinib	10.2	5.2	4%	68%	HFS: 17% Hypertension: 16% Increased AST: 12%	1%	16%	—
REACH-2	Ramucirumab	8.5	2.8	5%	NR	Hypertension: 8% Liver injury or failure: 4% Proteinuria: 2%	2%	11%	—

Abbreviation: HCC, hepatocellular carcinoma; OS, overall survival; PFS, progression-free survival; ORR, objective response rate; PR, partial response; DCR, disease control rate; NA, not applicable; HFS, hand-foot skin reaction; NR, not report.

survival benefit? Sorafenib plus DOX (*34*), sorafenib plus GEMOX (*66,67*), sorafenib plus tegafur-uracil (UFT)(*32*), and other combinations have been studied in clinical trials, but they all demonstrated a moderate benefit in terms of PFS versus sorafenib alone.

2.6. Chemotherapy and immune therapy

Immune therapy's mechanism of action is based on the TME around the malignant lesion and dysfunctional tumor-immune system interactions, which lead to immune evasion by reducing the recognition of tumor-associated antigens (TAAs) (*68*). HCC expresses immune checkpoint ligands, including co-inhibitory molecules (cytotoxic T lymphocyte-associated antigen 4 (CTLA4), PD1, T cell immuno-globulin and mucin domain containing molecule 3 (TIM3), and lymphocyte-activation gene 3 (LAG3)) (*68*). Immune checkpoints expressed by effector lymphocytes subsequently bind with the ligands to inhibit overwhelming activation (*68*). Interaction between receptors and their ligands needs to be blocked in order to sustain the activity of effector lymphocytes against the malignant proliferation of tumor cells. Immune checkpoint inhibitors (ICIs) and monoclonal antibodies can achieve this goal. PD1 and PDL1 inhibitors have demonstrated promising results in preventing the proliferation of HCC cells (*68*).

The efficacy of atezolizumab (a PDL1 inhibitor) plus bevacizumab (a VEGF inhibitor) was confirmed by the IMbrave150 trial; the combination resulted in a PFS of 6.8 months (95% CI, 5.7 to 8.3 months) and an ORR of 67.2% for one year among patients with unresectable HCC (*69*). The combination was also recommended by 2021 NCCN guidelines as first-line therapy (category 1) for patients with Child Pugh class A liver disease. After the IMbrave050 trial, more attention was paid to patients with a high risk of recurrence after curative resection or ablation (*70*). Although the combination displayed a benefit to an extent and it was superior to current systemic therapy, a subgroup analysis of a phase III study noted lower efficacy in NAFLD and HCC with activated Wnt/β-catenin signaling (*71*). The incidence of NAFLD has been increasing, and NAFLD has become a main etiological risk factor for HCC in the US (*71*) due to imperfect screening systems, leading to advanced tumor stages. Mahipal *et al*. found that about 50% of HCC cases were accompanied by overactivation of the Wnt/β-catenin signaling pathway, promoting proliferation and metastasis as well as sorafenib resistance (*72-74*).

The combination of GEMOX and bevacizumab resulted in an OS of 9.6 months OS (95% Cl, 8.0 months to not available) and a PFS of 5.3 months (95% Cl, 3.7 to 8.7 months) according to a phase II study (*33*), but further evidence is lacking. Atezolizumab and other chemotherapy agents have displayed efficacy in triple-negative breast cancer, but there have been few studies on the combination of chemotherapeutic drugs and immune agents to treat HCC.

3. Locoregional use of chemotherapy

3.1. TACE

Although systemic chemotherapy has failed to play a major role in the treatment of HCC, local use of chemotherapeutic agents is still considerable, and approaches include TACE and HAIC. The theoretical basis for these approaches is the vascular shift where benign lesions are supplied by branches of the portal system, while malignant nodules are nourished by the hepatic artery (*3,60*) according to CT and MRI (*3,60*). Patients with BCLC stage B HCC are eligible for TACE (*15*) according to the 2021 NCCN guidelines, which specify a reasonable level of liver function (Child-Pugh class A or B) or a multinodular tumor without vascular invasion or extrahepatic metastasis (*1,4,5*).

In addition to embolization, TACE can deliver a chemotherapeutic agent in a high concentration to a target lesion; this means less of the drug in the circulation, thus reducing adverse events (*1,4*). TACE includes conventional TACE (cTACE) and drug-eluting bead TACE (DEB-TACE) (1,75). Doxorubicin, mitomycin C, and cisplatin are usually used in cTACE. These drugs are suspended in lipiodol for delivery to the target location, followed by embolization with gelatin sponge particles (*75*). DEB-TACE involves agents similar to those used in cTACE but different carriers. In DEB-TACE, beads are implanted in the tumor vasculature, where they remain for a prolonged period (*75*) to maximize the duration of their presence and to attenuate systemic toxicity (*4,76*). A retrospective study initially indicated that DEB-TACE for unresectable HCC in all stages resulted in an OS of 610 days versus 284 days for cTACE (*77*). Moreover, a stratification analysis indicated that DEB-TACE resulted in a significant benefit for patients with Child-Pugh class A or B liver function since they potentially suffered liver failure when undergoing cTACE. Another retrospective study (*78*) and two prospective studies subsequently agreed with the earlier findings (*4,79*). However, the PRECISIONV trial suggested that the two procedures have equivalent efficacy and safety, possibly due to the inclusion of patients with all BCLC stages of liver disease (*4,80*).

Although widely used, chemoembolization remains highly controversial. A retrospective study (*81*) and 3 RCTs were optimistic about the benefits of TACE compared to symptomatic treatment of unresectable HCC (82,83). Moreover, a subsequent meta-analysis corroborated the survival advantage of TACE (*15*). However, two French studies reached the opposite conclusion, possibly because of the inclusion of a disproportionate number of patients with alcoholic cirrhosis (*15*). In addition, an RCT over 5 years compared the efficacy of TAE and transarterial DOX

embolization (*84*). The study indicated that TAE resulted in a median PFS of 6.2 months and an OS of 19.6 months versus a PFS of 2.8 months and an OS of 20.8 for the TACE arm, so the PFS and OS did not differ significantly. Does this mean that chemotherapy drugs have completely failed in both systemic and regional treatment of HCC? Fortunately, the answer is no. Despite those negative and disappointing results, the study in question did not stratify patients by BCLC stage, which led to a confounding bias. In addition, the study only used DOX and it did not consider other effective agents. Hence, a subsequent RCT compared the efficacy of cisplatin and epirubicin in TACE, and it have concluded that cisplatin was not significantly superior to epirubicin (*85*). Both cisplatin and mitomycin C have yielded consistent results according to a prospective study (*86*). One final aspect to consider is that the drug carrier may limit efficacy. HepaSpheres, which are vinyl alcohol-sodium acryl-ate microspheres, were the conventional carrier system, and they lasted almost 30 years in clinical practice. HepaSpheres provided superior absorption and release of a chemotherapeutic drug and they were pliant in blood vessels (*87*). However, micron-sized iron powder, barium ferrite (BaFe12O19), and carbon-coated iron nanocrystals (CCINs) are a novel carrier system (*88*). This new carrier enhances chemotherapy by maximizing the drug-loading capability and controlled-release, and this new carrier system has displayed great potential in animal experiments *in vitro* compared to the conventional carrier system (88). Together, these aspects play an essential role in the efficacy of chemotherapeutic drugs in TACE.

3.2. HAIC

HAIC has been widely used in Asia, and especially in Japan (*1,89*). HAIC's mechanism of action is to deliver a high concentration of a chemotherapeutic agent to a targeted lesion *via* the hepatic artery without embolization (*90*). There are two approaches to HAIC: single administration and continuous infusion with a subcutaneously sited reservoir system (*90*). Different chemotherapeutic agents involve different approaches to targeting in order for them to be most effective. In specific terms, epirubicin hydrochloride and miriplatin, which are concentration-dependent agents, can be used for bolus injection, while time-dependent agents, namely DOX and 5-FU, can be used for continuous HAIC. Notably, some agents, such as cisplatin and mitomycin C, are suitable for both bolus injection and continuous infusion (*90*).

Although HAIC has been routinely used in Asia, HAIC is not mentioned as a common locoregional therapy for advanced HCC in the 2021 NCCN guidelines or by the Asian Pacific Association for the Study of the Liver (*90*). Therefore, attention has focused on obtaining highly reliable clinical evidence of HAIC's efficacy. Although a systematic review and a retrospective study have indicated that HAIC conferred a survival benefit versus supportive therapy in advanced HCC (*91*), prolonging long-term survival was difficult due to the aggressive nature and rate of recurrence of HCC (*92*). Moreover, HAIC with 5-FU and cisplatin yielded a marginal survival benefit in patients with macrovascular invasion (MVI) but without extrahepatic metastasis (EHM) (*93*).

Would the combination of systemic therapy and HAIC result in increased efficacy? In theory, HAIC could compensate for MTAs failing to reach the expected dose at the targeted lesion because of PVTT (*94*), while systemic therapy could target extrahepatic lesions (*52*). However, the SILIUS study noted a similar OS of 11.8 months for combination therapy (HAIC (cisplatin)+ sorafenib) *vs*. 11.5 months for monotherapy (sorafenib) but with a higher likelihood of Grade 3-4 adverse events (*95*). Therefore, subsequent studies have focused on alternative agents for use in HAIC. As mentioned before, oxaliplatin is superior to cisplatin (96), so the question is whether oxaliplatin could avoid the adverse effects caused by cisplatin. A phase II and III trial on FOLFOX plus sorafenib in HAIC yielded positive results in terms of the OS (13.37 months *vs*. 7.13 months, $P < 0.001$), a higher RR (40.8% *vs*. 2.46%; $P < 0.001$), and a longer PFS (7.03 [95% CI, 6.05-8.02 months] *vs*. 2.6 [95% CI, 2.15-3.05 months]; $P < 0.001$), and especially when HAIC was combined with PVTT (*96*). Hence, FOLFOX has been the mainstay of HAIC (*97,98*). Nevertheless, a cost-effectiveness analysis found that SoraHAIC was moderately cost-effective in developing areas (*99*). Immune therapy is recommended in the 2021 NCCN guidelines on advanced HCC, so further studies need to be conducted to determine if HAIC is cost-effective.

Compared to TACE consistently administered *via* the hepatic artery, HAIC with FOLFOX resulted in a median OS (23.1 *vs*. 16.1 months, $P < 0.001$) and PFS (9.6 *vs*. 5.4 months, $P < 0.001$) superior to those of TACE for large HCC according to a prospective non-randomized study and a randomized study (*100,101*). However, TACE for preoperative and postoperative resection of large HCC has been studied, and its use remains controversial (*102,103)*. TACE can serve as a bridge therapy and downstaging therapy (*1*). That said, a point worth noting is that HAIC to treat HCC has yet to be fully examined.

4. Discussion and Conclusion

Conventional chemotherapy faces challenges from molecular therapy and immune therapy, but it plays an essential role in the treatment of hepatic malignancies. Over time, DOX was isolated from the pigment-producing Streptomyces peucetius and then DOX derivatives such as PLD and LTLD were examined. The efficacy of a combination of LTLD and RFA has been confirmed in the phase III HEAT study. As

chemotherapeutic drugs continue to advance, a variety of novel chemotherapeutic agents have been developed. in 2021, the NCCN recommended that FOLFOX serve as a first-line option (category 2B). Further advances in molecular therapy and immune therapy have challenged the dominance of conventional chemotherapy. Unlike the direct inhibition of DNA synthesis by chemotherapeutic agents, molecular strategies inhibit multi-kinases involved in cell proliferation. Molecularly targeted agents such as sorafenib and lenvatinib have demonstrated efficacy. That said, they are restricted to certain patients with Child-Pugh class A liver disease since they are not superior to conventional chemotherapy for patients with Child-Pugh class B or C liver disease. The appearance of immune agents has inaugurated a new era of systemic treatment of liver cancer, but immune therapy has a modest efficacy in circumstances involving overactivation of the Wnt/β-catenin signaling pathway, which occurs in 50% of HCC and which is related to a recurrence rate as high as 70% at 5 years. Immune therapy also offers a marginal survival benefit in hepatic virus infection-related HCC. Thus, alternatives to conventional chemotherapy, MTAs, and immune therapy are particularly controversial. What strategies should be adopted for patients with Child-Pugh class B or C liver disease? What is the nature of HCC recurrence? The current PFS for these strategies is less than one year according to clinical studies, and the current authors have justified questions about the costs and benefits of those strategies. All of the aforementioned topics need to be examined further.

Moreover, chemotherapeutic agents have been used to treat locoregional lesions through approaches such as TACE and HAIC. TACE includes cTACE and DEB-TACE. Various agents are used in TACE systems. In addition, HAIC is a consistent transcatheter arterial infusion strategy without embolization that is often used because it delivers a drug at a higher concentration. FOLFOX is a mainstay of HAIC. Moreover, HAIC is superior to TACE according to a recent prospective study. Although HAIC confers overwhelming advantages and is likely to have the most potential as a therapy, it is only used in Asia and is not mentioned in guidelines globally. Moreover, TACE can serve as a bridge therapy and downstaging therapy; whether HAIC can perform those roles is uncharted territory.

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Review

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Update on hormone therapy for the management of postmenopausal women

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SUMMARY Hormone therapy (HT) has been used in postmenopausal women for decades in clinical practice. With further analysis and newer studies, the benefits and risks of HT have been repeatedly verified and discussed. HT is recommended for the treatment of vasomotor symptoms (VMS), genitourinary syndrome of menopause (GSM) and the prevention of osteoporosis. However, the precise association between HT and the risks of cardiovascular diseases, venous thromboembolism, neurodegenerative diseases, breast cancer, and endometrial cancer remains controversial. Therefore, determining how to take advantage of and control the risks of HT by adjusting the initiation time, regimen, and duration is crucial. Recent studies have indicated that HT is not related to the risk of all-cause, cardiovascular, or breast cancer mortality although it might increase the incidence of some chronic diseases. For symptomatic postmenopausal women under the age of 60 without contraindications, early initiation of HT is safe and probably has a mortality benefit over the long term. Initiating HT close to menopause at the lowest effective dose is more likely to have maximal benefits and the lowest risks. Transdermal and vaginal HT may have a lower risk, but recent evidence suggests additional clinical benefits of oral HT formulations in relieving VMS and preventing osteoporosis. The pooled cohort risk equation for atherosclerotic cardiovascular disease (ASCVD) and the free app named Menopro can be used to perform individual risk assessments. In addition, Chinese herbal medicines have benefits in alleviating hot flashes, depression, and menopausal symptoms, although further data are needed to strongly support their efficacy. Acupuncture and electroacupuncture have definite efficacy in the treatment of postmenopausal symptoms with few adverse effects, so they are a reasonable option as an alternative therapy for high-risk women. This review discusses the history of, guidelines on, and strategies for HT in order to make suggestions based on the most up-to-date evidence for the management of postmenopausal women.

Keywords hormone therapy, post-menopause, menopausal management, gynecology

1. Introduction

Menopause is by definition amenorrhea for 12 consecutive months after the final menstrual period (FMP) (*1*). It is a permanent end to the menstrual cycle following the loss of ovarian follicular activity. The prominent decrease in estrogen production of the ovaries often leads to menopausal symptoms such as systemic vasomotor symptoms (VMS), vulvovaginal atrophy (VVA), and genitourinary syndrome of menopause (GSM). Menopause is also related to an increased prevalence and incidence of cardiovascular disease, stroke, Alzheimer's disease, dementia, and breast cancer (*2,3*). Post-menopause refers to the stage after the final menstrual period in a woman's life (*4*). As life expectancy increases, women will have a longer postmenopausal period. Menopausal symptoms and the risk of various related diseases are markedly higher in postmenopausal women compared to premenopausal women (*5*).

Hormone therapy (HT) is considered to be the most effective way to relieve menopausal symptoms. It has been used in clinical practice for over 60 years since the 1960s; however, the benefits and risks of HT have been controversial. In 2002, the Women's Health Initiative (WHI) found that HT increased the incidence of coronary heart disease and breast cancer, which led to a precipitous decline in the use of HT (*6*). Upon further analysis of the WHI data and with support from newer

studies (*7-9*), international societies and organizations such as the International Menopause Society (IMS), the North American Menopause Society (NAMS), the European Menopause and Andropause Society (EMAS) have formulated guidelines and announced consensus opinions on the use of HT. As understanding of HT improves, studies have found that HT is highly beneficial to symptomatic women who are younger than 60 years of age, within 10 years of menopause, and without contraindications such as active liver disease or thromboembolic disease (*10-12*).

This article reviews HT for the management of postmenopausal women by discussing various guidelines, strategies, and evidence. The purpose of this review is to organize the information on postmenopausal HT and to make suggestions according to the most up-to-date evidence for the management of postmenopausal women.

2. History of HT

In 1942, an estrogen product named Premarin was approved by the FDA for treatment of postmenopausal symptoms. With the feminist movement and the desire to be "feminine forever" in the 1960s, estrogen therapy (ET) was widely used to treat menopausal women (*13*). In the 1970s, a study found that estrogen supplements were related to an increased risk of endometrial cancer (*14*). Nevertheless, studies over the following years found that combining estrogen with progesterone could reduce the risk of endometrial cancer, so ET was switched to hormone replacement therapy (HRT) or HT (*15*). In the 1980s and 1990s, numerous clinical studies such as the Postmenopausal Estrogen/Progestin Intervention (PEPI) trial suggested that HT was safe for the treatment of menopausal symptoms and beneficial at preventing chronic diseases including cardiovascular disease (CVD) (*16,17*). The use of HT increased rapidly, reaching the second peak in its history. The first guideline on HRT management was published by the American College of Physicians in 1992, and it suggested the use of HT to prevent related chronic diseases (*18*). The FDA also approved HRT for the treatment of menopause and prevention of postmenopausal osteoporosis in 1995 (*19*).

To confirm the clinical benefits of HT, many organizations and scholars started large randomized trials to evaluate the effect of HT on related chronic diseases. In 1998, the Heart and Estrogen/progestin Replacement Study (HERS) found that HT did not reduce the overall rate of coronary heart disease (CHD) events but it did increase thromboembolic events and gallbladder disease in postmenopausal women with established coronary disease (*20*). The Women's Health Initiative (WHI) trial started in 1998 and preliminary results were published in 2002; the trial found that HT increased the incidence of CHD and breast cancer, with a reduction in colorectal cancer and osteoporotic fractures (*6*). The Oral Conjugated Equine Estrogens (o-CEE) plus Medroxyprogesterone Acetate (MPA) trial by the WHI was prematurely discontinued in 2002 and a trial of o-CEE alone was also stopped in 2004 because of the high risks of breast cancer and CVD. The unexpected results of the WHI trials increased panic and confusion among HRT recipients and doctors, which led to a precipitous decline in the use of HT.

After the results of the WHI trials were published, the safety and effectiveness of HT have been disputed. Many of the results of those trials have also been extensively debated. Professor Thomas Clarkson, DVM was a pioneer who demonstrated that the initiation time of HT may determine whether the benefits exceed the risks involved in coronary artery atherosclerosis (*21*). The timing hypothesis or "window of opportunity" theory attracted great attention in helping to explain the different results of various observational studies and the WHI trials (*7*). Subsequent clinical trials including the 2017 Kronos Early Estrogen Prevention Study (KEEPS) and the 2016 Early Versus Late Intervention Trial with Estradiol (ELITE) substantiated the safety of HT when initiated early in post-menopause (*8,9*).

In 2007, a secondary analysis of the WHI trials reported that women who began HT within 10 years of menopause tended to have a reduced risk of CHD and a favorable total mortality (*22*). Cumulative follow-up data from the WHI trials published in 2017 indicated that HT was not related to cause-specific mortality or all-cause mortality (*23*). Further follow-up data from the WHI trials focusing on the long-term risk of breast cancer were published in 2020 and led to the conclusion that CEE combined with MPA or CEE alone would not increase breast cancer mortality (*24,25*).

The history of HT development is complex and tortuous (Figure 1). As understanding of HT has improved, guidelines and consensus opinions have been published and updated to regulate its use.

3. Guidelines and consensus opinions

Based on the secondary analysis of the WHI trials and further studies, how HT can benefit postmenopausal women has become clearer. Several guidelines and consensus opinions have been updated in order to guide clinicians to make clinical decisions more appropriately and accurately (*1,10,11,26-31*). Nevertheless, there are similarities and differences among the guidelines that warrant attention (Table 1-2).

Guidelines and consensus opinions recommend HT in the treatment of postmenopausal symptoms including VMS and GSM, but none of them recommend its use to prevent CHD or breast cancer. Before making a clinical decision, risk factors such as CVD, breast cancer, obesity, age, and time from the onset of menopause should be taken into consideration. Most statements generally agree in recommending the use of HT in

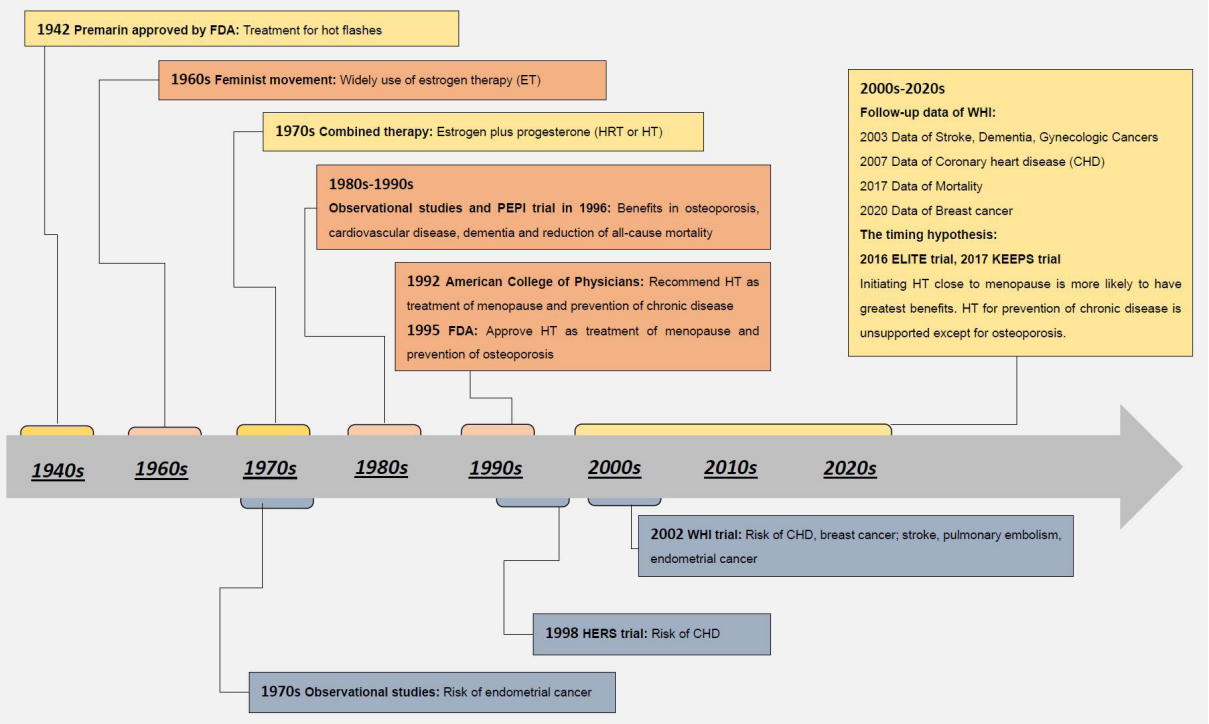

Figure 1. Timeline of development of hormone therapy for postmenopausal women. CHD: Coronary heart disease; ELITE: the Early versus Late Intervention Trial with Estradiol; FDA: the United States Food and Drug Administration; HERS: the Heart and Estrogen/progestin Replacement Study; HT: Hormone Therapy; HRT: Hormone Replacement Therapy; KEEPS: the Kronos Early Estrogen Replacement Study; PEPI: the Postmenopausal Estrogen/Progestin Intervention trial; WHI: the Women's Health Initiative trial.

symptomatic postmenopausal women younger than the age of 60 within 10 years of the onset of menopause. Use of HT is also encouraged in women with hypogonadism, early menopause, or primary ovarian insufficiency (POI) without discontinuation until they reach the mean age for the onset of menopause. Initiating HT close to menopause at the lowest effective dose is more likely to have the greatest benefits and the lowest risks, including short-term symptomatic relief and long-term prevention of chronic disease. Most guidelines also mention different routes and formulations. Compared to oral HT, transdermal therapy is recommended as a safer approach in women with an elevated risk of VTE because it is less likely to cause thrombotic risks such as stroke and coronary artery disease. Low-dose vaginal HT is recommended for genitourinary syndrome, and involvement of an oncologist is recommended for women who at risk of breast cancer. Compounded HT is not recommended due to the lack of evidence of its safety and effectiveness.

Most of the differing recommendations in the updated guidelines involve the duration of HT and opinions on related chronic diseases such as postmenopausal osteoporosis, CHD, dementia, obesity, and breast cancer. Some guidelines advocate individualized decisions regarding the duration based on consideration of the risk-benefit ratio without mandatory limits; some suggest that clinicians attempt to reduce or stop administration after symptoms have been relieved and then adjust the dose depending on the patient's quality of life. The NICE, AACE, and ACE advise a duration of 5 years or less. Although none of these latest guidelines support HT for the primary or secondary prevention of CHD, some still feature data supporting its potential cardiovascular benefits if given close to menopause. Recommendations for the use of HT to prevent osteoporosis and diabetes also vary in different guidelines. Individualized formulations are proposed in new recommendations, and the NAMS offers a mobile app called Menopro to help women and clinicians ascertain possible treatment options to make an appropriate decision together.

4. Indications for HT

4.1. Vasomotor symptoms (VMS)

VMS affects over 80% of postmenopausal women, with overwhelming symptoms including hot flashes, fatigue, muscle and joint aches, sleep disturbances, obesity, and depression (*32*). In postmenopausal women without contraindications, HT containing estrogen alone or together with progesterone in cyclic or continuous administration remains the recommended treatment for VMS. Combined progesterone with estrogen is used in women with an intact uterus to protect the endometrium while estrogen alone can be used in women without a uterus. Over 32 randomized, placebo-controlled trials currently recommend HT as a treatment for VMS (*31*).

Table 1. General agreement in guidelines on hormone therapy

Aspect of hormone therapy	General agreement
Indications	• **Menopausal symptoms** • **Primary ovarian insufficiency (POI) or early menopause** (ACOG, BMS, Endocrine Society, Global Consensus, IMS, NAMS, NICE)
Risk considerations	• **Age, time from onset of menopause** (AACE and ACE, BMS, Global Consensus, IMS, NAMS) • **CVD, breast cancer** (AACE and ACE, Endocrine Society) • **Lipid profile, Smoking history** (AACE and ACE) • **Individualized decisions based on the risk-benefit ratio** (ACOG) • Not evaluated (USPSTF)
Initiation	• **Age < 60 yr, within 10 years of the onset of menopause** (AACE and ACE, BMS, Global Consensus, IMS, NAMS) • **Possible benefit at preventing CVD when initiated close to menopause** (AACE and ACE, ACOG, BMS, Global Consensus, NAMS)
Duration	• **Individualized decisions** based on consideration of the risk-benefit ratio without mandatory limits (ACOG, BMS, Endocrine Society, Global Consensus, IMS, NICE,) • **Shortest total duration** for treatment goals and risk assessment (AACE and ACE, Endocrine Society, NAMS) • **5 years or less** (AACE and ACE, NICE) • Not evaluated (USPSTF)
Dosage	• **Lowest effective dose** needed to relieve symptoms and minimize risks of therapy (AACE and ACE, ACOG, Global Consensus, NAMS) or • **individualized dose to minimize risks** (BMS, IMS, NICE,)
Transdermal therapy	• **Recommend for women with an elevated risk of VTE** (AACE and ACE, ACOG, BMS, Endocrine Society, Global Consensus, IMS, NICE, NAMS,) • **obesity** (Endocrine Society, IMS, NICE) • **hypertension** (AACE and ACE; Endocrine Society) • **hypertriglyceridemia, or cholelithiasis** (AACE and ACE) • Not recommended (USPSTF)
Vaginal therapy	• **Recommend for genitourinary syndrome of menopause** (AACE and ACE, ACOG, Endocrine Society, Global Consensus, IMS, NICE, NAMS) • **An oncologist needs to be involved in treating woman at risk of breast cancer** (ACOG, Endocrine Society, IMS, NAMS) • Not addressed (USPSTF)
Compound hormone therapy	• **Recommend against** non-FDA approved compounded hormone therapy

Included Guidelines: AACE and ACE (*27*) 2017; ACOG (*28*) 2014; BMS (*31*) 2020; Endocrine Society (*29*) 2015; Global Consensus (*30*) 2016; IMS (*10*) 2016; NICE (*26*) 2016; NAMS (*11*) 2017; USPSTF (*1*) 2017.
AACE and ACE: American Association of Clinical Endocrinologists and American College of Endocrinology; ACOG: American College of Obstetricians and Gynecologists; BMS: British Menopause Society; IMS: International Menopause Society; NAMS: the North American Menopause Society; NICE: National Institute for Health and Care Excellence; USPSTF: United States Preventive Services Task Force.

Table 2. Different opinions on hormone therapy to prevent chronic disease

Items	Osteoporosis	CVD/CHD	Diabetes	Dementia
AACE and ACE (*27*) 2017	A	B	D	E
ACOG (*28*) 2016	D	B	E	E
BMS (*31*) 2020	A (First-line)	B	E	C
Endocrine Society (*29*) 2015	B	D	B	D
Global Consensus (*30*) 2016	A (Second-line)	B	E	B
IMS (*10*) 2016	A	D	A	B
NAMS (*11*) 2017	A	B	B	D
NICE (*26*) 2016	B	C	C	D
USPSTF (*1*) 2017	B	D	B	D

A Supported
B Not recommended but data suggest potential benefits
C Not recommended but safe
D Not recommended
E Not mentioned

AACE and ACE: American Association of Clinical Endocrinologists and American College of Endocrinology; ACOG: American College of Obstetricians and Gynecologists; BMS: British Menopause Society; CVD: Cardiovascular disease; CHD: Coronary heart disease; IMS: International Menopause Society; NAMS: North American Menopause Society; NICE: National Institute for Health and Care Excellence; USPSTF: United States Preventive Services Task Force.

Estrogen or combined estrogen/progestogen therapy can relieve the weekly frequency and severity of hot flashes by 75% and 87%, respectively (*33*).

Both oral and transdermal estrogens have been found to be clinically effective in relieving VMS, but opinions are divided in different studies regarding the optimal choice. The KEEPS trial suggested parallel and definite alleviation of hot flashes, night sweats, and poor sleep by oral or transdermal estrogen with a dosage lower than that commonly recommended (*34,35*). Transdermal estradiol (t-E2) plus intermittent micronized progesterone (IMP) has proven to be effective in prevention of depressive symptoms (*31*). Although oral and transdermal HT were both effective and did not differ significantly in efficacy, transdermal HT was thought to be more cost-effective and likely to cause fewer adverse events or result in a lower rate of discontinuation (*36*). However, a recent systematic review noted a cumulative amenorrhea rate ranging from 18% to 61% with oral HT and from 9% to 27% with transdermal HT. Oral HT formulations have a higher rate of amenorrhea and a lower rate of discontinuation than most transdermal HT formulations. A combination of oral 17 β-estradiol (E2) plus progesterone (P4) resulted in the lowest rate of bleeding and is probably an appropriate option for relieving moderate to severe VMS (*37*).

4.2. Genitourinary syndrome of menopause (GSM)/ vulvovaginal atrophy (VVA)

The concept of GSM was defined and formally endorsed by NAMS and the International Society for the Study of Women's Sexual Health (ISSWSH) in 2014 as more accurate and inclusive than the term VVA. GSM is defined as a collection of symptoms caused by estrogen deficiency, comprising changes in the genital area or the urinary tract such as a burning sensation or dryness of the vagina, dyspareunia, dysuria, and urinary tract infections (*38*).

Lubricants and moisturizers are considered the first-line treatment for the symptoms of vaginal dryness and painful sex, and especially for women whose symptoms primarily occur with coitus. Lubricants and moisturizers can be used alone or in combination with estrogen depending on the severity of GSM symptoms or the patient's preference (*26*). If vaginal lubricants or moisturizers are not effective at alleviating pain, systemic and local estrogen HTs are recommended to treat GSM in postmenopausal women without contraindications. Low-dose vaginal estrogen, the "gold standard" of therapy, remains the most effective way to treat postmenopausal women with only vulvovaginal symptoms. Systemic estrogen therapy is effective at treating concurrent symptoms of GSM, and it is approved or recommended for women with GSM as well as vasomotor symptoms (*26,39*).

Low-dose vaginal estrogen therapies include various preparations used vaginally such as tablets, creams, rings, and capsules with different compounds and doses, and studies have demonstrated that those therapies are an effective and safe treatment for GSM. Vaginal estrogen therapies can prevent recurring atrophy caused by estrogen deficiency, resulting in an enhancement of blood flow, vaginal wall thickness, and elasticity (*40-42*). The various forms of vaginal estrogen products are all effective and safe for the treatment of GSM; trials of these forms of topical estrogen products do not differ significantly in terms of objective endpoints, subjective endpoints, or adverse reactions (*41,42*). Estradiol vaginal cream in a very low-dose of 0.003% used twice a week is effective and well-tolerated (*43*). Conjugated estrogen tablets (0.625 mg) used vaginally have been found to adjust the vaginal pH and the vaginal maturation value (VMV) (*44*). Since clinical effects on objective signs and subjective symptoms will respectively wear off about 3 months and 1 month after the cessation of vaginal estrogen, long-term therapy rather than a short-term intervention with topical estrogen should be considered (*45*). Given that low-dose vaginal estrogens may restrict but not eliminate the systemic absorption of estrogen (*40*), discussing vaginal HTs with an oncologist is an appropriate approach for postmenopausal women with breast cancer or endometrial cancer that is refractory to non-hormonal therapies.

4.3. Osteoporosis prevention

Postmenopausal osteoporosis is a skeletal disorder characterized by low bone mass and micro architectural deterioration of bone tissue mainly resulting from a conspicuous deficiency of reproductive hormones in postmenopausal women (*46*). FDA-approved estrogen is used to prevent instead of treat postmenopausal osteoporosis. The Endocrine Society updated one of its guidelines in 2020 in order to provide professional advice for the pharmacological management of osteoporosis. The guideline recommends HT to prevent all categories of fractures in postmenopausal women with symptoms and a high risk of fracture, and especially in those who are not eligible for specific bone active medications/ bone-specific treatment (bisphosphonates, denosumab, *etc*.). In addition, estrogen-only therapy is appropriate for women who have undergone a hysterectomy. HT is not recommended as a treatment for postmenopausal osteoporosis while some osteoporosis drug treatments (ODT) such as bisphosphonates, denosumab, teriparatide, abaloparatide, romosozumab, and SERMs are recommended (*47*).

Studies have elucidated the mechanism by which estrogen protects bone (*48*). Estrogen is the major hormonal regulator in bone resorption and formation. It has a protective effect on bone by regulating osteocytes, osteoclasts, and osteoblasts. Receptors include ERα, osteoclast progenitors, and T-lymphocytes (*49*). OPG/

RANKL and the Sost/Dkk1/Wnt are important signaling pathways in bone metabolism (*48,50*).

Clinical trials have demonstrated the clear role of HT in the prevention of postmenopausal osteoporosis. The large WHI trials first indicated that a fixed composition of CEE and MPA may lead to a decreased risk of fractures. However, the WHI trials also reported an increased risk of breast cancer and cardiovascular and cerebrovascular events, which led to a sharp decline in the use of HT. After the decline in its use, parallel increased incidence of fractures was also reported (*6,51*). More recently, HT has proven to be effective in reducing the risk of hip, vertebral, and total fractures. However, this protection is attenuated when HT is discontinued or begun after the age of 60 (*52*). Individualized treatment should be used while considering different formulations, doses, and regimens of HT. Studies have indicated that low-dose and standard-dose sequential therapy with estrogen and progesterone are both safe and effective in increasing or maintaining bone mineral density (BMD) (*53,54*). Use of low-dose estrogens alone is not suggested for women with an intact uterus and ultra-low-dose transdermal estradiol is not recommended for women under the age of 60 (*55*). Moreover, low-dose HT and transdermal HT are considered to be safer than standard-dose oral HT because adverse events such as breast cancer or VTE are less likely while standard-dose HT displays more clinical efficacy in increasing the density of vertebrae and the femoral neck (*54,55*). Recent studies have also explored choosing HT, bone-specific treatment, or a combination of both. Estrogen therapy is a preferred choice compared to bone-specific treatment for women with premature menopause, which is related to increased fracture risk (*56*). However, significant differences in BMD were not found in postmenopausal women receiving HT alone or a combination of bone-specific treatment and HT (*57*).

Overall, HT has proven to have additional benefits of preventing fractures in postmenopausal women with VMS or GSM, and the combined use of HT and calcium plus vitamin D supplements has a synergistic effect on reducing hip fractures (*58*). Bone-specific treatment is needed for treatment of osteoporosis, and individual formulations should be used in light of the benefit-risk balance (*59*). Some of the main ingredients in medicinal plants, such as dioscin, have proven to be effective in animal models, but more clinical trials need to be conducted (*60*).

5. Controversies of HT

5.1. Cardiovascular disease (CVD) and coronary heart disease (CHD)

HT was found to prevent CVD and CHD in postmenopausal women in several observational trials and epidemiological studies such as the Nurses' Health Study (NHS) and PEPI trials (*61,62*). However, an increased risk of CVD was noted in a group receiving o-CEE+MPA in the HERS trial and the subsequent WHI trial (*63,64*). HT was not recommended for long-term prevention of cardiovascular disease, and a post hoc analysis of the WHI trial data as well as several clinical studies were initiated.

Postmenopausal women 10 years after menopause began and women ages 50-59 receiving HT have a lower risk of CHD, although these trends are not significant (*22*). The risks of CHD may possibly persist during CEE+MPA intervention and post-intervention, but cardiovascular disease mortality is not related to HT with CEE+MPA or CEE alone (*23,65*). Studies have also demonstrated that there is little increase in possible cardiovascular risks when initiating HT early during post-menopause, *i.e.*, within 10 years of onset (*8,66,67*).

Published guidelines emphasize that use HT for primary or secondary prevention of CVD is not evidence-based, and initiation before the age of 60 or within 10 years of the onset of menopause appears to reduce the risk of CVD (*68-70*). According to recent guidelines, HT is not recommended for prevention of CVD, and the risk of CVD should be assessed prior to initiation (*71*). The pooled cohort risk equation for atherosclerotic cardiovascular disease (ASCVD) from the ACC/AHA is recommended for individual risk assessment (*72,73*).

Recent studies have also assessed the effect of different formulations and routes of HT on CVD. Evidence shows that o-CEE appears to slow down accumulation of epicardial adipose tissue while transdermal 17 β-estradiol may increase the progression of coronary artery calcification related to epicardial and paracardial adipose tissue (PAT) (*74*). Moreover, o-CEE may also help reduce an increase in PAT as gauged by carotid intima-media thickness (CIMT) compared to transdermal 17 β-estradiol (*75*). o-CEE may be safer than transdermal 17 β-estradiol in women with cardiovascular risks. Nevertheless, further studies need to be conducted to explore the definite role of different forms, routes, and durations of HT in terms of cardiometabolic health.

5.2. Venous thromboembolism (VTE)

Observational and clinical studies including the WHI trials have noted increased VTE and stroke events in postmenopausal women receiving oral HT containing estrogen (*76*). Oral estrogen is associated with an increased risk of VTE whereas transdermal estrogen, micronized progesterone, and pregnane derivatives appear to be safe (*77*). Recent studies have indicated that transdermal estrogen alone or combined with micronized progesterone may possibly be the safest formulations for women at high risk of VTE, while oral HT is not recommended because of the relevant risk (*78,79*). Thus, the personal risk of VTE needs to be evaluated before making a decision, and transdermal

HT should be considered beforehand in postmenopausal women with a high risk of VTE. Additional trials need to be conducted to determine the effect of various routes and formulations of progestin and estrogen on the risk of VTE.

5.3. Alzheimer's disease (AD), Parkinson's disease (PD), and dementia

The WHI trials also mentioned that the use of o-CEE+MPA increased the risk of neurodegenerative diseases such as AD, PD, and dementia (*6,80*). The timing hypothesis and healthy cell bias hypotheses were proposed by scholars to explain those negative effects (*81,82*). The potential relationship between neurodegenerative diseases and HT is still hotly debated due to the incompatible results of different observational studies and randomized controlled trials (RCTs) (*83*).

A recent nationwide case-control study of 84,739 postmenopausal women in Finland indicated that systemic HT increased the risk of AD but vaginal estradiol did not affect that risk (*84*). However, the latest systematic reviews of various clinical studies have yielded disparate results. A meta-analysis of 25 case-control studies and cross-sectional studies suggested that estrogen replacement therapy reduced the incidence of AD and PD and that it had positive clinical effects, such as slowing their progression (*85*). Another meta-analysis of 14 observational and 11 controlled clinical trials indicated the HT and AD are not significantly related (*86*). A large systematic review of 28 case-controlled, cohort, and randomized-controlled studies found that HT was significantly associated with AD and all-cause dementia, but the review found no significant association between HT and PD. A non-linear time-response relationship between HT and AD has also been noted. In a subgroup analysis, estrogen-progestogen was related to a higher risk of AD compared to other formulations while progestogen and estrogen-progestogen may contribute to the development of PD. However, the relationship between HT and AD is restricted to the first 5 years of treatment and the association appears to reverse after that (*87*).

In summary, growing evidence supports the timing hypothesis in neurodegenerative diseases and suggests that HT be initiated early (*83,87*). The controversial results of different clinical studies and systematic reviews are possibly due to a small sample size or different HT formulations used in the studies analyzed. Therefore, studies with large sample sizes need to be conducted and formulations used in HT need to be clearly identified.

5.4. Breast cancer and endometrial cancer

The association between HT and breast cancer remains controversial. Based on the 5.2-year follow-up data from the WHI trial, an increased risk of breast cancer was noted in women with an intact uterus receiving o-CEE plus MPA, but a similar trend was not noted in women who underwent a hysterectomy and who received CEE alone (*6,80*). Moreover, the 10.7-year follow-up data from the WHI trial indicated a possible reduction in the group of taking CEE alone, in contrast to several observational studies suggesting CEE may increase the incidence of breast cancer, and especially in women with a lower BMI and receiving treatment for a longer duration (*88*). The latest follow-up data suggests that women who have undergone a hysterectomy and who are receiving CEE alone have a lower incidence of breast cancer and breast cancer-specific mortality, whereas women receiving CEE plus MPA have a higher incidence of breast cancer. Women who underwent a hysterectomy before the age of 60 and who received CEE seem to have a mortality benefit over the long term *(89*). Although taking CEE alone will not increase the incidence of breast cancer and HT will not increase the breast cancer mortality over time, HT should not be used for the sole purpose of reducing the risk of breast cancer risk because of complicated factors related to it (*24,25*). Estrogen-progestogen combinations are related to a higher risk of breast cancer compared to estrogen-only preparations, which indicates that the progestogen component is possibly the reason for the increased risk (*90,91*). The risk also increased with a longer duration of HT. HT for longer than 5 years was significantly related to an increased incidence of breast cancer in patients ages 50-69 (*91*). Recent data also indicated that HT containing micronized progesterone appears to be safe for the breasts with a markedly lower risk of breast cancer in comparison to HT with progestins (*92*). Although current thinking suggests that estrogen-progestogen combinations have a potential risk of promoting breast cancer, some scholars have contended that the practical risk is not as high as that for other endogenous factors or lifestyle factors (*93*). In addition, avoid use of HT in all patients is irrational because the all-cause mortality was not significantly affected and benefits may outweigh risks when initiated early (*24,25*).

The risk of endometrial cancer is also a concern for postmenopausal women receiving HT. An increased risk of endometrial cancer was reported in postmenopausal women receiving estrogen alone, tibolone, or sequential combined therapy (*94*). Since the balance between progestogen and estrogen is a key factor influencing the endometrium, the dose or duration of progestogen needs to be adjusted depending on the estrogen treatment. Continuous combined therapy at the right dose is safer than sequential administration in protecting the endometrium (*95*). Although studies have indicated that HT containing micronized progesterone is related to a lower risk of breast cancer, it may not be as efficient as therapies with synthetic progestins in terms of reducing the risk of endometrial cancer (*25,96*).

6. Complementary and alternative therapies

HT is recommended as the treatment of choice for relieving moderate-to-severe postmenopausal symptoms. However, many women still prioritize complementary and alternative therapies, and especially those worried about potential risks or with contraindications for HT. Alternative therapies such as herbal remedies, acupuncture, and mind-body therapies are also definite clinical options for postmenopausal women.

Herbal derivatives are commonly used for relief of mild-to-moderate postmenopausal symptoms. Black cohosh, Hypericum perforatum (St John's wort), and Dan quai have been found to be effective in relieving VMS (hot flashes, insomnia, and irritability). Schisandra chinensis is useful at relieving sweating and heart palpitations. Ginkgo is effective at treating attention disorders and ginseng can improve sexual function (*97*). Epimedium brevicornum Maxim can significantly increase BMD (*98*). In addition, individual RCTs have found that Chinese herbal medicines including compound capsules, granules, and oral herbal decoctions have benefits in alleviating hot flashes, depression, and menopausal symptoms (*99,100*). However, there is still insufficient data from large systematic reviews to conclude that Chinese herbal medicines are more effective than a placebo or HT (*101,102*).

Acupuncture has become more popular as a traditional Chinese therapy with few adverse reactions. Acupuncture and electroacupuncture have definite effects in treating postmenopausal symptoms such as insomnia but are ineffective in relieving hot flashes (*103-105*). Current evidence shows that manual acupuncture is safer than HT (106). For menopausal women with breast cancer, additional acupuncture significantly relieves relevant symptoms for at least 3 months. Acupuncture is an appropriate alternative to HT for women with breast cancer (*105*).

To date, although there is insufficient evidence to strongly support the effectiveness and safety of alternative therapies, but these therapies warrant exploration. Strictly regulated herbal supplements need to be used and standardized clinical studies need to be conducted to provide strong evidence.

7. Conclusion

HT has a tortuous and complex history with controversial risks and benefits. With further studies and analysis, the use of HT has gradually been standardized based on updated guidelines, and more preparations are available for patients to choose. Although many potential therapies are being developed, HT continues to be the most effective treatment available for postmenopausal women with VMS or GSM. It is not related to the risk of all-cause, cardiovascular, or breast cancer mortality although some formulations might increase the incidence of chronic diseases. For symptomatic postmenopausal women under the age of 60 without contradictions, early initiation of HT is safe and probably has a mortality benefit over the long term. Considering the long-term risks of HT, different types, doses, and durations of HT need to be devised for different individuals. The free app named Menopro and the pooled cohort risk equation for atherosclerotic cardiovascular disease (ASCVD) can be used to perform individual risk assessments. Depending on the willingness of patients and assessment of risks, non-hormone therapy and alternative therapy can also be choices. Chinese herbal medicine has benefits in alleviating hot flashes, depression, and menopausal symptoms. Acupuncture and electroacupuncture have definite efficacy in the treatment of postmenopausal symptoms with few adverse effects.

Current discussions of and issues with HT focus on the duration, the assessment of risks, and the optimal choice of regimens. Although there is insufficient data on how long HT can be safely used, clinicians are advised to reduce or stop therapy after menopausal symptoms have been relieved. The risks of HT mainly involve CVD, VTE, neurodegenerative diseases, breast cancer, and endometrial cancer. The relationship between HT and these chronic diseases remains controversial, and HT is not recommended to prevent them. Initiating HT within 10 years of menopause at the lowest effective dose is more likely to have maximal benefits and the lowest risks. Transdermal and vaginal HT may also have lower risks, but recent evidence suggests additional clinical benefits of oral HT formulations in relieving VMS and preventing osteoporosis. In terms of different formulations, o-CEE appears to help reduce the risk of CVD. Transdermal estrogen with or without micronized progesterone has a lower risk of VTE. HT containing micronized progesterone appears to be safe for the breasts while those containing synthetic progestin have stronger protective effects on the endometrium and a lower risk of endometrial cancer.

Therefore, more studies need to be conducted in the future to clarify the precise association between HT and related chronic diseases. The safety of specific durations and various preparations of HT needs to be examined in trials with a large sample size and specific formulations. Stronger evidence is needed to prove that Chinese herbal medicine and acupuncture have synergistic effects with HT.

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Review

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Effects and mechanisms of microenvironmental acidosis on osteoclast biology

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SUMMARY Due to continuous bone remodeling, the bone tissue is dynamic and constantly being updated. Bone remodeling is precisely regulated by the balance between osteoblast-induced bone formation and osteoclast-induced bone resorption. As a giant multinucleated cell, formation and activities of osteoclasts are regulated by macrophage colony-stimulating factor (M-CSF), receptor activator of nuclear factor-kappaB ligand (RANKL), and by pathological destabilization of the extracellular microenvironment. Microenvironmental acidosis, as the prime candidate, is a driving force of multiple biological activities of osteoclast precursor and osteoclasts. The mechanisms involved in these processes, especially acid-sensitive receptors/channels, are of great precision and complicated. Recently, remarkable progress has been achieved in the field of acid-sensitive mechanisms of osteoclasts. It is important to elucidate the relationship between microenvironmental acidosis and excessive osteoclasts activity, which will help in understanding the pathophysiology of diseases that are associated with excess bone resorption. This review summarizes physiological consequences and in particular, potential mechanisms of osteoclast precursor or osteoclasts in the context of acidosis microenvironments.

Keywords acidosis, microenvironment, osteoclasts, physiological consequences, acid-sensitive receptors/pathways

1. Introduction

The normal functioning of cells depends on proper maintenance of acid-base balance in the extracellular microenvironment (*1*). The pH of arterial blood ranges between 7.36 and 7.44, whereas the pH of venous blood is approximately 7.6 (*2*). In the human body, several intracellular and extracellular buffers help to keep the pH within this narrow range. In the blood, the HCO_3^-/CO_2 buffer system, plasma proteins, and histidine residues of hemoglobin provide buffering activity for H^+ and HCO_3^- (*2,3*). It is worth noting that the interstitial fluid lacks pH buffers, and for this reason, the pH of this fluid is determined by multiple complex factors (*3*). In this case, pH of the microenvironment greatly depends on the type of tissue, metabolic activity of different cells, and the status of blood supply in the local environment. For the musculoskeletal system, the pH of bone tissue microenvironment is affected by the following factors, such as tumors (*3,4*), inflammation (*5*), infection (*6*), wound healing (*7*) or fracture (*8*).

Generally, microenvironmental acidosis has a negative effect on the musculoskeletal system of the human body. Once the acid-base equilibrium is broken, the function of pH-dependent enzymes and membrane transporters in cells is impaired leading to bone malfunction and metabolic dysfunction (*9*). For osteoblasts, extracellular acidosis reduces the activity of alkaline phosphatase (ALP), decreasing formation of extracellular matrix, thereby inhibiting most of the biological functions of osteoblasts, decreasing trabecular bone formation, and reducing bone density (*10*). For bone marrow mesenchymal stem cells (BMSCs), although short-term acidic stimulation enhances the stem cell phenotype, cell proliferation and viability, it reduces the migration ability of BMSCs (*11*). More importantly, acidosis impairs the osteogenic differentiation of BMSCs (*12*).

However, osteoclasts are an exception. Osteoclasts, as non-proliferative polykaryons that differentiate from monocyte precursors, are responsible for bone remodeling and maintenance of the dynamic calcium homeostasis. Osteoclasts have the ability

to sense and respond to acidosis in the extracellular microenvironment, and osteoclasts require proton stimulation for differentiation, bone resorption activities and survival (*13*). Bone resorption activities of osteoclasts can be maximized and cause bone mineral loss when microenvironmental pH is 6.9, and a weak alkaline condition inhibits osteoclastogenesis (*14-16*). In addition, bone resorption functions of osteoclasts depend on excretion of H^+ to the sealing zone through vacuolar H^+-ATPase (V-ATPase), which helps dissolve the bone matrix. In turn, excess protons enter the cytoplasm from the sealing zone and are eventually discharged out of the cell through vesicles, leading to rhythmic pH oscillations and gradual intracellular acidification during the resorption process (*13,17,18*). Taken together, osteoclast biology is closely associated with protons (Table 1).

A better understanding of the functions and molecular mechanisms of acid-sensitive receptors/channels of osteoclasts under microenvironmental acidosis will help in establishing whether and how they can be used as drug targets for patients with bone metabolism disorders that are characterized by bone loss. Therefore, we summarize and explain the physiological consequences and underlying mechanisms of microenvironmental acidosis on osteoclasts.

2. Effects of microenvironmental acidosis on osteoclast biology

2.1. Differentiation

Osteoclasts are giant multinucleate cells that are derived from hematopoietic precursor cells of the monocyte/macrophage lineage (*19*). During this process, osteoclast differentiation is promoted by macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor-kappaB ligand (RANKL) secreted by osteoblasts. RANKL binds its receptor, RANK, located on osteoclast precursor plasma membrane, and further, recruits TNF receptor associated factor 6 (TRAF6). TRAF6 activates downstream signaling pathways of nuclear factor-kappaB (NF-κB) and mitogen-activated protein kinases (MAPKs). The nuclear factor of activated T-cells cytoplasmic 1 (NFATc1), a master regulator, is induced by NF-κB, MAPKs signaling cascades, calcium signal and autoamplification loop, which is indispensable for osteoclastogenesis (*13,19*). Extracellular protons, as a result of acidosis, play a significant role in fine-tuning osteoclastogenesis (*20-25*). Actually, extracellular acidosis has been shown to significantly elevate intranuclear NFATc1 levels in rat and rabbit osteoclast precursors through MAPKs (*20*) and Ca^{2+}/calcineurin pathways (*21-23*). Its effect of promoting osteoclastogenesis is comparable to that of RANKL (*21*). Kohtaro Kato *et al.* reported that one of the main action points for acidosis is in the final stages of osteoclastogenesis, especially during the 4-7 days of osteoclast precursor cultures. Moreover, protons can directly promote osteoclast differentiation, independently of bicarbonate ions (*24,25*).

Alkaline drugs or materials can counteract the negative effects induced by protons, such as K citrate or borosilicate glasses, which provide a new direction for clinical treatment of diseases with redundant osteoclastogenesis (*15,16*).

K citrate is commonly used to increase urine pH, thereby inhibiting solute precipitation and kidney stone formation. However, K citrate may also be beneficial in preventing the progression of bone loss. The potassium channel subfamily is an inhibitor of proton-induced osteoclastogenesis, and extracellular K^+ inhibits osteoclastogenesis in a dose-dependent manner. In addition, citrate has calcium-binding abilities, and it competitively binds intracellular Ca^{2+}, suppressing proton-induced NFATc1 signal transduction. Therefore, K citrate can counteract acidosis-induced negative effects, and can even overcome alendronate-associated drug resistance in a long-term acidic microenvironment (*15*). To rebuild bone regeneration balance, Wenlong Liu *et al.* established a local weakly alkaline microenvironment that was generated by biodegradation of borosilicate glasses, which further modulated osteoclast differentiation. Actually, the higher the pH, the lower the differentiation activity of osteoclasts. At a pH of 7.8, a threshold value for regulating osteoclast differentiation, this alkaline material almost completely shut down osteoclastogenesis (*16*).

2.2. Migration, adhesion and fusion

A prerequisite for bone resorption involves a series of complex events that osteoclast precursors must undergo, including attraction/migration, recognition, adhesion, membrane fusion, and finally, formation of giant multinucleated cells (*13*). These events are mainly attributed to DC-STAMP, osteopontin (OPN), Atp6v0d2 and CD47 among others, and defects in these genes inevitably lead to the generation of inactive osteoclasts (*26*). DC-STAMP, a membrane-bound receptor, with no definite ligand, is the main regulator of pre-osteoclast fusion (*26*). Some studies have reported that acidosis does not foster osteoclast precursor fusion, and that mRNA expression levels of DC-STAMP are rarely susceptible to pH changes (*24*). However, more reports showed that extracellular acidosis enhances the fusion of pre-osteoclasts and the largest surface area of mature osteoclasts (*27*), with redundant expression levels of DC-STAMP (*15,28*).

In addition to DC-STAMP, OPN, a matrix protein containing the Arg-Gly-Asp motif, and integrin avβ3, a subunit of the cell-surface receptor superfamily, coordinate to mediate the adhesion and migration of osteoclast precursors and osteoclasts (*26*). In fact, protons promote the expression of OPN, which in turn increases

its interaction with integrins avβ3, thereby inducing the activation of proline-rich tyrsosine kinase 2 (Pyk2) and Src protein-tyrosine kinase (Src) signals and the production of actin rings, which stimulate the migration and adhesion of osteoclast precursors (*20,22,29,30*).

Gap junction, a unique plasma membrane structure, is composed of two connexon hemichannels, connected to neighboring cells, and mediates the exchange of ions and small molecules (*31*). Osteoclast fusion is a multifactorial process that may involve gap junction communication (GJC) in addition to the above mentioned cytokines (*31*). To characterize the role of GJC, Elina Kylmäoja *et al.* showed that, at neutral pH (7.4), AAP10, a GJC agonist, was shown to promote the expression levels of connexin and maintained gap junctions in an open state, which led to the fusion of osteoclast precursors (*32*). Acidosis (pH 6.5) caused the gap junctions to close, offsetting the effects of AAP10, eventually inhibiting GJC-mediated fusion of osteoclast precursors (*32*). However, this will not affect osteoclastogenesis, which means that although GJC is an inhibitor of osteoclast fusion under acidic conditions, this inhibiting effect is insufficient to interfere with the promoter effect induced by cytokines mentioned above (*32*).

2.3. Bone resorption

After undergoing migration, adhesion and fusion of osteoclast precursors, giant multinucleated osteoclasts are involved in bone resorption and in deterioration of the skeletal microarchitecture (*13,33*). To improve efficiency of bone resorption, the bone-facing plasma membrane is transformed into a ruffled border, a specific late endosome-like domain, and releases H^+, Cl^-, cathepsin K and matrix metalloproteinase 9 (MMP-9) into the sealed area further acidifying the resorption lacuna and dissolving the bone matrix (*13,33*). This acidification is highly efficient and can reduce the pH to 3 within a few minutes of the resorption lacuna when osteoclasts exert bone resorption activity (*34*). Finally, products of bone resorption, including degraded collagen fragments, calcium, phosphate and extra protons, are released from the sealing zone to the extracellular matrix of the osteoclasts through transcytosis pathways (*18*).

Extracellular acidosis increases the size and number of bone resorption pits mediated by osteoclasts, and can even lead to calvarial bone perforations (*35*). In fact, the resorption area was shown to increase 14-fold as pH changed from pH 7.4 to 6.8, accompanied by a 3-fold increase in cathepsin K and V-ATPase activities (*27,36,37*). This response is highly sensitive, a decrease of 0.1 units in pH is enough to cause a two-fold trabecular bone loss (*38*). Interestingly, the relationship between the ability of bone resorption and pH is not linear, but curved. Bone resorption capacity reaches its peak when the extracellular pH drops to 6.8. Alkaline conditions and the peracid environment inhibits resorption activity (*27*). This effect is not desensitized in long-term cultures and bone resorption of osteoclasts continues (*27*).

Osteoclasts resorption activities are subject to paracrine regulation, including RANKL, OPN, Prostaglandin E2 (PGE2) and interleukin 6 (IL-6), which originate from surrounding stromal cells, including osteoblasts and its precursor (*39,40*). PGE2 redundantly expresses induction by osteoblasts under metabolic acidosis, which further stimulates bone resorption by osteoclasts (*39,40*). Cyclooxygenase-2 (COX-2), a cyclooxygenase that converts arachidonic acid into active prostaglandin metabolites, promotes PGE2 secretion. To further characterize paracrine effects on bone resorption, a series of studies were performed and it was found that acidosis specifically up-regulated $[Ca^{2+}]i$ levels, which induced the signal cascade from COX-2 to PEG2 in osteoblasts (*41*). PGE2 directly fosters RANKL expression in a paracrine manner, and RANKL binds the RANK receptor on plasma membranes of osteoclasts, which significantly increases bone resorption and net calcium efflux from the bone (*42-44*). This implies that not only do protons act on osteoclasts themselves, they also act on osteoblasts to promote the activities of osteoclasts in a paracrine manner.

NBCn1, an electrically neutral sodium bicarbonate cotransporter, is present in the ruffled border membrane of osteoclasts. NBCn1 removes bicarbonate from the sealing zone, and excretes bicarbonate into the upper space of osteoclasts through an electroneutral chloride-bicarbonate exchanger (*45*). This process contributes to maintenance of the acidic environment in the resorption cavity, therefore, inhibiting the activities of NBCn1 may provide a new method to inhibit the excessive bone resorption that osteoclasts suffer from acidosis (*45*).

2.4. Apoptosis

Osteoclasts are short-lived terminally differentiated cells. However, the acidic microenvironment can extend the lifespan of osteoclasts (*29*). Their survival rate was shown to have doubled at pH 7.0 when compared to pH 7.6 (*46*). To further define the mechanism of this phenomenon, Alexey Pereverzev *et al.* (*46*) found that these effects are due to Ca^{2+}/protein kinase C (PKC)/extracellular regulated protein kinases (ERK) signaling, rather than Ca^{2+}/calcineurin/NFAT signaling. Notably, when protons induce elevation of $[Ca^{2+}]i$ in osteoclasts, intracellular calcium signals stimulate the activation of PKC and ERK, two anti-apoptotic signals involved in multiple cell types, which promote the lifespan of osteoclasts (*47*).

3. The mechanism of microenvironmental acidosis on osteoclasts

Perception, response or adaptation of osteoclasts to extracellular acidosis is regulated by various proton-

Table 1. Effects of acidosis on osteoclasts biology

Author/year (*Ref.*)	pH value	Exposure time	Osteoclast source	Acidosis-induced actions	The effects of acidosis on osteoclast biology
T R Arnett 1986 (*36*)	6.8, 7.0, 7.2, 7.4	2h, 24h	long bones of rat pups	None	increase bone resorption
P Goldhaber 1987 (*95*)	6.94, 7.15, 7.28	7 d	neonatal mouse calvaria	increase net cell-mediated calcium release	increase bone resorption
A Teti 1989 (*30*)	6.5, 7.05, 7.4, 7.6	90 min	medullary bone of laying hens	increase formation of podosomes of osteoclasts	increase adhesion
T R Arnett 1994 (*94*)	6.76, 7.07, 7.20, 7.30	24h	long bones of rat pups	None	increase bone resorption
T R Arnett 1996 (*38*)	6.6, 6.8, 7.0, 7.2, 7.4	26h	long bones of littermate rat	None	increase bone resorption
T Nordström 1997 (*37*)	6.5, 7.0, 7.5	24, 48h	long bones of new zealand white rabbit	upregulation of vacuolar type H^+ ATPase activity	increase bone resorption
D M Biskobing 2000 (*95*)	6.5, 6.75, 7.4	4h	primary marrow cells of mice	upregulation of carbonic anhydrase II and calcitonin receptor genes	increase osteoclastogenesis and bone resorption
N S Krieger 2000 (*39*)	6.8, 7.1, 7.4	24, 48,51 h	calvariae of neonatal mouse	upregulation of PGE2 synthesis, increase net calcium efflux	increase bone resorption
S Meghji 2001 (*35*)	6.9, 7.0, 7.1, 7.2, 7.3,7.4	72h	calvariae of mice	increase net calcium efflux	increase bone resorption and even lead to bone perforations
D A Bushinsky 2001 (*40*)	7.1, 7.5	24, 48,51 h	calvariae of neonatal mice	upregulation PGE2 levels, increase net calcium efflux	increase bone resorption
Kevin K Frick 2003 (*43*)	7.1, 7.5	24h, 48h	calvariae of mice	increase net calcium efflux	increase bone resorption
Svetlana V Komarova 2005 (*21*)	7.0, 7.6	15, 45, 75, 90 min	RAW 264.7 mouse monocytic cell line	activation of Ca^{2+}/calcineurin/NFAT pathway	increase osteoclastogenesis and bone resorption
Kevin K Frick 2005 (*44*)	7.1, 7.4	24h, 48h	calvariae of mice	increase net calcium efflux	increase bone resorption
Jin-Man Kim 2007 (*20*)	7.0, 7.5, 8.0	1, 2, 3, 4 d	RAW 264.7 mouse monocytic cell line and bone marrow monocytes	activation of MAPK pathway, upregulation of osteopontin protein	increase osteoclastogenesis and migration
Nancy S Krieger 2007 (*41*)	7.1, 7.4	24h, 48h, 51h	calvariae of COX-2 wildtype($^{+/+}$), heterozygous(+/-) and homozygous knockout ($^{-/-}$) littermates	upregulation of COX-2 mRNA and protein, and net calcium efflux	increase bone resorption
Mariusz Muzylak 2007 (*27*)	6.92, 7.15, 7.25	7, 14 d	peripheral blood of cat	upregulation of the expression level of trap, cathepsin K and proton pump enzymes	increase osteoclastogenesis, fusion and bone resorption
Kaori Iwai 2007 (*96*)	7.0, 7.4	75min	bone marrow monocytes of C57BL/6J mouse and RAW 264.7 cell line	activation of OGR1/NFAT pathway	increase osteoclastogenesis
Alexey Pereverzev 2008 (*46*)	6.8, 7.0, 7.2, 7.4, 7.6	18h	long bones of neonatal Wistar rats and RAW 264.7 mouse monocytic cell line	activation of OGR1/Ca^{2+}/PKC signaling	increase osteoclasts survival and suppress osteoclast apoptosis
Riikka Riihonen 2010 (*45*)	6.0,7.2	none	CD14 positive cells from human peripheral blood	upregulation of NBCn1 protein expression	increase bone resorption
Nancy S Kriege 2011 (*42*)	7.1,7.4	24h, 48h	calvariae of neonatal CD-1 mouse	activation of OGR1/Ca^{2+}/COX2, PGE2/ RANKL signaling of osteoblast	increase bone resorption
Kohtaro Kato 2011 (*24*)	6.8, 7.0, 7.2, 7.4	pH 7.4 for 3 d and then pH 6.8 for 21 h	spleen mononuclear cells and bone marrow cells of male mice	upregulation of TRPV4 activity	increase osteoclastogenesis and fusion, especially in the last phase
Heejin Ahn 2012 (*29*)	7.0, 7.5	24h, 40h	bone marrow-derived macrophages of C57BL6 male mice	None	increase osteoclast adhesion, migration, bone resorption activity and survival
Kohtaro Kato 2013 (*25*)	6.8, 7.0, 7.2, 7.4, 7.6, 7.8, 8.0, 8.2	1, 2, 3, 7, 14 d	bone marrow cells of male ddY mice	upregulation of TRPV1, 4 mRNA	increase osteoclastogenesis
Xia Li 2013 (*22*)	6.0, 6.5, 7.0, 7.5	18 h	bone marrow-derived macrophages of rats	activation of [Ca^{2+}]i/NFATc1 signaling mediated by ASIC1a	increase osteoclastogenesis
Carlotta Reni 2016 (*23*)	7.2, 7.4	6d	bone marrow cells of mice	activation of TRPV1 ion channel	increase osteoclastogenesis

PGE2, Prostaglandin E2. NFAT, Nuclear Factors of activated T. MAPK, pathway mitogen-activated protein kinase pathway. COX-2, Cyclooxygenase-2. OGR1, ovarian cancer G protein coupled receptor 1. RANKL, Receptor Activator for Nuclear Factor-κ B Ligand. TRPV, transient receptor potential vanilloid. NFATc1, Nuclear factor of activated T-cells cytoplasmic. ASIC1a, acid-sensing ion channels 1a. Pyk2, proline-rich tyrsosine kinase 2. Src, Src protein-tyrosine kinase. RANK, receptor activator of NF-KB ligand. MMP-9, matrix metalloprotein-9. Tracp, tartrate resistant acid phosphatase. min, minute. h, hour. d, day.

Table 1. Effects of acidosis on osteoclasts biology (continued)

Author/year (Ref.)	pH value	Exposure time	Osteoclast source	Acidosis-induced actions	The effects of acidosis on osteoclast biology
X Li 2017 (*82*)	6.0,7.4	6, 12, 18 h	bone marrow cells of rat femurs	upregulation the expression of αvβ3 integrin and phosphorylation of Src and Pyk2 mediated by ASIC1a	increases osteoclast migration and adhesion
Donatella Granchi 2017 (*97*)	6.9,7.4	2, 4, 7, 14d	RAW 264.7 cell line and peripheral blood mononuclear cells of health volunteers	upregulation of the expression level of RANK, CD44, DC-STAMP, Cathepsin K and MMP-9 mRNA	increase osteoclastogenesis, fusion and bone resorption activity
Elina Kylmäoja 2018 (*32*)	6.5, 7.4	14d	bone marrow mononuclear cells and peripheral blood mononuclear cells of human	None	increase osteoclastogenesis, fusion and bone resorption activity
Wenlong Liu 2019 (*16*)	7.21-7.32,7.43-7.50,7.59-7.64,7.70-7.78,7.9-8.04	5d	RAW 264.7 cell line	None	increase osteoclastogenesis and bone resorption activity
Pedro Henrique Imenez Silva 2020 (*98*)	7.05, 7.4	none	bone marrow of both $OGR1^{-/-}$ and $OGR1^{+/+}$ mice	None	increase bone resorption
Nancy S Krieger 2021 (*28*)	7.1,7.4	45min, 1d, 2d, 4d	bone marrow or spleen cell of both $OGR1^{-/-}$ and $OGR1^{+/+}$ mice	upregulation of the expression level of Cathepsin K, MMP-9, Tracp, Dc-stamp, NFATc1 and RANK RNA mediated by OGR1	increase osteoclastogenesis and bone resorption activity

PGE2, Prostaglandin E2. NFAT, Nuclear Factors of activated T. MAPK, pathway mitogen-activated protein kinase pathway. COX-2, Cyclooxygenase-2. OGR1, ovarian cancer G protein coupled receptor 1. RANKL, Receptor Activator for Nuclear Factor-κ B Ligand. TRPV, transient receptor potential vanilloid. NFATc1, Nuclear factor of activated T-cells cytoplasmic. ASIC1a, acid-sensing ion channels 1a. Pyk2, proline-rich tyrsosine kinase 2. Src, Src protein-tyrosine kinase. RANK, receptor activator of NF-KB ligand. MMP-9, matrix metalloprotein-9. Tracp, tartrate resistant acid phosphatase. min, minute. h, hour. d, day.

sensitive receptor/channels, which fall into three overall categories, including G-protein-coupled receptor (GPCR), transient receptor potential vanilloid (TRPV) and acid-sensing ion channel (ASIC) (Table 2).

3.1. GPCR

Currently, four subfamilies of GPCRs are known, which are ovarian cancer G protein coupled receptor 1 (OGR1), G protein coupled receptor 4 (GPR4), T cell death associated gene 8 (TDAG8), and G2 accumulation protein (G2A) (*48*). Compared to TRPV and ASIC, OGR1 is sensitive to weak acids, approximately at pH 6-8. For the musculoskeletal system, OGR1 signaling was initially reported in the plasma membrane of osteoblasts (*48*). $[Ca^{2+}]$i and inositol phosphate concentrations have been directly associated with the degree of OGR1 and subsequently Gq activation in osteoblasts (*49*). $[Ca^{2+}]$i is a fundamental second messenger, which can lead to a series of signaling cascades, one of which is COX2/PEG2 in osteoblasts, which stimulate bone resorption of osteoclasts and calcium release from bones *via* the paracrine system (*50*).

Similarly, OGR1 causes calcium mobilization in osteoclasts (Figure 1). On the one hand, $[Ca^{2+}]$i promotes osteoclastogenesis (*51*), especially in the early stages (*52*), and bone resorption through the $[Ca^{2+}]$i -calcineurin- NFAT signal (*21*). On the other hand, it inhibits osteoclasts apoptosis through $[Ca^{2+}]$i/PKC/ERK1/2 signaling (Figure 1) (*46*). To further investigate the functions of OGR1, Nancy S. Krieger *et al.* (*53*) established mice with a genetic null mutation in OGR1, and found that both trabecular bone and cortical bone volume increased in $OGR1^{-/-}$ mice when compared to wild-type mice. *In vitro*, the number of $OGR1^{-/-}$ mice-derived osteoblasts increased, and expressions of alkaline phosphatase, type I collagen, osterix, runx2, and RANKL were up-regulated. However, interestingly, the number of tartrate-resistant acid phosphatase (TRAP) stained-positive osteoclasts derived from $OGR1^{-/-}$ mice similarly increased, which is contrary to evidence from OGR1 studies currently. There are two probable explanations for this controversial phenomenon. One, the function of osteoblasts is enhanced so much that the function of osteoclasts that should be suppressed is promoted through paracrine signals in OGR1 global knockout mice. However, this explanation is less likely, because the activity of osteoblasts should also decrease after OGR1 is globally knocked out, but this possibility cannot be fully excluded. Two, there are other proton-sensing channels that play a compensatory role for osteoclasts, such as GPR4 and TRPV4 among others, although OGR1 is inhibited. To explain this discrepancy, this team specifically deleted OGR1 in osteoclasts, and found that $OGR1^{-/-}$ mice bone densities increased (*54*), which is comparable to the previous study (*53*). However, *in vitro*, $OGR1^{-/-}$ mice-derived osteoclast

Table 2. The acid-sensitive mechanism of osteoclasts

Author/year (Ref.)	Receptor/Channels	Cell type	Downstream	The effects of acidosis on osteoclasts biology
Svetlana V Komarova 2005 (*21*)	OGR1	osteoclasts derived from RAW 264.7 cells	PLC- Ca^{2+}-calcineurin- NFATc1	increase osteoclastogenesis, bone resorption
Meiheng Yang 2006 (*52*)	OGR1	osteoclasts derived from long bones osteopetrotic rats	none	increase osteoclastogenesis at early stages
Kaori Iwai 2007 (*96*)	OGR1	osteoclasts derived from RAW264.7 and bone marrow cells	$[Ca^{2+}]i$ - NFATc1	increase osteoclastogenesis
Alexey Pereverzev 2008 (*46*)	OGR1	osteoclasts derived from long bones of neonatal Wistar rats	$[Ca^{2+}]i$- PKC- ERK1/2	increase survival
Hideaki Tomura 2008 (*50*)	OGR1	osteoblasts derived from human osteoblastic cell line	Gq/11-PLC-Ca^{2+}	increase osteoclastogenesis
Kevin K Frick 2008 (*49*)	OGR1	primary bone cells derived from CD-1 mouse calvariae	none	increase $[Ca^{2+}]i$
Hui Li 2009 (*51*)	OGR1	osteoclasts derived from $OGR1^{+/+}$ or $OGR1^{-/-}$ mice	none	increase osteoclastogenesis
Nancy S Krieger 2016 (*53*)	OGR1	osteoclast derived from $OGR1^{+/+}$ or $OGR1^{-/-}$ mice	none	increase osteoclastogenesis, bone resorption
Nancy S Krieger 2021 (*54*)	OGR1	osteoclasts derived from osteoclast-specific deletion of OGR1 mice	none	increase osteoclastogenesis, bone resorption
Asuka Okito 2015 (*55*)	GPR4	osteoblasts derived from mouse bone marrow cells	cAMP/PKA	increase osteoclastogenesis
	TDAG8	osteoclasts derived from mouse bone marrow cells	cAMP	
Hisako Hikiji 2014 (*56*)	TDAG8	osteoclasts derived from bone marrow cells	none	decrease osteoclastogenesis, bone and calcium resorption
Bram C J van der Eerden 2005 (*57*)	TRPV5	osteoclasts derived from $TRPV5^{+/+}$ or $TRPV5^{-/-}$ mice	none	decrease osteoclastogenesis and the number of nuclei per osteoclasts, increase bone resorption
Ritsuko Masuyama 2008 (*77*)	TRPV4	osteoclasts derived from WT and $Trpv4^{-/-}$ mice	$[Ca^{2+}]i$-NFATc1	increase osteoclasts terminal differentiation, bone resorption and survival
Tom Nijenhuis 2008 (*60*)	TRPV5	osteoclasts derived from $TRPV5^{+/+}$ or $TRPV5^{-/-}$ mice	none	increase the number of resorption pits
Rossi F 2009 (*74*)	TRPV1	osteoclasts derived from healthy subjects	$[Ca^{2+}]i$	increase osteoclastogenesis
Aymen I Idris 2010 (*67*)	TRPV1	osteoclasts derived from long bones of mice	IκB and ERK1/2	increase osteoclastogenesis, bone resorption and decrease apoptosis
Estelle Chamoux 2010 (*62*)	TRPV5	osteoclasts derived from human cord blood	$[Ca^{2+}]i$ - RANKL	decrease bone resorption
Francesca Rossi 2011 (*68*)	TRPV1	osteoclasts derived from peripheral blood of menopausal women and healthy subjects	$[Ca^{2+}]i$	increase the number of nuclei per osteoclast and osteoclasts activity of healthy subjects
Kohtaro Kato 2011 (*24*)	TRPV4	osteoclasts derived from male mice	Ca^{2+}-calcineurin-myosin IIa	increase osteoclastogenesis, fusion and migration of osteoclasts
Peng Yan 2010 (*61*)	TRPV5	osteoclasts derived from bone marrow cells of the tibiae and femurs of SD rats	$[Ca^{2+}]i$	increase bone resorption
E Verron 2012 (*99*)	TRPV5	osteoclasts derived from RAW 264.7 cell line	none	None
Ritsuko Masuyama 2012 (*78*)	TRPV4	osteoclasts derived from Trpv4 null mice	$[Ca^{2+}]i$ -calmodulin	increase osteoclastogenesis, migration and bone resorption
Kainat Khan 2012 (*75*)	TRPV1	osteoclasts derived from bone marrow cells of Balb/cByJ mice	$[Ca^{2+}]i$	increase osteoclastogenesis
B C J van der Eerden 2013 (*79*)	TRPV4	osteoclasts derived from $TRPV4^{+/+}$ or $TRPV4^{-/-}$ mice	none	increase osteoclastogenesis and bone resorption for male mice instead of female mice
Fangjing Chen 2014 (*65*)	TRPV6	osteoclasts derived from $TRPV6^{+/+}$ or $TRPV6^{-/-}$ mice	none	decrease osteoclastogenesis and bone resorption
Fangjing Chen 2014 (63)	TRPV5	osteoclasts derived from SHAM and ovariectomy operation mice	none	decrease osteoclastogenesis and bone resorption
F Rossi 2014 (*69*)	TRPV1	osteoclasts derived from $TRPV1^{+/+}$ or $TRPV1^{-/-}$ mice	none	increase osteoclastogenesis and bone resorption
Francesca Rossi 2014 (*72*)	TRPV1	osteoclasts derived from β-thalassemia major patient	none	decreases osteoclastogenesis
Bram C J van der Eerden 2016 (*58*)	TRPV5	osteoclasts derived from $Trpv5^{+/+}$ and $Trpv5^{-/-}$ mice	none	increase osteoclastogenesis and bone resorption

OGR1, ovarian cancer G protein coupled receptor 1. PLC, phospholipase C. NFATc1, Nuclear factor of activated T-cells cytoplasmic 1. PKC, protein kinase C. ERK1/2, extracellular regulated protein kinases 1/2. COX-2, cyclooxygenase-2. PGE2, Prostaglandin E2. cAMP, cyclic adenosine monophosphate. PKA, protein kinase A. RANKL, receptor activator of nuclear factor-kappaB ligand. TGFβ-3, transforming growth factorβ-3. GPR4, G protein-coupled receptor 4. TDAG8, T cell deathassociated G protein 8. TRPV, transient receptor potential vanilloid. WT, Wild Type. IκB, inhibitor of NF-κB. TRAP, tartrate-resistant acid phosphatase. IGF, insulin like growth factor. ASIC1, acid sensing ion channel 1. PI3K, phosphatidylinositol 3-kinase. Pyk2, proline-rich tyrsosine kinase 2. Src, Src protein-tyrosine kinase.

Table 2. The acid-sensitive mechanism of osteoclasts (continued)

Author/year (Ref.)	Receptor/Channels	Cell type	Downstream	The effects of acidosis on osteoclasts biology
Carlotta Reni 2016 (*23*)	TRPV1	osteoclasts derived from type 1 diabetic mice	none	increase osteoclastogenesis
Giulia Bellini 2017 (*70*)	TRPV1	osteoclasts derived from dperipheral blood mononuclear cells of healthy subjects	none	increase osteoclastogenesis and bone resorption
Lin-Hai He 2017 (*73*)	TRPV1	osteoclasts derived from Trpv1 $^{+/+}$ and Trpv1 $^{-/-}$ mice	none	increase osteoclastogenesis
Tengfei Song 2018 (*64*)	TRPV5	osteoclasts derived from RAW 264.7 cell line and bone marrow-derived macrophages	none	decreases bone resorption activity and promote osteoclast apoptosis
Boran Cao 2019 (*80*)	TRPV4	osteoclasts derived from RAW 264.7 cell line	[Ca^{2+}]i-calcineurin-NFATc1	increase osteoclastogenesis and autophagy of osteoclasts
Shu Yan 2019 (*76*)	TRPV1	osteoclasts derived from bone marrow of C57/BL6 mice	none	increase osteoclastogenesis
Haruki Nishimura 2020 (*100*)	TRPV1, 4	osteoclasts derived from TRPV1/TRPV4 double knockout mice and wild type mice	none	increase osteoclastogenesis
Jun Ma 2021 (*66*)	TRPV6	osteoclasts derived from wild type and Trpv6 $^{-/-}$ mice	IGF-PI3K-AKT	decrease osteoclastogenesis, and bone resorption
Xia Li 2013 (*22*)	ASIC1	osteoclasts derived from bone marrow-derived macrophages of rats	[Ca^{2+}]i-calcineurin-NFAT	increase osteoclastogenesis
Xia Li 2017 (*82*)	ASIC1	osteoclasts derived from bone marrow of rats	αvβ3 integrin-Pyk2-Src	increase migration and adhesion

OGR1, ovarian cancer G protein coupled receptor 1. PLC, phospholipase C. NFATc1, Nuclear factor of activated T-cells cytoplasmic 1. PKC, protein kinase C. ERK1/2, extracellular regulated protein kinases 1/2. COX-2, cyclooxygenase-2. PGE2, Prostaglandin E2. cAMP, cyclic adenosine monophosphate. PKA, protein kinase A. RANKL, receptor activator of nuclear factor-kappaB ligand. TGFβ-3, transforming growth factorβ-3. GPR4, G protein-coupled receptor 4. TDAG8, T cell deathassociated G protein 8. TRPV, transient receptor potential vanilloid. WT, Wild Type. IκB, inhibitor of NF-κB. TRAP, tartrate-resistant acid phosphatase. IGF, insulin like growth factor. ASIC1, acid sensing ion channel 1. PI3K, phosphatidylinositol 3-kinase. Pyk2, proline-rich tyrsosine kinase 2. Src, Src protein-tyrosine kinase.

Figure 1. The acid-sensitive mechanism of osteoclasts mediated by OGR1. OGR1 activated by protons promotes the release of calcium from the endoplasmic reticulum. The calcium signal activates calmodulin-dependent kinase, dephosphorylates NFATc1 and promotes NFATc1 entry into the nucleus, thereby promoting osteoclastogenesis. Moreover, elevated [Ca^{2+}]i promotes the survival of osteoclasts through PKC signaling. (OGR1: ovarian cancer G protein-coupled receptor 1; CaM: calmodulin; IP3: inositol 1,4,5-trisphosphate; IP3R: inositol 1,4,5-trisphosphate receptor; CaMK: calmodulin-dependent kinase; PKC: protein kinase C; ERK1/2: extracellular regulated protein kinases 1/2.)

precursors significantly inhibited differentiation and pit formation, as well as the expression of cathepsin, MMP-9, tartrate resistant acid phosphatase (TRACP), DC-STAMP, NFATc1 and RANKL. This implies that, in the absence of interference from osteoblasts, through OGR1, protons promote osteoclastogenesis and activities of osteoclasts (*54*).

In addition to OGR1, other GPCR subfamily members, such as GPR4 and TDAG8, exhibit proton sensing abilities in the musculoskeletal system. OGR4, and the subsequent cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) signals promote the release of RANKL from osteoblasts, and osteoblasts exhibit a phenotype that promotes osteoclast mineralization under acidic conditions, thereby modulating the generation and activity of osteoclasts (*55*). In contrast, TDAG8 is the only known member of GPCRs that inhibits bone resorption activities. A lack of TDAG8 leads to a significant increase in osteoclast formation, osteoclastic calcium resorption, and prevents abnormal morphologic changes of osteoclasts (*56*). Characterization of the role of TDAG8 in inhibition of bone resorption may help in elucidating the equilibrium mechanism of bone remodeling in the acidic condition.

3.2. TRPV

The transient receptor potential (TRP) family consists of several subfamilies, one of which is TRPV. TRPV1/2, TRPV3, TRPV4, and TRPV5/6 are four members in TRPV. Moreover, TRPV1/2, TRPV4 and TRPV5/6 are expressed in osteoclasts.

3.2.1. TRPV5/6

At the amino acid level, TRPV5 and TRPV6 share a 75% homology. TRPV5/6 are regulated strictly by Ca^{2+} because they are the only high-Ca^{2+} selective channels in the TRPV family. TRPV5/6 is a universal gatekeeper for epithelial cell Ca^{2+} transport (*57*). It is well known that the Ca^{2+} signal is also essential for osteoclast formation and activities.

TRPV5 is the first acid-sensitive TRPV channel member discovered in osteoclasts. However, the effects of TRPV5 on bone resorption by osteoclasts have not been conclusively determined. TRPV5-deficient mice showed gross phenotypic dysregulation of Ca^{2+} homeostasis, severe hypercalciuria and excess bone loss in the musculoskeletal system (*57-59*). Interestingly, bone resorption was significantly inhibited in *in vitro* cell cultures from TRPV5-deficient mice *(57,58,60)*. Apparently, contradictory phenotypes of reduced bone resorption and excessive bone loss concurrently appearing in the same mice are inconsistent. This implies that, *in vivo*, TRPV5-deficient mice have bone resorption compensatory mechanisms. $1,25(OH)_2D_3$ and TRPV6, as potential candidates, may contribute to this mechanism (*57,58,60*). $1,25(OH)_2D_3$ is a powerful regulator that maintains Ca^{2+} homeostasis and compensates for the loss of Ca^{2+} in the absence of TRPV5, but at the expense of enhanced bone resorption (*57*). This means that bone loss should be attributed to $1,25(OH)_2D_3$, rather than TRPV5 deletion, *in vivo*. Especially, in long-term bred TRPV5-deficient mice, old TRPV5-deficient mice bone resorption function was found to be essentially the same as that of wild mice, which is attributed to over expression of $1,25(OH)_2D_3$ in old mice compared to young ones (*58*). In short, this evidence has confirmed that TRPV5 promotes bone resorption in wild type mice (*57,58,60,61*).

The above research also has another puzzling phenomenon (*57*). Although the bone resorption capacity of osteoclasts is weakened, the number of osteoclasts increases in the absence of TRPV5. Estelle Chamoux *et al.* (*62*) postulated that there are two explanations for this puzzling phenomenon. One is that the lack of TRPV5 promotes survival of osteoclast precursors, which are TRAP-positive cells, but they exhibit low resorption activities, while the other is that a lack of TRPV5 produces dysfunctional mature osteoclasts. These are closely related to the differentiation of osteoclasts. In order to reduce disruption of differentiation, differentiated osteoclasts were used to establish study models by Estelle Chamoux *et al.* (*62*), and Estelle Chamoux *et al.* reported that TRPV5 promotes stable Ca^{2+} influx at the ruffled border and significantly inhibits human osteoclast-mediated bone resorption, inconsistent with the above research results. Subsequent research further confirmed that, TRPV5, as a target of estrogen (E2), is able to suppress osteoclastogenesis, formation of F-actin ring and an increase in osteoclast apoptosis, which diminishes bone loss. This implies that TRPV5 is a potential option for inhibiting hyperabsorption (*63,64*).

Although the function of TRPV5 has not been conclusively determined, current evidence for TRPV6 is unified. TRPV6 is a negative regulator of osteoclast differentiation and activity. In fact, TRPV6 exhibits several similar characteristics to TRPV5. For example, TRPV6 is located on the ruffled border and is associated with calcium homeostasis, and mice lacking TRPV6 were shown to exhibit a bone loss phenotype and an apparent increase in the expression of $1,25(OH)_2D_3$ (*57*). However, unlike TRPV5, TRPV6 is capable of inhibiting osteoclast formation and bone resorption activities, and these moderating effects have nothing to do with RANKL-induced calcium oscillations, therefore, other signals are involved (*65*). Differentiations and activities of osteoclasts are susceptible to some non-Ca^{2+}-independent pathways, and insulin like growth factor (IGF) is one of the candidates. NVP-AEW540, an inhibitor of IGF-1R/InsR, inhibits the increase of osteoclastogenesis induced by TRPV6-deletion. Further exploration of downstream signal transduction of IGF revealed that ratios of p-P85/P85, p-phosphoinositide dependent kinase-1(PDK1)/PDK1 and p-AKT (also known as PKB, protein kinase B)/AKT were elevated in osteoclasts isolated from TRPV6-deficient mice. Apparently, Trpv6 may aid in reducing the ratio of phosphoprotein/total protein in the IGF- phosphatidylinositol 3-kinase (PI3K)-AKT signaling pathway and lead to unfavorable functions of osteoclasts (Figure 2) (*66*).

3.2.2. TRPV1/2

TRPV1, as a non-selective cation channel, is activated by its agonists capsaicin and resiniferatoxin (RTX) as well as heat (thermal threshold > 43°C) or microenvironmental acidosis (protons) (*67*). TRPV2 is sensitive to noxious heat (thermal threshold > 52°C) and mechanical stimulation, but not to protons. Therefore, we discuss the pathological mechanisms of osteoclast overactivation under the induction of TRPV1 in acidic environments.

TRPV1 is involved in the pathophysiological processes of certain bone metabolic diseases, such as menopause (*67-69*), type 1 diabetes (*23*), glucocorticoids (*70*), or disuse (*71*) induced osteoporosis, thalassemia (*72*), and non-union after fracture (*73*). This implies that TRPV1 exerts potential impacts on osteoclasts. $TRPV1^{-/-}$ mice were found to exhibit higher bone densities, bone volume/total volume, trabecular thickness and trabecular number (*73*), and prevented bone loss associated with over-differentiation of osteoclasts in ovariectomized mice (*67,72,74*). *In vitro*, $[Ca^{2+}]i$ concentrations were found to be significantly up-regulated in osteoclasts (*69,72-74*), and TRAP-positive cells, cathepsin K expressions were also found to be elevated after administration of TRPV1 agonists, such as RTX (*74-76*). However, it is worth

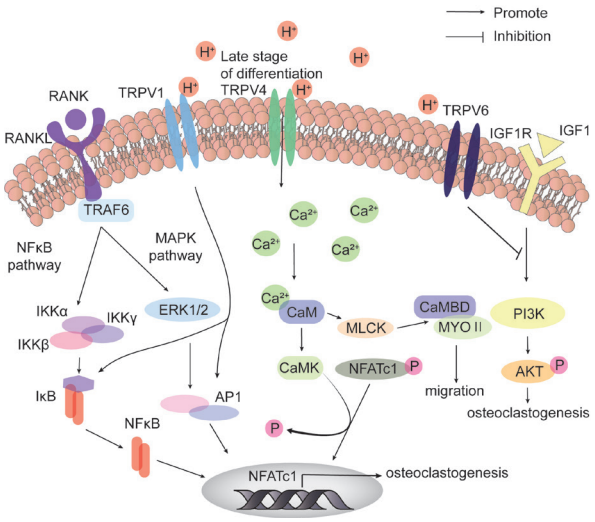

Figure 2. The acid-sensitive mechanism of osteoclasts mediated by TRPV1, 4 and 6. RANKL and its receptor RANK activates two classic pathways through TRAF6, namely the NF-κB pathway and the MAPK pathway. TRPV1 stimulated by protons respectively promotes the NF-κB pathway and the MAPK pathway, and ultimately promotes osteoclastogenesis. In the late stage of osteoclast differentiation, TRPV4 activated by protons induces an increase in the concentration of $[Ca^{2+}]i$. On the one hand, $[Ca^{2+}]i$ promotes the nuclear translocation of NFATc1 and osteoclastogenesis. On the other hand, $[Ca^{2+}]i$ and CaM forms a complex and promotes migration of osteoclast precursors under the mediation of MLCK. As a non-calcium-dependent pathway, the activation of IGF signal promotes osteoclastogenesis, however, the TRPV6 ion channel stimulated by protons inhibits the signal cascade of IGF, thereby reducing differentiation of osteoclasts. (RANKL: receptor activator of nuclear factor-kappaB ligand; RANK: receptor activator of nuclear factor-kappaB; TRAF6: TNF receptor associated factor 6; NF-κB: nuclear factor-kappa B; MAPK: mitogen-activated protein kinase; IKK: i kappaB kinase; IκB: inhibitory κB; ERK: extracellular regulated protein kinases; AP1: activator protein 1; TRPV: transient receptor potential vanilloid; CaM: calmodulin; CaMK: calmodulin-dependent kinase; MLCK: myosin light chain kinase; CaMBD: CaM-binding domain; MYO II: myosin II; IGF: insulin like growth factor; PI3K: phosphatidylinositol 3-kinase. NFATc1: nuclear factor of activated T-cells cytoplasmic 1.)

noting that some evidence proves that activated TRPV1 exerts adverse effects on the physiology of osteoclasts, which may be a consequence of desensitization of TRPV1 after long-term activation, such as in long term osteoporosis patients, or after administration of an excessive dose of agonists (*67-69,72*). Moreover, this does not imply that TRPV1 is absolutely insensitive to external stimulus after desensitization. Francesca Rossi *et al.* found that, under RTX stimulation, desensitized TRPV1 from osteoporosis patients could still promote the expression of NFκB, although it was not strong enough to affect osteoclast biology. On the contrary, with TRPV1 genetic ablation, desensitization or when subjected to its antagonists, such as capsazepine or 5′-iodo-resiniferatoxin (I-RTX), $[Ca^{2+}]i$ concentrations, peak and wave numbers of intracellular calcium oscillations were weakened (*73*), TRAP-positive cells as well as expression levels of NFATc1, cathepsin K were suppressed, RANKL-induced NFκB and MAPKs signaling were inhibited, fracture healing was impaired while caspase-3 induced apoptosis of osteoclasts was enhanced (*67-74*). Therefore, TRPV1 promotes osteoclast activities, and this effect is not compensated by osteoblasts (Figure 2) (*67*).

Interestingly, in addition to TRPV1 functions, a clear relationship between the endovanilloid and endocannabinoid system has been determined, which may relate to the mechanisms involved in osteoclasts overactivity. Cannabinoids (CB) exert their physiological activities through CB1 and CB2 cannabinoid receptors, which are co-localized with TRPV1 in osteoclast plasma membranes, and they share some endogenous agonists, such as anandamide (AEA), and some endogenous antagonists, such as URB597. When osteoclasts are exposed to the TRPV1 agonist for 48 h, CB1 and CB2 were found to be significantly up-regulated, and the CB2 gene transcription level was increased 10-fold. Vice versa, the CB1 receptor antagonist tends to enhance the expression levels of TRPV1 (*68*). Therefore, it is considerably meaningful to study the cross-talk of these receptors or channels for bone microenvironmental acidosis and metabolic disorders. Indeed, TRPV1 and CB1 play a synergistic role in the promotion of osteoclast activity, while CB2 inhibits osteoclast activities (*68-70,72,74*). Therefore, when TRPV1 is agonized and CB2 is antagonized, TRAP-positive osteoclasts and expression levels of cathepsin K are significantly increased, compared to pure agonism or antagonism (*68-70,72,74*). This implies that drugs that are characterized by inhibition of TRPV1 and promotion of CB2 may aid in the treatment of diseases that are characterized by excess osteoclast activity. However, the cross-talk between endovanilloid/endocannabinoid systems is modulated by multifactorial items, and PKCβII is one of them. Glucocorticoids, a common inducer of osteoporosis, increase in TRPV1 expression and decreases in CB2 expression in osteoclasts, however, these outcomes can be counteracted by inhibition of PCKβII. The combination of TRPV1 and PCKβII was shown to induce PCKβII activation, the activated PCKβII further phosphorylates TRPV1 and reinforces osteoclast reactivity. Therefore, PCKβII is a positive regulator of the crosstalk between the endovanilloid/endocannabinoid system (*70*).

Sirtuin 1 (SIRT1), a nicotinamide adenine dinucleotide dependent lysine deacetylase, can inhibit bone resorption. TRPV1 is a key component in SIRT1 inhibition of bone resorption (*76*). When SIRT1 is silenced, TRPV1 and its ligands are up-regulated and osteoclastogenesis is enhanced. Therefore, SIRT1 inhibits osteoclast activities by weakening TRPV1 channel activities, in other words, TRPV1-associated activation of osteoclasts is attributed to suppression of SIRT1 (*76*).

3.2.3. TRPV4

TRPV4, a non-selective Ca^{2+} channel, is stimulated

by mechanical stress, protons, heat, and its agonists, such as 4a-PDD (*77*). Unlike TRPV5, which is located on the apical side of osteoclasts, it is involved in Ca^{2+} transport during bone resorption, TRPV4, located on the basolateral membrane, is responsible for Ca^{2+} uptake during osteoclast differentiation (*24,77*). Maintaining $[Ca^{2+}]i$ concentration is an important determinant of osteoclastogenesis, and subsequently bone resorption. Sources of $[Ca^{2+}]i$ are classified into two categories, including intracellular organelles, such as endoplasmic reticulum, while the second involves the influx of $[Ca^{2+}]o$ through the Ca^{2+} channel in the plasma membrane. In the early stages of differentiation, spikes in calcium oscillation depend on the supply of intracellular organelles rather than TRPV4, however, in the latter stages, the persistent influx of Ca^{2+} induced by TRPV4 can stabilize the high concentrations of $[Ca^{2+}]i$ and induce osteoclastogenesis *via* $[Ca^{2+}]i$-NFATc1 signaling (*77,78*). Ca^{2+} binds the Calmodulin (CAM) binding domain to form a Ca^{2+}/CAM complex when $[Ca^{2+}]o$ enters pre-osteoclasts under the induction of TRPV4. As a result, the Ca^{2+}/CAM complex stimulates the expression of calmodulin kinase and further potentiates dephosphorylation and nuclear translocation of NFATc1. In addition, the Ca^{2+}/CAM complex also exerts its effects on phosphorylation of CAM-dependent myosin light chain intermediates by the myosin light chain kinase (MLCK), which initiates cytoskeletal contraction and migration of pre-osteoclasts or osteoclasts (Figure 2) (*78*). Consistent with *in vitro* experiments, $TRPV4^{-/-}$ mice exhibited a phenotype of increased bone mass, decreased osteoclastogenesis and bone resorption, however, decreased stress resistance on long bones. This outcome may partially be attributed to compensatory mechanisms of osteocytes, although it has not been confirmed (*79*).

Interestingly, *in vivo* TRPV4 studies, male animals, instead of females, are the most common models. Regarding whether gender affects the function of TRPV4, B C J van der Eerden *et al.* reported that TRPV4 predisposes males to disorders related to bone metabolic perturbation and increases the risk of fractures (*79*). Notably, male mice lacking TRPV4 showed a decrease in the number of osteoclasts and bone resorption, but, interestingly, these were not observed in female mice. In line with this would be that adult men are more at risk of fractures associated with TRPV4 compared to women. Taken together, TRPV4-induced osteoclast activity is associated with a distinct sexual dimorphism, and TRPV4 can be used as a predictor of male-specific bone mass and bone strength (*79*).

Autophagy, as a control system for maintaining cell homeostasis, significantly facilitates the production of ruffled borders, secretion of protons and degradation of the bone matrix of osteoclasts. Moreover, autophagy is involved in TRPV4-induced osteoclastogenesis (*80*). The autophagy-related protein, LC3, is redundantly expressed, and the LC3-I/LC3-II ratio is elevated when TRPV4 is overexpressed. Mechanistically, Ca^{2+} attributed to TRPV4 activation is involved in calcineurin-NFATc1 signaling, and thereby, contributes to the yield of autophagy-related proteins, which modulate osteoclastogenesis (*80*).

3.3. ASIC

ASICs are ligand-gated cation channels that are responsible for perception and response to extracellular protons by osteoclasts when pH is lower than 7.0 (*81*). ASICs contain four subunits, ASIC 1-4. The expression level of ASIC2 is the highest, followed by ASIC1, and ASIC3, while ASIC4 is rarely expressed in osteoclasts (*81*). During osteoclast differentiation, expression levels of ASIC2 are significantly suppressed, and its expression in mononuclear osteoclast precursors is 4-fold that of mature multinuclear osteoclasts.

Bone resorption of osteoclasts involves multiple steps, including differentiation of mononuclear osteoclast precursors, migration, adhesion to the bone surface, and finally, resorption of the bone matrix. ASIC1a are involved in all processes, exhibit acid sensitivity, and regulate the activities of osteoclasts in cases of acidosis (*22,82*). To further define the potential mechanisms, as expected, ASIC1a enhances the concentrations of $[Ca^{2+}]i$ in osteoclasts, leading to transcriptional as well as nuclear translocation of NFATc1 and differentiation of osteoclast precursors in acidic conditions (Figure 3) (*22*). Besides differentiation, activation of ASIC1a potentiates the expression of αvβ3 integrins, and elevates phosphorylation levels and protein interactions of Pyk2 and Src, which enhances the formation of actin rings that are required for migration and adhesion of pre-

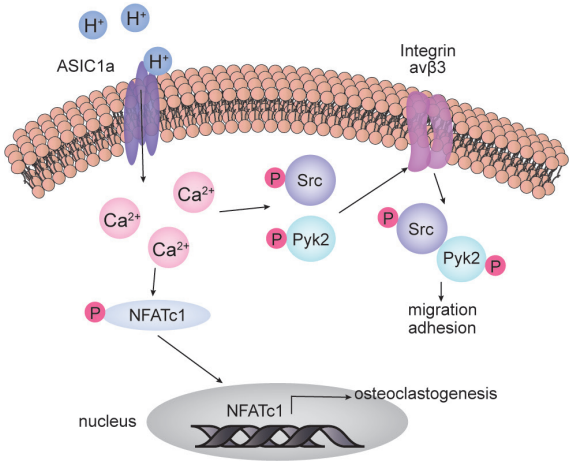

Figure 3. The acid-sensitive mechanism of osteoclasts mediated by ASICs. The acid-activated ASICs1a not only increases the concentration of $[Ca^{2+}]i$, promotes osteoclastogenesis by increasing the nuclear translocation of NFATc1, but also enhances expression of αvβ3 integrin, and its signal cascade is essential for migration and adhesion of osteoclast precursors. (ASICs1a: acid-sensing ion channels 1a; NFATc1: nuclear factor of activated T-cells cytoplasmic 1; Src: Src protein-tyrosine kinase; Pyk2: proline-rich tyrsosine kinase 2.)

osteoclasts or osteoclasts (Figure 3) (*82*).

4. The effects of microenvironmental acidosis on bone metastases

Bone metastasis is a common and serious complication in patients with multiple myeloma, breast cancer, lung cancer, prostate cancer and kidney cancer (*83*). Once tumor cells develop in bone tissue, they disrupt the balance between osteoblasts and osteoclasts, leading to osteogenic or osteolytic lesions. Osteolytic bone metastasis results in higher morbidity compared with osteogenic bone metastasis (*83*). When osteoclast-mediated bone resorption dominates, it leads to excessive remodeling of local bone and lytic lesions.

Microenvironmental acidosis is an important factor that promotes osteolytic bone metastasis (*84*). Protons in the osteolytic bone metastasis microenvironment come from two categories of sources. The first source is glycolysis in tumor cells. In fact, proliferating tumor cells exhibit a high degree of glycolysis, which produces a large amount of protons or lactic acid in the extracellular matrix, and this is known as the Warburg effect (*85*). The second source of protons is excessive bone resorption by osteoclasts (*13,17,18*).

The V-ATPase is considered to be the primary pH regulator of bone metastasis because it is exists both in tumor cells and osteoclasts (*13,17,18*). In addition to V-ATPase, other ion/proton pumps contribute to bone metastases, such as the Na^+/H^+ exchanger, monocarboxylate transporters, and carbonic anhydrase 9 (*84*). These ion/proton pumps collectively lead to acidosis in the microenvironment of osteogenic bone metastasis.

Extracellular acidification of bone metastasis generally has three consequences. The first consequence is that it enhances invasion and aggressiveness of tumor cells (*86*). The second outcome is the promotion of differentiation and activity of osteoclasts. As mentioned above, osteoclasts are sensitive to protons, hence extracellular acidification stimulates various proton sensing pathways/receptors of osteoclasts leading to excessive bone remodeling (*27,36-40*). The third consequence is cancerous bone pain (*87*).

Of note, once tumor cells settle in the bone tissue, they are stimulated by protons thereby affecting the functions of osteoclasts through parathyroid hormone related protein (PTHrP), interleukin (IL)-11, and Jagged 1 (*88*). PTHrP and IL-11 enhance the production of RANKL, which stimulates the formation and activation of osteoclasts. On the other hand, Jagged 1 promotes the fusion of osteoclast precursors by directly binding to monocytes (*88*). Moreover, being a multinucleated giant cell with complex functions, osteoclasts consume a lot of energy during bone resorption (*33*). The acidified bone metastasis microenvironment improves mitochondrial function, thereby promoting the survival of osteoclasts and maintaining bone resorption (*89*).

Bone pain is a common symptom in patients with osteolytic bone metastases. Protons can directly stimulate acid-sensitive ion channels (such as TRPV1 and ASIC3) expressed on bone sensory neurons, thereby triggering pain-causing signals (*87*). Studies have indicated that suppressing acid-sensitive ion channels using specific ASIC3 antagonist APETx2 and specific TRPV1 inhibitor JNJ-17203212 can inhibit cancer-induced bone pain (*90,91*).

Taken together, the extracellular microenvironment of osteolytic bone metastasis is protonated by glycolytic tumor cells and excessive activity of osteoclasts (*13,17,18,85*). Extracellular acidosis of osteolytic bone metastasis can, not only enhance the invasion and aggressiveness of tumors, but also evoke osteolysis, and cancer-induced bone pain (*27,36-40,86,87*).

5. Conclusion

Bone remodeling is precisely regulated by the dynamic balance between bone formation induced by osteoblasts and bone resorption induced by osteoclasts, which occurs in the bone microenvironment (*13,17,25,73*). Virtually, it will inevitably elicit perturbation of the musculoskeletal system homeostasis and emergence of osteogenic or osteolytic disorders when this equilibrium is broken (*13,17,73*). Osteoclasts are multinucleated giant cells that are activated by M-CSF and RANKL, which play a pivotal role in diseases that are characterized by bone loss, such as osteoporosis, multiple myeloma and Paget's disease among others (*13,14,19*). The activity of osteoclasts is also influenced by the pathological state of the extracellular microenvironment, such as hypoxia, inflammation, mechanical stress and particularly, acidosis (*14*).

Protons are closely associated with osteoclast formation and functions. When osteoclasts are directed to move to resorption sites, H^+, Cl^-, and certain acid proteases are pumped into the sealing zones, leading to regional dissolution of the bone matrix (*13,33*). H^+ are obtained from three sources, the first is that they are byproducts of high mitochondrial metabolism in osteoclasts. In fact, in the process of bone resorption, osteoclasts require mitochondrial hyperactivation to maintain their high metabolic activities (*33*). The second is carbonic acid produced by carbonyl anhydrase II (CA II), which tends to split into protons and bicarbonate (*92,95*). Finally, they are obtained from extracellular acidosis. In *in vitro* simulation of osteoclasts, [pH]i was shown to drop to 6.8 as a consequence of [pH]o droping to 6.5, implying that osteoclasts are the local responding units for protons (*38*).

Numerous acid-sensitive channel subunits provide the basis for the response to pH of osteoclasts. Most of them share the feature with specificity increases $[Ca^{2+}]$i of osteoclast induced by protons. Ca^{2+} is a

common second messenger that induces various cellular biological functions, especially in osteoclasts. The [Ca^{2+}]i and the subsequent signaling cascade is essential for osteoclastogenesis (*46,80*). Although calcium oscillations are initially regulated by organelles that store Ca^{2+}, at late stages of osteoclast differentiation, elevated concentrations of [Ca^{2+}]i rely on the acid-sensitive channel, such as TRPV4 (*77*). Furthermore, for acid-sensitive Ca^{2+} channels, their functions correspond with membrane positioning. For example, high quantities of degradation products, including broken collagen, Ca^{2+} and phosphate are generated in the sealing zone during bone resorption (*13,33*), TRPV5/6, located on the apical side, is responsible for mediating Ca^{2+} translocation into the cytoplasm, thereby promoting the pumping out of Ca^{2+} (*57*). In contrast, TRPV4, located on the basolateral membrane, is involved in Ca^{2+} uptake in the late stages of osteoclast differentiation (*77*), furthermore, TRPV1, located on the ruffled border membrane, is involved in calcium mobilization along with the endocannabinoid system (*73,74*), and promotes the formation of osteoclasts. In addition to osteoclasts themselves, the paracrine elements of osteoblasts are also involved in activation of osteoclasts in acidic microenvironments, among them, PEG2 and RANKL are involved in facilitation of osteoblasts to osteoclasts (*39-42*).

Acid-sensitive channels promote multiple pivotal physiological activities of osteoclasts, however, evidence suggests that some inhibit, at least in part, osteoclast activities, such as TDAG8 and TRPV6 (*56,65*). Therefore, the acidosis microenvironment dynamically and bidirectionally regulates the activity of osteoclasts instead of invariably and unidirectionally, however, the members of inhibitory receptors and especially its mechanism are incomplete. In addition, genetic ablation of certain acid-sensitive receptors/pathways leads to contradictory bone phenotypes (*57,67,79*), implying that the relationship between the acidosis microenvironment and bone remodeling is multifactorial and complex *in vivo*. Although 1,25 $(OH)_2D_3$ or parathyroid hormone (PTH) are involved in compensation after certain acid-sensitive pathways are suppressed as far as we know (*57,60*), it is still incompletely understood to a large extent, which requires additional new insights into this issue.

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Review

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Revealing the magic of acupuncture based on biological mechanisms: A literature review

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SUMMARY Acupuncture has been used to treat various disease for more than 3,000 years in China and other Asian countries. As a complementary and alternative therapy, it has gained increasing popularity and acceptance among public and healthcare professionals in the West. Over the past few decades, basic and clinical research on acupuncture has made considerable progress. Internationally recognized evidence from clinical studies has been published, a preliminary system to clinically evaluate acupuncture has been created, and some clinical guidelines have been formulated. Moreover, scientists have strived to explore the physiological and biological mechanisms of acupuncture. Some basic studies have indicated that acupuncture has various actions, such as analgesic, muscle relaxing, anti-inflammatory, mild anxiolytic, and antidepressant actions, with possible biological mechanisms such as central sensitization, neurotransmitters, the intestinal flora, immune regulation, oxidative stress, and neuroinflammation. The current review describes the common indications for acupuncture recommended by the WHO and the use of acupuncture in China, the United States, Australia, and several other countries. This review then summarized the mechanisms by which acupuncture treats common conditions including lower back pain (LBP), ischemic stroke, depression, and irritable bowel syndrome (IBS) and it also cited specific acupuncture points for treating these conditions. The hope is that this review will provide useful information for both acupuncturists and researchers to better understand the mechanisms of acupuncture and reasons for its usage.

Keywords acupuncture, electroacupuncture, indications, biological mechanisms, common acupuncture points

1. Introduction

Acupuncture has been used to treat various disease for more than 3,000 years in China and other Asian countries, and it spread to Europe and America from the sixteenth to the nineteenth century. As one of the most popular complementary and alternative therapies, it has gained increasing popularity and acceptance among public and healthcare professionals in the West. The World Health Organization (WHO) listed 43 diseases and conditions that can be treated with acupuncture in 1979, and that number increased to 63 in 1996, as shown in Table 1 (*1*). Moreover, data from the WHO indicated that 103 of its 194 member countries use acupuncture as a treatment and that 29 had enacted laws to regulate traditional Chinese medicine (TCM), while 18 had included acupuncture in their medical insurance system (*2*). Thus, acupuncture plays an important role in the healthcare system worldwide.

In China, acupuncture is a longstanding legal medical practice widely used by doctors in Chinese medicine, with an estimated 18,404 doctors in acupuncture in Mainland China in 2018 (*3*). In Japan, acupuncture has been a part of Chinese medicine since 562 AD; it was introduced from China to Japan and is still used today (*4*). In South Korea, the government enacted the National Medicine Act in October 1951, which stipulates that Eastern and Western medicine have the same status (*4*). In United States, the National Institutes of Health (NIH) approved acupuncture as a treatment for patients in 1991, and the Food and Drug Administration (FDA) approved acupuncture needles as a medical device in 1996. Moreover, 47 states and Washington, D.C. in the United States have successively passed acupuncture laws, ensuring the legal use and development of acupuncture since 2014 (*4*). In Canada, acupuncture legislation

Table 1. Common indications for acupuncture recommended by WHO

Indications	Examples
Nervous system disease	Migraine, Tension headache, Trigeminal neuralgia, Facial nerve palsy, and Ischemic stroke.
Musculoskeletal diseases	Osteoarthritis (knee), Fibromyalgia, LBP, Neck pain, sciatica, and Postoperative pain.
Gastrointestinal diseases	Nausea and vomiting, Constipation, Postoperative ileus, and IBS.
Gynecological/reproductive diseases	Dysmenorrhea, Premenstrual Syndrome, Menopausal syndrome, and Infertility,
Respiratory diseases	Common cold, Acute bronchitis, Acute and chronic pharyngitis, Asthma, and Chronic obstructive pulmonary disease.
Oral disease	Toothache, Post tooth extraction pain, and Gingivitis.
Mental diseases	Anxiety, Depression, and Insomnia.
Addictions	Nicotine dependence and Alcohol dependence.
Endocrine diseases	Obesity.

Abbreviations: lower back pain (LBP), irritable bowel syndrome (IBS).

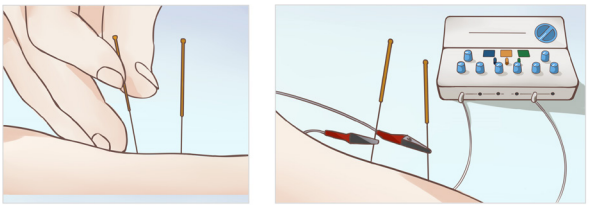

Figure 1. Different types of acupuncture.

has been approved by the governments of 5 provinces between 1988 and 2014 (*5*). In Australia, a national registration standard for TCM has been implemented since July 1, 2012, and Australia is the first Western country to legislate TCM. In addition to the countries discussed above, South Africa, Canada, France, Brazil, and Germany also have legislation on acupuncture (*4*). In all, the development of acupuncture has entered a new era worldwide.

Acupuncture is defined as the insertion of fine needles through the skin into specific body sites, known as acupuncture points, on meridians according to the ancient theory of TCM to improve the body's energy flow and maintain overall health and vitality (Figure 1A). Acupuncture points have been emphasized as key elements that account for the efficacy of acupuncture. TCM uses approximately 361 acupuncture points on 14 traditional channels and 34 extra points (*6*). The required efficacy is achieved only by the precise stimulation of specific acupuncture points. Electroacupuncture is a modification of acupuncture in which needles with attached electrodes are inserted to deliver a pulsed electrical current (Figure 1B). Although it is still controversial, electroacupuncture has been shown to achieve similar or even better effects compared to acupuncture (*7*).

Until now, numerous efforts have been made to determine the clinical efficacy and specificity of acupuncture. Internationally recognized evidence from clinical studies has been published. These clinical studies have indicated that acupuncture and electroacupuncture are effective for the management and treatment of various conditions including various types of pain, ischemic stroke, anxiety disorders, irritable bowel syndrome (IBS), and inflammatory bowel disease, which are common and tricky clinical conditions (*1,8,9,10*). Moreover, clinical practice guidelines on acupuncture have been formulated in countries such as China, Japan, South Korea, the United States, the United Kingdom, Australia, and Malaysia (as shown in Table 2) (*4,11*).

With the development of modern advanced science and technology, scientists have strived to explore the physiological and biological mechanisms of acupuncture. Some basic studies have indicated that acupuncture has various actions, such as analgesic, muscle relaxing, anti-inflammatory, mild anxiolytic, and antidepressant actions (*12*). Acupuncture might involve biological mechanisms such as central sensitization, neurotransmitters, the intestinal flora, immune regulation, oxidative stress, and neuroinflammation.

With increasing evidence of its efficacy, acupuncture is now a magic and widely practiced treatment modality in complementary and integrative medicine. The aim of the current review is to reveal the magic of acupuncture. The underlying mechanisms by which acupuncture treats common conditions including lower back pain (LBP), ischemic stroke, depression, and IBS will be discussed based on studies that have been published over the last two decades.

2. Acupuncture for LBP

LBP is defined as pain or discomfort localized between the costal margin and buttocks. It is one of the most common health problems in adults, with an average lifetime prevalence as high as 39% (*13*). Chronic LBP is a major cause of disability, absenteeism, and costly medical expenses, which place a great economic burden on society. Commonly, non-pharmacologic therapies are recommended as first-line treatment, which included acupuncture, massage, spinal manipulation, and yoga. Acupuncture, and pain relief in particular, is a characteristic non-pharmaceutical option to combat

Table 2. The clinical practice guidelines for acupuncture to treat common conditions in countries around the world

Countries/Numbers	Name /(Language)
China/20	1. Evidence-Based Clinical Practice Guidelines on Acupuncture for Adult Bronchial Asthma; /(in Chinese) 2. Evidence-Based Clinical Practice Guidelines on Acupuncture for Diabetic Peripheral Neuropathy; /(in Chinese) 3. Evidence-Based Clinical Practice Guidelines on Acupuncture Chronic Atrophic Gastritis; /(in Chinese) 4. Evidence-Based Clinical Practice Guidelines on Acupuncture for Knee Osteoarthritis; /(in Chinese) 5. Evidence-Based Clinical Practice Guidelines on Acupuncture for Depression; /(in Chinese) 6. Evidence-Based Clinical Practice Guidelines on Acupuncture for Primary Dysmenorrhea; /(in Chinese) 7. Evidence-based Clinical Practice Guidelines on Acupuncture for Insomnia; /(in Chinese) 8. Evidence-based Clinical Practice Guidelines on Acupuncture for Herpes Zoster; /(in Chinese) 9. Evidence-based Clinical Practice Guidelines on Acupuncture for Pseudobulbar Palsy after Stroke; /(in Chinese) 10. Evidence-based Clinical Practice Guidelines on Acupuncture for Lower Back Pain; /(in Chinese) 11. Evidence-Based Clinical Practice Guidelines on Acupuncture for Chronic Constipation; /(in Chinese) 12. Evidence-Based Clinical Practice Guidelines on Acupuncture for Migraine; /(in Chinese) 13. Evidence-based Clinical Practice Guidelines on Acupuncture for Cervical Spondylosis; /(in Chinese) 14. Evidence-based Clinical Practice Guidelines on Acupuncture for Frozen Shoulder; /(in Chinese) 15. Evidence-based Clinical Practice Guidelines on Acupuncture for Sudden Deafness; /(in Chinese) 16. Evidence-based Clinical Practice Guidelines on Acupuncture for Simple Obesity; /(in Chinese) 17. Evidence-Based Clinical Practice Guidelines on Acupuncture for Primary Trigeminal Neuralgia; /(in Chinese) 18. Evidence-based Clinical Practice Guidelines on Acupuncture for Allergies; /(in Chinese) 19. Evidence-based Clinical Practice Guidelines on Acupuncture for Bell's Facial Palsy; /(in Chinese) 20. Evidence-based Clinical Practice Guidelines on Acupuncture for Sciatica. /(in Chinese)
South Korea/4	1. Clinical Practice Guidelines on Acupuncture for Post-stroke Spasticity; /(in Korean) 2. Clinical Practice Guidelines on Acupuncture for Post-stroke Urinary Retention; /(in Korean) 3. Evidence-Based Clinical Practice Guidelines on Medical Manual Acupuncture for Shoulder Pain; /(in English) 4. Clinical Practice Guidelines for Acupuncture to Treat Adults with Acute Ankle Sprains. /(in English)
Japan/1	Clinical Practice Guidelines for the Management of Lower Back Pain. /(in Japanese)
United States/1	Diagnosis and Treatment of Low Back Pain: A Joint Clinical Practice Guideline from the American College of Physicians and the American Pain Society. /(in English)
United Kingdom/1	Guidelines for Providing Acupuncture Treatment for Cancer Patients. /(in English)
Australia/1	Practice Guidelines for Acupuncturists Using Acupuncture-assisted Treatment of Anorexia Nervosa. /(in English)
Malaysia/1	Practice Guidelines for Traditional Acupuncture and Complementary Medicine. /(in English)

the opioid crisis (*14*). LBP was the leading indication in American acupuncture clinics according to a cross-sectional study (*15*).

2.1. Acupuncture points frequently used for LBP

Appropriate selection of acupuncture points is fundamental to the efficacy of clinical acupuncture. Numerous studies have been conducted to investigate the acupuncture points most frequently used to treat LBP. According to a network analysis, acupuncture points BL 23, BL 25, BL 60, GB 30, and BL 26 appeared to be widely used to treat LBP (*16*). Lee *et al*. found that the bladder meridian (BL 23, BL 24, BL 25, BL 26, BL 32, BL 40, and BL 60) and the gall bladder meridian (GB 30, GB 40) were most frequently used to treat LBP based on 53 treatment regimens in clinical trials (*17*). These findings were highly consistent with the theories of traditional Chinese medicine, in which some acupuncture points, including BL 23, BL 25, GV 3, BL 40, GB 30, and KI 3, are commonly used to treat chronic LBP (*18*). Based on these studies and the current authors' own clinical experience, BL 23, BL 24, BL 25, BL 26, BL 32, BL 40, BL 60, GB 30, GB 40, KI 3, and GV 3 are the acupuncture points frequently used for LBP (Figure 2). These acupuncture points are stimulated to relax entrapped nerves and myofascia and to improve local blood circulation.

2.2. Mechanisms by which acupuncture treats LBP

Despite its widespread use, the underlying mechanisms by which acupuncture induces analgesia are still not fully understood. In terms of current studies, acupuncture may have analgesic action by reducing inflammation, relieving central sensitization, and regulating adenosine triphosphate (ATP) metabolism. A brief description of the mechanisms by which acupuncture treats LBP is provided here (Table 3).

2.2.1. Anti-inflammatory action

Pro-inflammatory cytokines are known to be involved in the development of inflammatory pain. Endogenous cannabinoids and peripheral cannabinoid CB2 receptors (CB2Rs) are involved in the antinociceptive effect of

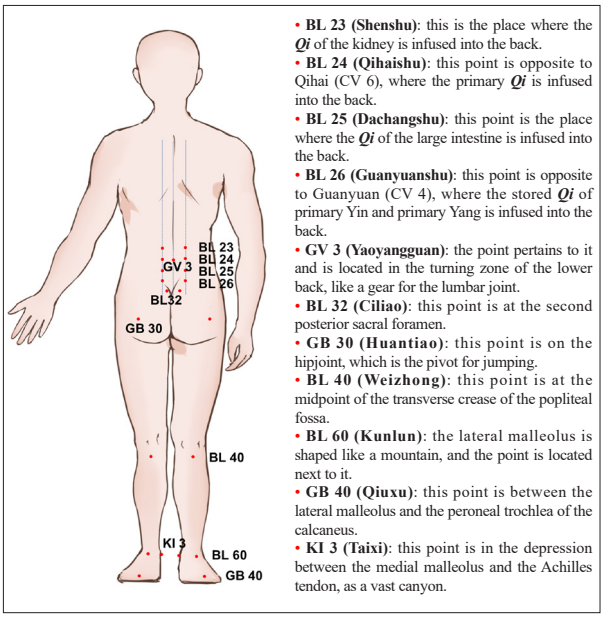

Figure 2. Acupuncture points frequently used for lower back pain (LBP) and their location on the human body according to the clinical trials reviewed.

electroacupuncture on inflammatory pain. Su *et al*. found that electroacupuncture applied to acupuncture points GB 30 and GB 34 significantly reduced thermal hyperalgesia and mechanical allodynia induced by tissue inflammation. Electroacupuncture reduced the levels of the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α and it alleviated inflammatory pain in inflamed tissues *via* activation of CB2Rs (*19*).

Endogenous opioid peptides such as β-endorphin and met-enkephalin may also contribute to acupuncture-induced analgesia. They activate opioid receptors both at the level of the spinal cord as well as on peripheral sensory neurons at the site of inflammation. Wang *et al*. found that electroacupuncture applied to acupuncture point GB 30 stimulated opioid peptide release in inflamed tissue by regulating chemokine CXCL10 (IP-10, interferon gamma inducible protein 10) production. Electroacupuncture can induce an anti-inflammatory cytokine profile with increased expression of interferon-gamma (IFN-γ) and CXCL10 and increased numbers of opioid peptide-containing $CXCR3^{+}$ macrophages (*20*).

2.2.2. Relieving central sensitization

Central sensitization is defined as an amplification of neural signaling within the central nervous system (CNS) that elicits pain hypersensitivity. Hyperalgesia and allodynia are the two main characteristics of central sensitization (*21*). Many musculoskeletal disorders, including LBP, appear capable of triggering the phenomenon of central sensitization with an associated increase in sleep disturbance, fatigue, and widespread pain. Resting-state (RS) functional magnetic resonance imaging (fMRI) data have revealed deficient

Table 3. Proposed mechanisms and evidence for acupuncture to treat lower back pain (LBP)

Authors/*Ref.*	Species/model	Treatment	Acupuncture points	Manipulation	Course	Results of molecular expression	Mechanisms
Su *et al.* /(*19*), 2012	SD rats CFA injection	EA	GB 30 and GB 34	1 mA, 2 Hz, 30 min	Once every other day for 6 days	IL-1β, IL-6, and TNF-α ↓	Alleviating inflammatory pain in inflamed tissue through activation of CB2Rs.
Wang *et al.* /(*20*), 2014	Wistar rats CFA injection	EA	GB 30	2-2.5-3 mA, 100 Hz, 20 min	Every day for 2 days	IFN-γ and CXCL10 ↑	Stimulating opioid peptide release in inflamed tissue by regulating chemokine CXCL10 production.
Kim *et al.* /(*23*), 2020	Patients with LBP (n = 102)	MA	GV 3, BL 23, BL 40, and KI 3	0.20-0.25 mm diameter, 25-50 mm length, 20 min	4 weeks	S1-back gray matter volume and FA in the white matter adjacent to the S1-back subregion ↓	Improving tactile acuity over the back of patients with LBP by regulating somatotopically-specific structural S1 neuroplasticity.
Yu *et al.* /(*24*), 2020	Patients with LBP (n = 79)	MA	GV 3, BL 23, BL 40, KI 3, and 1-3 Ashi points bilaterally on the lower back and legs	25 min	6 times for 4 weeks	VTA/PAG rsFC ↑	The amygdala might be a key node linking the descending pain modulation and reward systems to produce an antinociceptive effect.
Ren *et al.* /(*27*), 2012	Rats with neuropathic pain	EA	—	—	—	A1 receptor ↑ P2X3 receptor ↓	Alleviating neuropathic pain *via* promoting the degradation of ATP to adenosine and regulating purinergic A1 and P2X3 receptors

Abbreviations: complete Freund's adjuvant (CFA), fractional anisotropy (FA), resting state functional connectivity (rsFC), periaqueductal gray (PAG), ventral tegmental area (VTA), P2X purinergic receptor 3 (P2X3), adenosine 5'-triphosphate disodium (ATP), Electroacupuncture (EA), Manual acupuncture (MA).

mesocorticolimbic connectivity in patients with LBP, with mesolimbic dysconnectivity potentially mediating the contribution of pain sensitization to chronic pain (*22*).

While multiple brain-based mechanisms of action for acupuncture have been proposed, a possible mechanism for LBP may involve neuroplasticity in somatosensory pathways and manifest in improvements in tactile acuity. According to a longitudinal neuroimaging study, Kim *et al*. found that after 4 weeks of acupuncture therapy, patients with LBP had improved tactile acuity over the back, and improvement was associated with a reduced S1 (primary somatosensory cortex)-back gray matter volume and increased fractional anisotropy (FA) in the white matter adjacent to the S1-back subregion (*23*). Yu *et al*. found that acupuncture may simultaneously modulate the resting state functional connectivity of key regions in the descending pain modulation and reward systems and that the amygdala may be a key node linking the two systems to produce an antinociceptive effect (*24*).

2.2.3. Regulating ATP metabolism

ATP is an important source of energy but it also plays an important role in regulating the biological activities of cells. Adenosine, the core molecule of ATP, is recognized by specific receptors that regulate neuronal and non-neuronal cellular functions. As a neurotransmitter, adenosine regulates pain transmission in both the spinal cord and in the periphery (*25*).

Numerous studies have indicated that acupuncture can trigger an increase in the extracellular concentration of ATP and its metabolite adenosine near acupuncture points. Acupuncture can induce the release of ATP from keratinocytes, the major type of cell in the skin, and from subcutaneous mast cells, and it can stimulate nociceptive terminals of sensory ganglia (*e.g*., dorsal root ganglion (DRG)) neurons. The signaling message is then relayed *via* the DRGs to the spinal cord and subsequently through ascending pathways to the brain stem, which contains motor neurons. Signals also travel to certain centers in the cortex that perceive pain and localize painful stimuli in the body. These centers can be modulated by locally released adenosine to deliver a message to inhibit pain (*26*).

According to a rat model, after acupuncture ATP in the extracellular space was broken down into adenosine, which in turn inhibited pain transmission by means of an adenosine A1 receptor-dependent process. Moreover, acupuncture might simultaneously act *via* adenosine A1 and P2X purinoceptor 3 (P2X3) receptors to have an analgesic effect on neuropathic pain (*27*). Yao *et al*. found that acupuncture can lead to an increase in intracellular Ca^{2+} in mast cells and release of ATP, which can activate nerve cells and modulate pain-processing pathways in response to acupuncture (*28*).

3. Acupuncture for ischemic stroke

Stroke is the second leading cause of death and the leading cause of disability worldwide. It affects 15 million people per year around the world, 5 million of whom die, and 5 million of whom are permanently physically disabled (*29*). In most cases, stroke is caused by an abrupt blockage of an artery (ischemic stroke), which accounts for 71% of all stroke cases, while in some instances stroke may be caused by bleeding into brain tissue when a blood vessel ruptures (hemorrhagic stroke) (*30*). Ischemic stroke is caused by cerebral vascular occlusion, and the decreased blood flow causes a shortage of oxygen and glucose in brain tissue, resulting in impairment of normal neurologic function. Numerous studies have revealed complex primary and secondary brain injuries after ischemic stroke, including blood-brain barrier (BBB) damage, brain edema, neuronal death, and neurological dysfunction (*31*).

Acupuncture, considered to be a promising strategy to prevent stroke, has been used to treat stroke for thousands of years in Asia. It is widely used to improve motor, sensory, speech, and other neurological functions in patients after a stroke. The WHO has recommended acupuncture as an alternative and complementary strategy for stroke and post-stroke rehabilitation. Numerous clinical trials and meta-analyses have indicated the efficacy of acupuncture in improving balance, reducing spasticity, and increasing muscle strength and general well-being post-stroke (*32*). Moreover, many basic studies have indicated that acupuncture is effective at facilitating ischemic stroke rehabilitation and reducing post-stroke infarct volume and neurological deficits (*9*).

3.1. Acupuncture points frequently used for ischemic stroke

Acupuncture points may be excitable muscle/skin-nerve complexes containing a high density of nerve endings. Acupuncture at particular points activates afferent fibers that send signals to the spinal cord (*33*). Numerous studies have been conducted to investigate the acupuncture points most frequently used to treat ischemic stroke. According to a review of 40 basic studies, Chavez *et al*. found that the acupuncture points most frequently used for ischemic stroke included GV 20, ST 36, LI 11, GV 26, GV 14, and LI 4 (*9*).

BBB disruption and tissue inflammation jointly provoke brain edema/swelling after cerebral ischemia/reperfusion injury (CIRI), while acupuncture and electroacupuncture can alleviate CIRI symptoms. Acupuncture/electroacupuncture at GV 20 and ST 36 similarly provided neuroprotection in a rat model of middle cerebral artery occlusion (MCAO) by modulating matrix metalloproteinase 2 (MMP2), aquaporin (AQP) 4, and AQP9 expression and inflammatory cell infiltration (*34*). Laser acupuncture at GV 20 significantly decreased the brain infarct volume and malondialdehyde level and increased catalase, glutathione peroxidase, and superoxide-

dismutase activity in rats with cerebral ischemia (*35*). Moreover, electroacupuncture at GV 20, GV 14, LI 11, and KI 1 dramatically ameliorated neurologic damage and alleviated degenerative changes to the ultrastructure of cortical neurons in rats with hypoxic-ischemic encephalopathy (*36*). Based on these studies and the current authors' own clinical experience, GV 20, GV 26, GV 14, ST 36, LI 11, and LI 4 are the acupuncture points frequently used for ischemic stroke (Figure 3).

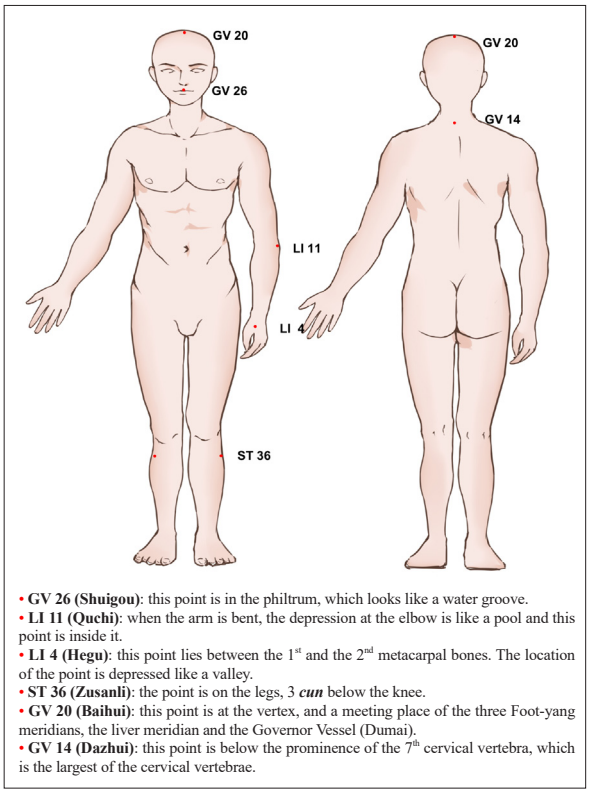

Figure 3. Acupuncture points frequently used for ischemic stroke and their location on the human body according to the clinical trials reviewed.

3.2. Mechanisms by which acupuncture treats ischemic stroke

Acupuncture is widely used for ischemic stroke, but the exact mechanisms underlying the beneficial effects of acupuncture in the treatment of stroke remain unclear. In terms of current studies, acupuncture is effective at facilitating ischemic stroke rehabilitation and reducing post-stroke infarct volume and neurological deficits; the mechanisms of those actions might involve neurogenesis, neuroinflammation, neuronal cells apoptosis, and oxidative stress (*9*). A brief description of the mechanisms by which acupuncture treats ischemic stroke is provided here (Table 4).

3.2.1. Promoting neurogenesis

Most stroke patients may have residual neurological deficits. The brain is reported to have the capability to generate new neurons, so neurogenesis has become a topic of interest. Promoting neurogenesis in a brain damaged by stroke will help stroke patients with chronic disabilities. Lu *et al*. conducted a systematic review and meta-analysis of preclinical studies to assess the current evidence for acupuncture's effect on neurogenesis in treating ischaemic stroke (*37*). Their findings indicated that acupuncture ameliorated neurological deficits and reduced brain edema in experimental ischemic stroke and that the mechanisms correlated with endogenous neurogenesis. Acupuncture may promote the proliferation, migration, and differentiation of neural stem cells (NSCs).

Electroacupuncture at GV 20 and ST 36 significantly reduced the cerebral infarct size, improved neuronal behavior, and alleviated ultrastructural injury to the hippocampus in a rat model of cerebral ischemia/reperfusion injury (*38*). The mechanism of that action might be by down-regulation of the RhoA/ROCK signaling pathway that regulates myelin-associated inhibitors (MAIs) and by promoting the expression of growth-associated protein 43 (GAP43) and brain-derived neurotrophic factor (BDNF) to protect against cerebral ischemia/reperfusion injury. In addition, electroacupuncture at GV 20 and GV 14 and mesenchymal stem cell (MSCs) transplantation reduced prominent atrophic changes in the striatum and induced proliferation of neural progenitor cells in the subventricular zone and the surrounding areas of the striatum in mice with middle cerebral artery occlusion (MCAO) (*39*). Electroacupuncture and MSC transplantation may activate the expression of neurotrophic factors such as BDNF and neurotrophin-4 (NT4), which are associated with neurogenesis in the ischemic brain.

In addition, exosomes released by the neural cells are reported to regulate the development and progression of nervous system diseases but to also play an important role in regeneration and remodeling of the nervous system after neural injury (*40*). Zhang *et al*. indicated that electroacupuncture enhanced the differentiation of endogenous neurogenesis and mitigated neurological deficits after ischemic stroke (*41*). Exosomal miR-146b is an important neuromodulator of neurogenesis that promotes endogenous neural stem cell differentiation into neurons in peri-ischemia after stroke. Electroacupuncture may promote endogenous neural stem cell differentiation into neurons in the peri-ischemic striatum and subventricular zone of the ischemic hemisphere *via* the up-regulation of miR-146b after ischemic stroke.

3.2.2. Alleviating neuroinflammation

Neuroinflammation plays a key role in the pathogenesis of ischemic stroke, and it has become a target for therapeutic intervention. Mounting evidence has indicated that electroacupuncture effectively attenuates inflammatory responses during the early stage of cerebral ischemia.

Table 4. Proposed mechanisms and evidence for acupuncture to treat ischemic stroke

Authors/*Ref.*	Species/model	Treatment	Acupuncture points	Manipulation	Course	Results of molecular expression	Mechanisms
Chen *et al.* /(*38*), 2020	SD rats MCAO/R model	EA	GV 20 and ST 36	1 mA, 2 Hz, 30 min	Once a day for 7 days	GAP43 and BDNF ↑	Promoting axonal regrowth by down-regulating the myelin-associated inhibitor-induced RhoA/ROCK pathway.
Kim *et al.* /(*39*), 2018	C57BL/6 mice MCAO model	EA + MSCs trs	GV 20 and GV 14	2 V, 2 Hz, 20 min	Once a day for 12 days	BDNF and NT4 ↑	Facilitating the amelioration of neurological impairment by enhanced neurogenesis.
Zhang *et al.* /(*41*), 2020	SD rats MCAO model	EA	LI 11 and ST 36	1 mA, 1/20 Hz, 30 min	Once a day for 21 days	Exosomal miR-146b ↑	Promoting the differentiation of endogenous neural stem cells *via* exosomal miR-146b.
Liu *et al.* /(*42*), 2016	SD rats MCAO/R model	EA	LI 11 and ST 36	0.2 mA, 1-20 Hz, 30 min	Once a day for 3 days	(*i*) TNF-α, IL1β, IL-6, NF-κB p65, p38 MAPK, and MyD88 ↓; (*ii*) IκB-α ↑	Mitigating motor impairment *via* inhibition of microglia-mediated neuroinflammation in the peri-infarct sensorimotor cortex.
Xu *et al.* /(*43*), 2018	SD rats MCAO model	EA	GV 20, LI 4, and LR 3	1 mA, 2/20 Hz, 30 min	Once a day for 3 days	(*i*) TNF-α, IL1β, and IL-6 ↓; (*ii*) IL-10 and Arg-1 ↑; (*iii*) TREM2 ↑	Attenuating neuroinflammation by promoting microglial TREM2 expression *via* the PI3K/Akt and NF-kB signaling pathways.
Sha *et al.* /(*44*), 2019	SD rats MCAO model	EA	TE 5 and ST 36	1 mA, 20 Hz, 30 min	Once a day for 7 days	(*i*) Neurological deficits and infarct volume ↓; (*ii*) miR-223 ↑; (*iii*) NLRP3, caspase-1, IL-1b, and IL-18 ↓	Alleviating neuroinflammation by inhibiting the miR-223/NLRP3 pathway.
Xing *et al* /(*45*), 2018	SD rats MCAO/R model	EA	LI 11 and ST 36	1 mA, 4/20 Hz, 30 min	Once a day for 3 days	(*i*) Neurological deficits and infarct volume ↓; (*ii*) caspase-3 ↓ and Bim and Bcl-2 ↑; (*iii*) p-ERK1/2, p-JNK, and p-p38 ↓; (*iv*) MK ↑	Alleviating neuronal apoptosis *via* the MK and ERK/JNK/p38 signaling pathways.
Xing *et al* /(*46*), 2018	SD rats MCAO/R model	EA	LI 11 and ST 36	4 V, 4 or 20 Hz, 30 min	Once a day for 2 days	(*i*) Neurological deficits, infarct volume, and the proportion of apoptotic cells ↓; (*ii*) PDK1, Akt, and GSK-3β ↑; (*iii*) p-PTEN and p-Akt ↓; (*iv*) Caspase-3 and cleaved-caspase-3 ↓ and Bim and Bcl-2 ↑	Providing neuro-protection by inhibiting apoptosis *via* the PTEN pathway.
Liu *et al.* /(*47*), 2018	SD rats MCAO/R model	EA	GV24 and GV20	1 or 20 HZ, 1 mA, 30 min	Once a day for 10 days	(*i*) Infarct volume ↓; (*ii*) JNK and p38 ↓; (*iii*) ERK1/2 ↑; (*iv*) Bcl-2/Bax ↑	Mediating neuronal cell apoptosis *via* multiple cellular pathways such as JNK, ERK, and P38.
Jittiwat /(*50*), 2017	Wistar rats MCAO model	LA	GV20	30 min	Once a day for 14 days	(*i*) Infarct volume and malondialdehyde level ↓; (*ii*) Catalase, glutathione peroxidase, and superoxide dismutase activity ↑	Mitigating brain damage and oxidative stress.
Liu *et al.* /(*51*), 2013	Wistar rats Cerebral multi-infarction model	MA	CV 17, CV 12, CV 6, ST 36, and SP 10	210 s	Once a day for 21 days (with a rest every 7 days)	Ref-1 ↑	Improving reference memory and displaying anti-oxidative action.
Jung *et al.* /(*52*), 2016	C57BL/6 mice MCAO model	EA	GV 20 and GV 14	20 min	Once a day for 3 days	(*i*) Infarct volume ↓; (*ii*) Neurological function ↑; (*iii*) ROS ↓; (*iv*) NOX4 ↓	Reducing ROS generation by down-regulating NOX4 and ameliorating blood-brain barrier disruption.
Sun *et al.* /(*53*), 2016	C57BL/6 mice MCAO model	EA	GV 20	1 mA, 2/15 Hz, 30 min	One time, 2 h before surgery	Mn-SOD ↑	Mitigating oxidative injury by activation of the Mn-SOD signaling pathway *via* CB1R-dependent STAT3 phosphorylation.

Note: abbreviations: ischemia/reperfusion (I/R) injury, middle cerebral artery occlusion (MCAO), middle cerebral artery occlusion and reperfusion (MCAO/R), growth-associated protein 43 (GAP43), mesenchymal stem cells (MSCs), brain-derived neurotrophic factor (BDNF), neurotrophin-4 (NT4), triggering receptor expressed on myeloid cells 2 (TREM2), midkine (MK), redox effector factor (Ref-1), reactive oxygen species (ROS), NADPH oxidase 4 (NOX4), manganese superoxide dismutase (Mn-SOD), cannabinoid receptor type 1 receptor (CB1R), Electroacupuncture (EA), MSCs transplantation (MSCs Trs), Laser acupuncture (LA), Manual acupuncture (MA).

Electroacupuncture at the acupuncture points LI 11 and ST 36 mitigated motor impairment by inhibiting microglia-mediated neuroinflammation in the peri-infarct sensorimotor cortex (*42*). The possible mechanism for this was because electroacupuncture attenuated the over-activation of microglia, which suppressed the release of pro-inflammatory cytokines by inhibiting NF-κB p65 nuclear translocation and by inactivation of p38 MAPK and MyD88 in the peri-infract sensorimotor cortex after middle cerebral artery occlusion and reperfusion (MCAO/R) injury. Triggering receptor expressed on myeloid cells 2 (TREM2) is a microglia-specific receptor in the CNS that is involved in regulating neuroinflammation in cerebral ischemia. Xu *et al*. found that electroacupuncture at the acupuncture points GV 20, LI 4, and LR 3 up-regulated TREM2 expression by regulating the PI3K/Akt and NF-kB signaling pathways (*43*). Moreover, electroacupuncture at the acupuncture points TE5 and ST36 markedly increased miR-223 levels, and this effect was accompanied by decreased NLRP3, caspase-1, IL-1β, and IL-18 levels in the peri-infarct cortex, which resulted in alleviated inflammatory injury associated with brain ischemia/reperfusion (*44*).

3.2.3. Inhibiting neuronal cell apoptosis

Apoptosis, the genetically programmed process of cell death, is reported to play an important role in the progression of cerebral ischemia-reperfusion injury. Several studies have confirmed that inhibition of cell apoptosis can reduce ischemia-reperfusion injury. Electroacupuncture has a beneficial effect by reducing neurological deficits and by restoring injured cerebral cells after cerebral ischemic stroke by inhibiting neuronal apoptosis.

Xing *et al*. found that electroacupuncture at the acupuncture points LI 11 and ST 36 reduced the infarct volume and neurological deficits and it reduced apoptotic cells in the peri-infarct cortex in rats with cerebral ischemia (*45*). Electroacupuncture's mechanism of mitigating apoptosis after ischemic stroke might be associated with up-regulation of growth factor midkine (MK) and mediation of the ERK/JNK/p38MAPK pathway. In another study, Xing *et al*. also found that electroacupuncture at the acupuncture points LI 11 and ST 36 inhibited neuronal cell apoptosis, and they posited that these effects were probably regulated by the PTEN pathway (*46*). In addition, Liu *et al*. found that electroacupuncture at the acupuncture points GV 20 and GV 24 promoted functional recovery in post-stroke rats by inhibiting neuronal cell apoptosis (*47*). Electroacupuncture may mediate neuronal cell apoptosis *via* multiple cellular pathways such as JNK, ERK, and P38.

3.2.4. Regulating oxidative stress

Oxidative stress is defined as a pathologic state in which cells are subjected to excessive reactive oxygen or nitrogen species (ROS/RNS) and they cannot counterbalance the deleterious effects with the antioxidant defense system, commonly resulting in cellular damage and tissue destruction (*48*). In ischemic stroke, oxidative stress can cause neuronal apoptosis, activation of inflammatory signaling pathways, and impairment of the BBB, all of which promote neurodegeneration and cell death (*49*). Recently, several studies have indicated that acupuncture has the potential to alleviate oxidative stress caused by cerebral ischemia, which may be linked to the neuroprotective effect of acupuncture. By regulating a battery of molecular signaling pathways involved in redox modulation, acupuncture activates the inherent antioxidant enzyme system and it also inhibits the excessive generation of ROS.

Laser acupuncture at the acupuncture point GV 20 significantly decreased the brain infarct volume and malondialdehyde levels and it increased catalase, glutathione peroxidase, and superoxide-dismutase activity in rats with cerebral ischemia (*50*). These findings indicated that acupuncture displayed antioxidative action in ischemic stroke. Moreover, acupuncture increases the expression of redox effector factor (Ref-1), a sensitive marker of oxidative injury within the hippocampus, consequently producing antioxidative action in rats with multiple cerebral infarcts (*51*). In addition, Jung *et al*. found that electroacupuncture at the acupuncture points GV 20 and GV 14 delayed or mitigated the development of ischemic brain edema, which might be achieved *via* down-regulation of ROS generation and NADPH oxidase 4 (NOX4) expression in mice with MCAO (*52*). The overproduction of mitochondrial ROS is a key mechanism of injury during neurodegeneration damage, and especially in the context of ischemia/reperfusion injury. Sun *et al*. indicated that electroacupuncture at GV 20 induced up-regulation of manganese superoxide dismutase (Mn-SOD) *via* the cannabinoid receptor type 1 receptor (CB1R)-dependent signal transducer and activator of transcription 3 (STAT3) phosphorylation, thus attenuating oxidative stress and resulting in neuroprotection (*53*).

4. Acupuncture for depression

Depression is a serious neuropsychiatric disorder that involves symptoms such as a persistent feeling of sadness, loss of interest or pleasure in activities, changes in weight, difficulty sleeping or oversleeping, energy loss, feelings of worthlessness, psychomotor changes, and thoughts of death or suicide. There are several types of depression including major depressive disorder, adolescent depression, antenatal depression, postpartum depression, perimenopausal depression, drug-induced depression, and post-stroke depression. Depression is recognized as a major public health problem that has a serious impact on individuals and on society, affecting approximately 400 million people worldwide (*54*). In 2008, the WHO ranked

major depression as the third leading cause of the disease burden worldwide, and it projected that the disease would rank first by 2030. Antidepressants, psychotherapy, or both are all reasonable treatment options for depression (*55*). However, patients often report intolerable adverse reactions to antidepressants such as weight gain, sedation, dry mouth, nausea, blurred vision, constipation, and tachycardia. Psychotherapy appears to produce equivalent outcomes to those obtained with antidepressants, but it is not uniformly accepted thus far, and the rate of withdrawal from treatment is similar to that with antidepressants (*56*). In recent years, numerous randomized controlled trials (RCTs) and meta-analyses have indicated that acupuncture was effective at treating depression. Moreover, many basic studies have indicated that the mechanisms by which acupuncture treats depression may be through regulation of the hypothalamic-pituitary-adrenal (HPA) axis, neurotransmitters, anti-inflammatory, and signaling pathways.

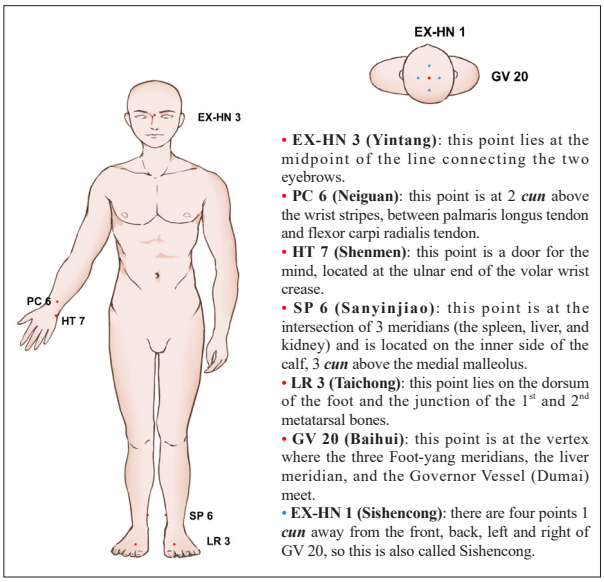

Figure 4. Acupuncture points frequently used for depression and their location on the human body according to the clinical trials reviewed.

4.1. Acupuncture points frequently used for depression

Available evidence regarding the ability of acupuncture to reduce the severity of depression is of low quality, but acupuncture is still widely used to treat depression as a complementary and alternative therapy around the world (*57*). Acupuncture appears to be more effective and safer than no treatment, control acupuncture, antidepressants, or psychotherapy. Numerous studies have been conducted to investigate the acupuncture points most frequently used to treat depression. Pilkington concluded that the four acupuncture points most frequently used for depression were GV 20, EX-HN 3, LR 3, and HT 7 (*58*). In addition, Smith *et al*. conducted a Cochrane review of 64 studies (7,104 participants) and they indicated that GV 20, EX-HN 3, PC 6, HT 7, EX-HN 1, LR 3, and SP 6 were the acupuncture points frequently used for depression (*57*). Based on these studies and the current authors' own clinical experience, GV 20, EX-HN 1, EX-HN 3, LR 3, PC 6, SP 6, and HT 7 are the acupuncture points frequently used for depression (Figure 4).

4.2. Mechanisms by which acupuncture treats depression

In recent years, research on the efficacy of acupuncture in treating depression has progressed considerably. Acupuncture involves multiple mechanisms, including inhibition of hypothalamo-pituitary-adrenal (HPA) axis hyperactivity, regulation of neuropeptides and neurotransmitters, promotion of signaling pathways, modulation of the expression of particular genes, a reduction in levels of proinflammatory cytokines, and restoration of hippocampal synaptic plasticity (*59*). A brief description of the mechanisms by which acupuncture treats depression is provided here (Table 5).

4.2.1. Regulating the HPA axis

The HPA axis is considered to be the final common pathway in the stress response and the symptoms of depression. Altered activity of the HPA axis is one of the most commonly observed neuroendocrine abnormalities in patients suffering from depressive disorder. Abnormal functioning of the HPA axis will affect behavior and physical function and induce the release of multiple hormones, including cortisol and pro-inflammatory cytokines, corticotropin-releasing factor (CRF), adrenocorticotropin hormone (ACTH), and glucocorticoids (GCs) (*60*). Several recent studies have suggested that acupuncture modulates the HPA axis, the stress response, and depression. Acupuncture may relieve the excessive excitation of the HPA axis induced by stress.

Lee *et al*. found that acupuncture at the acupuncture point PC 6 inhibited chronic corticosterone (CORT)-induced depression disorder, probably by modulating the HPA axis (*61*). Acupuncture significantly reduced depression- and anxiety-like behavior and increased NPY expression in the hypothalamus. Le *et al*. found that electroacupuncture acted on depression by modulating the HPA axis and enhancing hippocampal 5-HT/5-HT1AR in rats with chronic unpredictable mild stress (CUMS) (*62*). They indicated that electroacupuncture reversed the behavioral defects induced by CUMS in rats, it decreased expression of corticotropin-releasing hormone (CRH) mRNA in the hypothalamus, it decreased ACTH and CORT levels in plasma, and it markedly increased 5-HT levels and 5-HT1AR (mRNA and protein) expression in the hippocampus. In addition, Liu *et al*. found that electroacupuncture in the auricular concha region induced cardioinhibitory action similar to that of vagus nerve stimulation (VNS) and that electroacupuncture significantly mitigated depression induced by CUMS in rats (*63*). The antidepressant action of electroacupuncture

Table 5. Proposed mechanisms and evidence for acupuncture to treat depression

Authors/*Ref.*	Species/model	Treatment	Acupuncture points	Manipulation	Course	Results of molecular expression	Mechanisms
Lee *et al.* /(*61*), 2009	SD rats CORT injection	MA	PC 6 or TE 5	5 min	Once a day for 19 days	(*i*) Depression- and anxiety-like behavior ↓; (*ii*) NPY ↑	Stimulation of PC 6 suppressing the symptopathology of the hypoactivated HPA axis
Le *et al.* /(*62*), 2016	SD rats CUMS model	EA	ST 36 and CV 4	2/100HZ, 1 mA, 20 min	Once a day for 14 days	(*i*) CRH mRNA in the hypothalamus ↓; (*ii*) ACTH and CORT levels in plasma ↓; (*iii*) 5-HT and 5-HT1AR in the hippocampus ↑	Stabilizing the HPA axis and increasing hippocampal 5-HT/5-HT1AR
Liu *et al* /(*63*), 2013	Wistar rats CUMS model	EA	Auricular concha region	2 HZ, 1 mA, 20 min	Once a day for 14 days	Plasma cortisol ↑ and ACTH levels ↓	*Via* normalization of HPA axis hyperactivity
Guo *et al.* /(*65*), 2014	SD rats Chronic restraint stress model	EA	GV 20 and GV 29	2 HZ, 1 mA, 20 min	Once a day for 21 days	IL-1β and IL-6 in the hippocampal CA3 region ↓	Alleviating depression *via* a potential mechanism of immunological modulation
Yue *et al.* /(*66*), 2018	SD rats CUS model	EA	GV 20 and GB 34	2/100 Hz, 0.3 mA, 30 min	Every other day for 4 weeks	(*i*) NLRP3 and IL-1β ↓; (*ii*) P2X7 receptor, Iba-1, IL-18, TNF-α, and IL-6 ↓	Reversing depression-induced IL-1β-related microglial activation *via* P2X7-NLRP3 inflammatory signaling.
Zhang *et al.* /(*68*), 2020	Wistar rats LPS injection	EA	GV 20 and GV 29	2 HZ, 20 min	Once a day for 7 days	(*i*) IL-1β, IL-6, and TNF-α ↓; (*ii*) IDO ↓; (*iii*) 5-HT ↑; (*iv*) NR2B ↓	Inhibiting the inflammatory response, regulating the tryptophan degradation pathway mediated by IDO, and inhibiting NR2B activation
Han *et al.* /(*70*), 2018	Wistar-Kyoto rats depression model	EA	GV20 and EX-HN3	2 HZ, 4 mA, 15 min	Once a day for 3 weeks	5-HTT and 5-HT1A in the hippocampus CA1 region ↓	Restoring hippocampal synaptic plasticity *via* modulation of 5-HT receptors
Chen *et al.* /(*71*), 2020	Wistar rats CUMS model	EA	GV 20 and GV 29	2 HZ, 0.6 mA, 30 min	Once a day for 14 days	5-HT1A ↑	Promoting the expression of 5-HT1A receptor mRNA and protein, thereby improving synaptic plasticity in the hippocampus
She *et al.* /(*73*), 2015	Wistar-Kyoto rats depression model	EA	GV20 and EX-HN3	2 HZ, 3 mA, 15 min	Once a day for 21 days	(*i*) LTP ↑; (*ii*) GluN2B ↑	Alleviating depression-like behavior and reversing the impairment of LTP by regulating GluN2B
Zhang *et al.* /(*74*), 2021	Wistar rats CUMS model	EA	GV 20 and GV 29	2 HZ, 20 min	Once a day for 21 days	(*i*) MAP-2, PSD-95, and SYN ↑; (*ii*) GluN2B and CaMKII ↓; (*iii*) p-CREB ↑	Alleviating depression-like behavior and hippocampal plasticity *via* the GluN2B/CaMKII/CREB pathway
Kang *et al.* /(*76*), 2021	Rats Model of post-stroke depression	EA	LI 4 and LR 3	2-20 HZ, 30 min	Once a day for 21 days	BDNF and TrkB ↑	Relieving depression by regulating BDNF and its receptor TrkB

Abbreviations: corticosterone (CORT), hypothalamic-pituitary-adrenal (HPA) axis, neuropeptide Y (NPY), chronic unpredictable mild stress (CUMS), corticotropin-releasing hormone (CRH), chronic unpredictable stress (CUS), lipopolysaccharide (LPS), indoleamine-2,3-dioxygenase (IDO), long-term potentiation (LTP), microtubule-associated protein 2 (MAP-2), postsynaptic density 95 (PSD-95), synaptophysin (SYN), N-methyl-D-aspartic acid receptor (NMDAR), brain-derived neurotrophic factor (BDNF), tyrosine receptor kinase B (TrkB), Manual acupuncture, Electroacupuncture (EA).

in the auricular concha region was possibly mediated by the normalized activity of the HPA axis.

4.2.2. Alleviating neuroinflammation

Neuroinflammation is defined as the brain's response to physical injury or infection. Over the last few years, a number of studies have suggested that patients who had major depressive disorder had changes in immunologic markers including increased activity of pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α and increased inflammation. Moreover, chronic low-grade inflammation may result in activation of brain immune cells such as microglia, astrocytes, and oligodendroglia and changes in brain structure and synaptic plasticity, leading to neurodegeneration in patients with depression (*64*). Several recent studies have suggested that acupuncture might inhibit neuroinflammation and hence have a beneficial effect on pathological changes in the hippocampus and depressive symptoms.

Guo *et al*. reported that electroacupuncture at acupuncture points GV 20 and GV 29 mediated the onset of depressive symptoms and down-regulated the levels of IL-6 and IL-1β in the hippocampus of depressed rats, suggesting that electroacupuncture may potentially alleviate depression through a mechanism involving neuroinflammation and immunological modulation (*65*). Yue *et al*. conducted a study to assess the effectiveness of electroacupuncture at acupuncture points GV 20 and GB 34 on depressive-like behavior in rats with chronic unpredictable stress (CUS) (*66*). They found that electroacupuncture significantly attenuated behavioral deficits caused by CUS. Moreover, the antidepressant action of electroacupuncture was accompanied by a markedly decrease in IL-1β-related microglial activation induced by depression, which might be mediated by P2X7-NLRP3 inflammatory signaling.

Proinflammatory cytokines have been reported to activate indoleamine 2,3-dioxygenase (IDO) as a critical event in the switch from sickness to depression. IDO is a key enzyme responsible for tryptophan degradation along the kynurenine pathway (*67*). Zhang *et al*. found that electroacupuncture successfully corrected depressive-like behaviour induced with lipopolysaccharides (LPS) and that it reduced levels of inflammatory factors such as IL-1β, IL-6, and TNF-α in the blood and hippocampus, prevented over-activation of IDO and restoring NR2B expression after a challenge with LPS (*68*). The antidepressant action of electroacupuncture might be related to inhibition of the inflammatory response, regulation of the tryptophan degradation pathway mediated by IDO, and inhibition of NR2B activation.

4.2.3. Restoring hippocampal synaptic plasticity

The hippocampus is a key anatomical brain region associated with depression. Numerous studies have confirmed that changes in hippocampal plasticity including hippocampal volume, the number of synapses, synaptic plasticity, changes in glutamate receptors, neurogenesis, and glial cell plasticity are evident in both human patients with depression and rodent models of depression (*69*). Several recent studies have indicated that acupuncture is involved in the regulation of synaptic plasticity in the hippocampal region and that it alleviates the symptoms of depression.

According to various studies, the 5-HT system plays an important role in the treatment of depression. Han *et al*. found that electroacupuncture at acupuncture points GV 20 and EX-HN 3 in a model of depression involving Wistar-Kyoto (WKY) rats ameliorated depressive-like behavior by restoring hippocampus CA1 synaptic plasticity, which might be mainly mediated by regulating 5-HT receptor levels (*70*). Moreover, Chen *et al*. found that electroacupuncture at acupuncture points GV 20 and GV 29 alleviated depression-like symptoms in rats with CUMS (*71*). The underlying mechanism might include promotion of the expression of 5-HT1A receptor mRNA and protein, thereby improving synaptic plasticity in the hippocampus.

N-methyl-D-aspartic acid receptor (NMDAR) is an ionic glutamate receptor, and acute or chronic stress increases the levels of glutamate around synapses in the hippocampus and thereby leads to NMDAR over-activation. The interaction of death-associated protein kinase 1 (DAPK1) with the 2B subunit (GluN2B) C-terminus of NMDAR plays a critical role in the pathophysiology of depression and is considered to be a potential target for the structure-based discovery of new antidepressants (*72*). She *et al*. found that electroacupuncture at acupuncture points GV 20 and EX-HN 3 significantly alleviated depression-like behavior in the WKY rat model of depression (*73*). This effect might be related to the increased NMDAR subunit expression of GluN2B and enhanced long-term potentiation (LTP) in the hippocampus. Moreover, Zhang *et al*. indicated that electroacupuncture at acupuncture points GV 20 and GV 29 mitigated depression-like behaviour and improved synaptic plasticity in the hippocampal neurons of rats with CUMS and that these effects were potentially related to the GluN2B/CaMKII/CREB signalling pathway (*74*).

Brain-derived neurotrophic factor (BDNF), a neurotrophic factor, plays a key role in promoting synaptic plasticity and neuronal growth and it has been put forward as a biological marker of brain neuroplasticity. Over the last decade, mounting evidence has highlighted BDNF as a key player in antidepressant action. BDNF serves as a transducer, acting as the link between an antidepressant and the neuroplastic changes that result in the alleviation of depressive symptoms (*75*). Kang *et al*. indicated that electroacupuncture at Siguan acupoints (LI 4 and LR 3) had the same antidepressant action as fluoxetine and that electroacupuncture was more effective than fluoxetine in

relieving depression in rats with post-stroke depression (PSD) (*76*). The mechanism for this might be related to activation of the expression of BDNF and its receptor TrkB.

5. Acupuncture for IBS

IBS is a chronic, relapsing, remitting functional disorder of the gastrointestinal tract, and it is the most prevalent of those disorders. It is a symptom-based condition defined by the presence of abdominal pain or discomfort, with altered bowel habits that lack a known structural or anatomic explanation (*77*). According to the WHO DMS-IV code classification for IBS and its subcategories, IBS can be classified as either diarrhea-predominant (IBS-D), constipation-predominant (IBS-C), or with an alternating stool pattern (IBS-A) or pain-predominant. A debilitating gastrointestinal disorder, IBS affects 9-23% of the population worldwide, and women are two to four times more likely to develop IBS than men (*78*). IBS can impact the quality of an individual's daily life, cause socioeconomic problems, and potentially damage the patient-physician relationship.

The exact pathophysiology of IBS is still not completely understood, but low-grade inflammation, alterations in the gut-brain axis, visceral hypersensitivity, altered gastrointestinal motility, altered serotonin levels, microbial changes, and genetics may all contribute to symptom development (*79*). Traditionally, first-line therapies for IBS have focused on alleviating diarrhea (*e.g.*, loperamide and probiotics) or constipation (*e.g.*, fiber supplements and laxatives). Moreover, dietary and lifestyle changes are also considered as first-line treatment for all IBS subtypes (*77*). However, in the United States, only one-third of IBS patients are satisfied with their current therapy (*80*). Lack of effectiveness and associated adverse effects are common reasons for dissatisfaction. Given these gaps in treatment, some patients turn to traditional, complementary, and integrative medicine. Evidence of the efficacy of complementary and integrative treatment approaches, including behavioral therapy, herbal medicines, moxibustion, and acupuncture, is emerging. According to a Delphi expert consensus study conducted by Su *et al*., most experts (> 90%) agreed that acupuncture might be used to relieve clinical symptoms and improve quality of daily life in mild and moderate IBS (*81*). In addition, several recent studies have indicated that acupuncture alleviated the clinical symptoms of IBS and reduced recurrence *via* mechanisms such as gastrointestinal (GI) motility, visceral hypersensitivity, the immune system, neurotransmitters, and the brain-gut axis (*82*).

5.1. Acupuncture points frequently used for IBS

Selection of specific acupuncture points is critical to treatment of a given condition and can result in greater efficacy. A Delphi expert consensus study conducted by Su *et al*. recommended the acupuncture points ST 25, ST 36, and CV 12 for IBS (*81*). A systematic review and meta-analysis of 21 relevant RCTs indicated that the top six most commonly used acupuncture points for IBS-D were ST 36, ST 25, LR 3, SP 6, ST 37, and CV 12; these points were considered to have played an important role in invigorating the spleen, mitigating diarrhea, relieving the liver, and alleviating pain (*83*). Moreover, Zhu *et al*. conducted a network meta-analysis which found that acupuncture at acupuncture points such as ST 25, ST 36, ST 37, SP 6, GV 20, and EX-HN 3 might alleviate IBS-D more than drugs and with fewer adverse reactions (*84*). According to a pragmatic trial by Stuardi *et al*., the acupuncture points most frequently used for IBS included CV 12, LR 3, LI 4, ST 36, and SP 6 (*85*). Based on these studies and the current authors' own clinical experience, ST 25, ST 36, ST 37, SP 6, CV 12, LI 4, LR 3, GV 20, and EX-HN 3 are the acupuncture points frequently used for IBS (Figure 5).

5.2. Mechanisms by which acupuncture treats IBS

Numerous studies have noted the efficacy of acupuncture in attenuating the symptoms of IBS without causing obvious adverse effects. Moreover, several recent studies indicated that the mechanisms by which acupuncture treats IBS involved GI motility, visceral hypersensitivity, the brain-gut axis, the neuroendocrine system, and the immune system (*82*). A brief description of the mechanisms by which acupuncture treats IBS is provided here (Table 6).

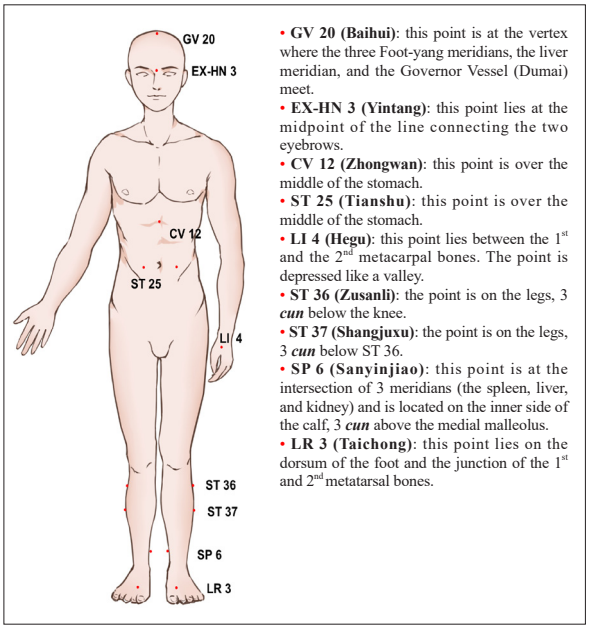

Figure 5. Acupuncture points frequently used for irritable bowel syndrome (IBS) and their location on the human body according to the clinical trials reviewed.

Table 6. Proposed mechanisms and evidence for acupuncture to treat irritable bowel syndrome (IBS)

Authors/*Ref.*	Species/model	Treatment	Acupuncture points	Manipulation	Course	Results of molecular expression	Mechanisms
Yang *et al.* /(*88*), 2019	Visceral hypersensitivity rat model	EA	ST 36	5/100 HZ, 1 mA, 30 min	Once a day for 5 days	TLR4, MCT, IL-1β, and IL-8 ↓	Ameliorating visceral hypersensitivity by decreasing the levels of pro-inflammatory cytokines and regulating TLR4 expression
Ma *et al.* /(*91*), 2009	IBS rat model	EA	ST 25 and ST 37	2/50 Hz, 15 min	Once a day for 7 days	(*i*) The number of mucosal MC ↓; (*ii*) SP and SPR ↓; (*iii*) CRH ↓	Decreasing the number of MC, the expression of SP and SPR in colon, and the CRH level in the hypothalamus
Tian *et al.* /(*93*), 2008	IBS rat model	EA	ST 36 and ST 37	4/100 Hz, 1 mA, 30 min	Once a day for 3 days	pNR1 ↓	Attenuating chronic visceral hypersensitivity by regulating spinal cord NMDA receptor phosphorylation
Sun *et al.* /(*96*), 2015	D-IBS rat model	EA	ST 25, ST 36, and LR 3	2/15 Hz, 0.8-1.3 mA, 15 min	Once a day for 14 days	(*i*) 5-HT and CGRP ↓; (*ii*) NPY ↑	Restoring the balance of the brain-gut axis
Song *et al*, /(*99*), 2020	TNBS-induced IBS rat model	EA	ST 25 and ST 36	2/15 Hz, 0.5-1.0 mA, 30 min	Once a day for 10 days	IL-18 ↓	Regulating IL-18 and gut microbial dysbiosis

Abbreviations: toll-like receptor 4 (TLR4), mast cell tryptase (MCT), mast cells (MC), substance P (SP), substance P receptor (SPR), corticotropin-releasing hormone (CRH), N-methyl-D-aspartic acid (NMDA), phosphorylated NMDA receptor subunit 1 (pNR1), diarrhea-predominant irritable bowel syndrome (D-IBS), calcitonin gene-related peptide (CGRP), neuro-peptide Y (NPY), trinitrobenzene sulfonic acid (TNBS), Electroacupuncture(EA).

5.2.1. Alleviating visceral hypersensitivity

Visceral hypersensitivity is an important hallmark feature of IBS and is the main mechanism underlying abdominal pain in patients with IBS. The pathogenesis of visceral hypersensitivity has yet to be fully elucidated, but several mechanisms have been proposed, such as inflammation, psychosocial factors, and altered sensorimotor function of the gut, a major component of which is believed to be peripheral and central sensitization of visceral afferent neuronal pathways (*86*). Especially importantly is that immune cells in the mucosal wall, such as mast cells, and enterochromaffin cells may sensitize afferent nerves *via* the release of their mediators. Several recent studies have indicated that acupuncture effectively reduced visceral hypersensitivity in IBS.

The expression of TLR4 is up-regulated in the colonic mucosa of patients with IBS and rat models of visceral hypersensitivity (*87*). Yang *et al*. indicated that electroacupuncture at acupuncture point ST36 ameliorated visceral hypersensitivity in a model of colon sensitization (*88*). The potential mechanism for this involved inhibition of the expression of TLR4 in the mast cells of colonic tissues and reduction of the levels of inflammatory factors IL-1β and IL-8 in serum. These results suggest that acupuncture can regulate visceral hypersensitivity by alleviating inflammation in patients with IBS.

Mental stress is considered as one of the factors for the induction or aggravation of the symptoms of IBS. CRH, which plays an important role in the stress response, can induce a higher level of ACTH, profound enhancement of GI motility, and visceral hypersensitivity in patients with IBS (*89*). Substance P (SP) is a gastrointestinal peptide hormone found in the CNS and gastrointestinal tract and a signaling molecule connecting the nervous system to the immune system (*90*). Ma *et al*. indicated that electroacupuncture at ST 25 and ST 37 decreased the number of mucosal mast cells, it down-regulated the expression of CRH in the hypothalamus, and it decreased the expression of SP and substance P receptor (SPR) in the colon of rats with IBS (*91*). These findings suggest that acupuncture might regulate visceral hypersensitivity by alleviating mental stress in patients with IBS.

The occurrence of chronic visceral hypersensitivity is closely related to the phenomenon of central sensitization at the spinal level. Pivotal in the development of spinal cord central sensitization is the activation of the N-methyl-d-aspartate receptor (NMDAR) (*92*). NMDAR is an ionotropic glutamate receptor widely expressed in the nervous system that plays key roles in excitatory synaptic transmission. Tian *et al*. indicated that electroacupuncture at ST 36 and ST 37 significantly inhibited hyperphosphorylation of spinal cord NMDAR in a rat model of chronic visceral hypersensitivity (*93*). This finding suggests that the activity of spinal cord

NMDAR can be affected by electroacupuncture and that acupuncture can be a promising physical therapy to alleviate chronic visceral hypersensitivity in patients with IBS by regulating central sensitization.

5.2.2. Modulating the gut-brain axis and gut microbiota

The gut-brain axis is a bidirectional communication system that integrates brain and GI functions, such as gut motility, appetite, and weight, and the microbiota plays a critical role in the gut (*94*). The gut-brain axis includes the enteric nervous system (ENS), the CNS, the gut wall in the periphery, and the HPA axis. Mounting evidence has suggested that the pathogenesis of IBS is associated with an abnormality of the gut-brain axis as well as gut microbiota. Changes in the gut microbiota alter the immunity and integrity of the gut and further modulate the gut-brain axis and the gut neuromuscular junction (*95*). Several recent studies have indicated that acupuncture seems to be a specific therapy that alleviates IBS symptoms and that also restores the balance of the gut-brain axis and gut microbiota (*82*).

5-HT is a major neurotransmitter in the gut-brain axis. Calcitonin gene-related peptide (CGRP) and its receptors are enriched in DRG and correlate with visceral hypersensitivity. Neuro-peptide Y (NPY) is a major neurotransmitter in the enteric plexus, and an increase in NPY may affect cholinergic transmission in the inferior mesenteric ganglion and regulate stress and mood by affecting the hippocampus and hypothalamus. Sun *et al*. found that electroacupuncture at ST 25, ST 36 and LR 3 alleviated IBS-D symptoms, and they encouraged its clinical use in patients with IBS (*96*). Moreover, electroacupuncture decreased the levels of 5-HT, CGRP, and NPY in the gut-brain axis, which indicated that electroacupuncture can restore the balance of the brain-gut axis in IBS-D. In addition, a randomized controlled clinical trial indicated that both electroacupuncture and mild-warm moxibustion treatment at ST 25 and ST 37 significantly alleviated some of the most intrusive symptoms in patients with IBS-C and that electroacupuncture was more effective than mild-warm moxibustion (*97*). The efficacy of these two therapies might be through modulation of the gut-brain axis (Registration No. ChiCTRTRC-11001349).

IBS is closely linked to alterations in gut microbiota composition, which can lead to increased permeability of the intestinal mucosal barrier and modulation of cytokine secretion, thus playing an important role in the pathophysiology of IBS. IL-18 is an important pro-inflammatory factor in the GI tract. It can excite macrophages, differentiate Th1 cells, induce the production of IL-1β and TNF-α by T-cell subtype 1 (Th1) and NK cells, and promote the synthesis of TNF and other chemokines (*98*). Song *et al*. indicated that post inflammatory-IBS was associated with a significant increase in IL-18 levels as well as changes in microbiota diversity and that electroacupuncture at ST 25 and ST 37 in a rat model reversed those changes (*99*). Electroacupuncture appeared to alleviate IBS symptoms by decreasing IL-18 levels and altering the composition of microbiota, and especially Fusobacteria.

6. Conclusion

With the increasing availability of acupuncture around the world, patients are increasingly seeking and using acupuncture to treat a multitude of symptoms and conditions to maintain health and prevent illness. Over the past few decades, basic and clinical research on acupuncture has made considerable progress. Internationally recognized evidence from clinical studies has been published and a preliminary system to clinically evaluate acupuncture has been created. Moreover, scientists have strived to explore the physiological and biological mechanisms of acupuncture. Some basic studies have indicated that acupuncture has various actions, such as analgesic, muscle relaxing, anti-inflammatory, mild anxiolytic, and antidepressant actions, with possible biological mechanisms such as central sensitization, neurotransmitters, the intestinal flora, immune regulation, oxidative stress, and neuroinflammation. The current review described the common indications for acupuncture recommended by the WHO and the use of acupuncture in China, the United States, Australia, and several other countries. This review then summarized the mechanisms by which acupuncture treats common diseases including LBP, ischemic stroke, depression, and IBS and it also cited specific acupuncture points for treating these conditions.

The hope is that this review will provide useful information for both acupuncturists and researchers to better understand the mechanisms of acupuncture and reasons for its usage. However, most current research on acupuncture is still in its infancy, and much of the scientific evidence surrounding it is fragmentary. There are still great challenges on how to fully integrate acupuncture into the Western medical paradigm. Therefore, both acupuncturists and researchers must continue to conduct studies to further investigate and provide more evidence of the merits of acupuncture.

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Brief Report

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HIV/AIDS strategies should focus on outcomes and the psychological status of older patients diagnosed with HIV

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SUMMARY In the context of an aging global population, the aging of patients with HIV is an issue that society will have to face. Data indicate that between 2011 and 2019, the proportion of patients age 60 and over who were newly diagnosed with HIV in China increased from 12% to 25%. In contrast to younger groups, the special characteristics of older patients pose major challenges to the management of their disease. The current study examined the clinical outcomes and psychological status of patients age 50 and over who were diagnosed with HIV. Out of a total of 566 older patients from eastern China, viral suppression was achieved in 446 (78.8%), treatment was immunologically effective in 410 (72.4%), and treatment was effective in 324 (57.2%). Thirty-nine patients (6.9%) had significant anxiety and 143 (25.3%) exhibited depressive tendencies. Level of education and the time from diagnosis to treatment were associated with the effectiveness of treatment. Age, sleep quality, chronic illness, exercise, and travel time to medical appointments were associated with depressive symptoms. These findings suggest that the burden of HIV among the older population remains high in more economically developed areas. The urgent need for HIV education and screening programs, as well as follow-up visits and early initiation of treatment in older patients, is called for.

Keywords effectiveness of HAART, HIV/AIDS, older patients, depressive symptoms

1. Introduction

In recent years, aging has further intensified in China (*1*). According to data from China's 7th National Census, the country's population totaled 141.78 million, of which 18.70% were age 60 and over and 13.50% were age 65 and over. The proportions of these two age groups increased by 5.44% and 4.63%, respectively, compared to 2010 (*2*). As the older population continues to grow, society will be confronted by the aging of patients infected with HIV. Relatively stable viral suppression has been achieved in an increasing number of patients with HIV through highly active antiretroviral therapy (HAART). This is reflected at the epidemiological level in the reduction in mortality and prolonged survival for patients with HIV. According to a report in the United States and dependent areas, the number of people over the age of 55 years with HIV increased from 2014-2018 (Figure 1) (*3*). A point worth mentioning is that most older patients with HIV are currently infected and diagnosed in midlife (*4*). Data indicate that between 2011 and 2019 the proportion of patients age 60 and over who were newly diagnosed with HIV in China increased from 12% to 25% (Figure 2) (*5*). This indicates that the incidence of new HIV infections in older people in China is also increasing each year.

Compared to the general population, older patients have some specific problems, such as reduced immune function, a high risk of chronic comorbidities, and high levels of anxiety and depression. All of these problems pose significant challenges to health management. An increase in the $CD4^+$ T lymphocyte count (CD4) over 2 years in patients receiving HAART is negatively correlated with age (*6*). Despite receiving HAART, older patients have a worse prognosis and more rapid disease progression compared to younger patients. In older patients, HIV is often transmitted through heterosexual sex, and a higher proportion of those patients are males (*7*). Older men need not consider contraception, and they often do not use condoms when having sex with their spouse or sexual partners. These behaviors increase the risk of HIV infection for themselves and their sexual

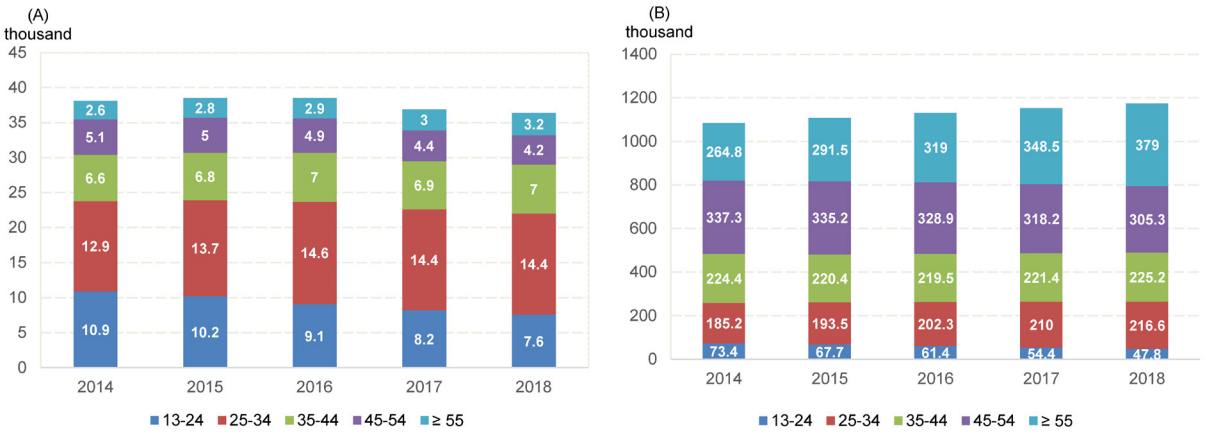

Figure 1. HIV incidence and prevalence among persons ≥ the age of 13 in the United States from 2014 to 2018. Based on data from the Center for Disease Control and Prevention, the number of patients newly diagnosed with HIV ≥ the age of 55 increased from 26,000 in 2014 to 32,000 in 2018 and the number of people living with HIV age 55 or over increased from 264,800 in 2014 to 379,000 in 2018.

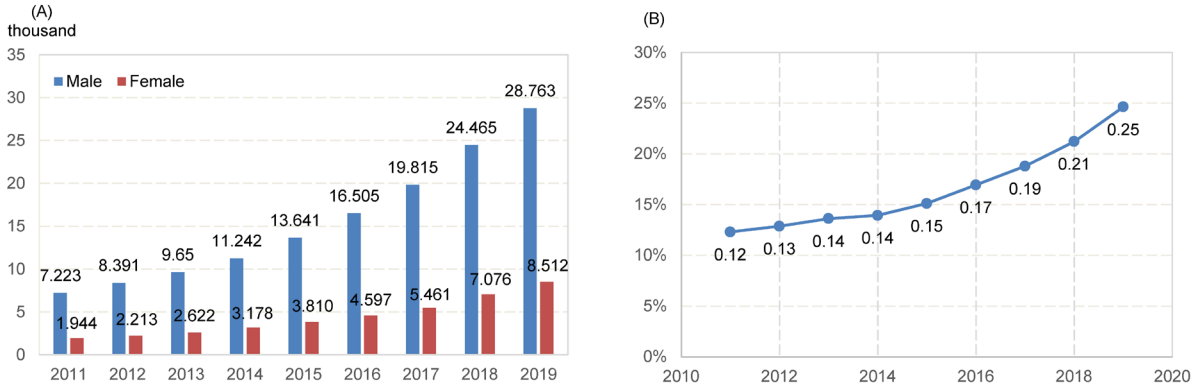

Figure 2. Gender distribution and proportion of patients age 60 and over newly diagnosed with HIV in China from 2011-2019. Based on data from the Chinese CDC and Public Health Science Data Center, the number of male patients newly diagnosed with HIV ≥ the age of 60 in China increased from 7,223 in 2011 to 28,763 in 2019 and the number of female patients increased from 1,944 to 8,512. The ratio of male to female patients was relatively consistent during the 9 years in question, with more than three times as many male patients as female patients. The proportion of patients age 60 and over newly diagnosed with HIV increased from 12% in 2011 to 25% in 2019.

partners (*8*). A study has found that older patients may face more complex physical, psychological, and social adjustment challenges due to the double whammy of aging and AIDS (*9*). Havlik *et al.* (*10*) reported that more than 50% of older patients had depressive symptoms. Therefore, the factors associated with infection and treatment need to be promptly ascertained in order to control the spread of outbreaks among older patients.

Shanghai is a typical large metropolis with one of the largest elderly populations in China. Although the prevalence of HIV is low, data indicate that the prevalence of HIV and the proportion of older patients with HIV are increasing in Shanghai (*5,11*). The current study was conducted in patients age 50 and over who were newly diagnosed with HIV in Shanghai. Their clinical outcomes and their virological and immunological status were described and analyzed in a real-world context. Their anxiety and depression profiles were also analyzed to ascertain the characteristics of their distribution and factors influencing those conditions in order to provide a scientific basis for the formulation of prevention and treatment strategies targeting older patients.

2. Materials and Methods

2.1. Study design

A cross-sectional study was conducted by administering a one-on-one questionnaire in an outpatient clinic. Data on outcome measures were obtained from clinic files or the hospital information system (HIS). The questionnaire consisted of three parts: the first part concerned demographic characteristics (age, gender, marital status, level of education, *etc.*); the second part concerned information related to HAART (route of infection, chronic disease, CD4 count, viral load, *etc.*); and the third part concerned psychological status (anxiety/depression) and the Hospital Anxiety and Depression Scale (HADS). This study was approved by the Ethics Committee of the Shanghai Public Health Clinical Center (approval no. 2019-S054-02). Informed

consent was obtained from all participants.

2.2. Study population

Potential participants were patients seen at the outpatient clinic of the Department of Infection and Immunology, Shanghai Public Health Clinical Center from September 2019 to September 2020. These patients were positive for HIV antibodies according to primary screening *via* an enzyme-linked immunosorbent assay, and those results were confirmed using Western blotting. Participants were all age 50 years or over at the time of HIV diagnosis and they had received HAART for three years or longer. Patients over 50 years of age who were newly diagnosed with HIV were deemed to be "older" while those <the age of 50 were deemed to be "younger" (*7*). Potential participants with a history of substance abuse or drug use or who were unable to clearly provide consent were excluded. Potential participants who for any reason might not be able to complete this study were also excluded.

2.3. Outcomes

2.3.1. Clinical outcomes and the effectiveness of treatment

(*i*) Viral suppression: According to Chinese guidelines on treatment of HIV (*12*), virological indicators are the most important indicators. Viral load is mainly used to evaluate the effectiveness of viral suppression after HAART in patients with HIV. A recent viral load < 20 copies/mL was deemed to be complete viral suppression.

(*ii*) Immunological indicators: In this study, an increase in CD4 cells served as a supplementary indicator to further corroborate the effectiveness of treatment. According to the aforementioned guidelines, an increase in CD4 cells > 100 cells/uL or an increase > 30% after 1 year of HAART suggests that immunotherapy has been effective (*12*).

(*iii*) Immune reconstitution: Levels of CD4 cells are mainly used to evaluate the immune status of patients with HIV. Considering the specificity of the population, CD4 cells of 350 cells/uL or more after three years of HAART was deemed to be immune reconstitution.

In this study, treatment was deemed to be effective with an increase in CD4 cells > 100 cells/uL or > 30% after 1 year of HAART and a viral load < 20 copies/mL.

2.3.2. Psychological status

The HADS can rapidly screen anxious/depressed patients and is now widely used to assess depression and anxiety status in inpatients (*13*). In the current study, the HADS was used to evaluate psychological symptoms of anxiety/depression with 14 items, including two subscales for anxiety (7 items) and depression (7 items). A study participant was considered positive for anxiety/depression if his or her score was ≥ 8, with a maximum score of 21.

2.4. Statistical analysis

IBM SPSS Statistics 23.0 was used for data analysis. The normality of data was tested. The mean ± standard deviation was used to describe data with a normal distribution, quartiles were used to describe data with a non-normal distribution, and the percentage (%) was used to describe numerical data. Differences between groups were analyzed using ANOVA for normally distributed data. Non-parametric tests were used to analyze non-normally distributed data, and the chi-square test and Fisher's exact probability test were used to analyze numerical data. Logistic regression analysis was used to analyze influencing factors. A P value < 0.05 was considered significant.

3. Results and Discussion

3.1. Descriptive statistics overall and by HIV status

Table 1 summarizes the demographic of characteristics of the sample of older patients from 1994 to 2017. This study involved 566 patients infected with HIV (496 males (87.6%) and 70 females (12.4%)) from eastern China. As is apparent from the table, most participants (57.2%) were 60-70 years of age, and 18.7% were over 70 years of age. Most participants (64.0%) had a BMI from 18.5 to 23.9 kg/m^2. Few of the participants (7%) lived in rural areas. Most participants (409 patients, 72.3%) were married, most (458 patients, 80.9%) had a junior high school or high school level of education, and most (446 patients, 78.8%) were retired. Few participants (158 patients, 27.9%) lived alone. This study found that a slightly higher percentage of participants had same-sex HIV infections than heterosexual infections. And the ratio of male to female patients differed significantly (more than 7 times as many males as females). This reflects the seriousness of the HIV status among elderly men who have sex with men in Shanghai. Elderly males may have a greater desire for sex than females. In addition, the sex life and risky sexual behavior of elderly males are key reasons for HIV infection (*14,15*).

3.2. Clinical outcomes and psychological characteristics of older patients

Table 2 summarizes the clinical outcomes and psychological characteristics of older patients. Viral suppression was achieved in a total of 446 participants (78.8%). Levels of viral suppression were similar across three age groups, with the highest rate of suppression being 82.4% among participants under the age of

60. Treatment was immunologically effective in 410 participants (72.4%). Treatment was effective in a total of 324 participants (57.2%). In this study, immunological nonresponse (INR) after long-term HAART was defined as a CD4 cell count < 350 cells/μl in patients who had received HAART for more than 36 months. An INR was present in 257 older patients (45.4%), and the rate of INR was similar across all age groups. Thirty-nine patients (6.9%) exhibited symptoms of anxiety and 143 (25.3%) had depressive tendencies, while the younger group (under 60 years of age) had the lowest percentage of both psychological symptoms.

Table 1. Descriptive statistics overall and by HIV status (*n* = 566)

Characteristics	*n*	%
Gender		
Male	496	87.6
Female	70	12.4
Age (years)		
< 60	136	24
60 ~	324	57.2
≥ 70	106	18.7
BMI (Kg/m^2)		
< 18.5	32	5.7
18.5-23.9	362	64.0
24-28	146	25.8
≥ 28	26	4.6
Residence		
Rural	40	7
Non-rural	526	93
Annual household income ($)		
< 10,700	318	56.2
≥ 10,700	248	43.8
Personal medical expenses annually ($)		
< 466	217	38.3
≥ 466	349	61.7
Living conditions		
Living alone	158	27.9
Living with others	408	72.1
Level of education		
Primary School or below	52	9.2
Middle School or High School	458	80.9
University or above	56	9.9
Marital status		
Married	409	72.3
Unmarried	52	9.2
Divorced or widowed	105	18.6
Employment status		
Working	92	16.3
Retired	446	78.8
Unemployed	28	4.9

3.3. Analysis of factors influencing the effectiveness of treatment and symptoms of depression

3.3.1. Effectiveness of treatment

Table 3 shows the results of an analysis of the association between each background variable and the effectiveness of treatment in older patients from among the entire sample. Treatment was effective in a total of 324 participants (57.2%). Univariate analysis indicated that significant variables associated with effectiveness of treatment included age, marital status, level of education, living conditions, time from HIV diagnosis to treatment, and anxiety and depression. Multivariate analysis indicated that treatment was more likely to be effective in patients who were college-educated and who underwent treatment within one month of being diagnosed with HIV.

Public education about the HIV epidemic has spread nationwide. HIV screening and treatment have been heavily promoted *via* continued updates of guidelines on HIV prevention and treatment (*16-18*). Although HIV testing and HAART are free, treatment was not entirely effective in 42.8% of the older patients in the current study. Moreover, nearly half of the participants developed INR. This may be due to a progressive decline in immune function with age, suggesting that advanced age may be a risk factor for INR and adverse outcomes (*19*). Like a previous study (*20*) found, our study found that treatment was more likely to be

Table 2. Clinical outcomes and psychological characteristics of older patients

Clinical indicators	*n* (%)				*P*
	< 60	60-70	> 70	Total	
Virologic suppression					
Recent viral load < 20 copies/mL	112 (82.4)	250 (77.2)	84 (79.2)	446 (78.8)	0.46
Immunological indicators					
Increase in CD4 cells > 100 cells/uL or an Increase > 30% after 1 year of HAART	103 (75.7)	237 (73.1)	70 (66)	410 (72.4)	0.22
Effective treatment*	83 (61.0)	187 (57.7)	54 (50.9)	324 (57.2)	0.28
Immune reconstitution					
CD4 cells ≥350 cells/uL after 3 years of HAART	76 (55.9)	172 (53.1)	61 (57.5)	309 (54.6)	0.72
Psychological conditions					
Anxiety	6 (4.4)	26 (8.0)	7 (6.6)	39 (6.9)	0.37
Depression	26 (19.1)	82 (25.3)	35 (33.0)	143 (25.3)	0.04

*Treatment was deemed to be effective with an increase in CD4 cells > 100 cells/uL or > 30% after 1 year of HAART and a viral load < 20 copies/mL.

Table 3. Univariate and multivariate logistic regression analysis of the effectiveness of treatment in older patients (*n* = 566)

Characteristics	Total	Univariate *P*-value	OR (95%CI)	Multivariate *P*-value	OR (95%CI)
Age (years)					
< 60	83 (61.0)	0.01	1		
60 ~	187 (57.7)	0.51	0.87 (0.58-1.31)		
≥ 70	54 (50.9)	0.12	0.66 (0.40-1.11)		
Gender					
Male	282 (56.9)		1		
Female	42 (60.0)	0.62	1.14 (0.68-1.90)		
BMI (Kg/m)					
< 18.5	14 (43.8)	0.25	1		
18.5-23.9	211 (58.3)	0.12	1.80 (0.87-3.72)		
24-28	81 (55.5)	0.23	1.60 (0.74-3.46)		
≥ 28	18 (69.2)	0.05	2.89 (0.98-8.58)		
Marital status					
Married	246 (58.9)	0.003	1		
Unmarried	19 (44.2)	0.97	1.01 (0.56-1.81)		
Divorced or widowed	59 (56.2)	0.82	0.95 (0.62-1.46)		
Level of education					
Primary school or lower	24 (46.2)	0.58	1	0.06	1
Middle school or High school	261 (57.0)	0.14	1.55 (0.87-2.75)	0.11	1.62 (0.90-2.93)
University or higher	39 (69.6)	0.01	2.68 (1.22-5.89)	0.02	2.64 (1.12-5.91)
Employment status					
Working	58 (63.0)	0.45	1		
Retired	251 (56.3)	0.23	0.76 (0.48-1.20)		
Unemployed	15 (53.6)	0.37	0.68 (0.29-1.60)		
Route of infection					
Heterosexual transmission	106 (61.3)	0.43	1		
Homosexual transmission	100 (54.9)	0.23	0.77 (0.51-1.18)		
Unknown	118 (55.9)	0.29	0.80 (0.53-1.21)		
Living conditions					
Living alone	77 (48.7)		1		
Living with others	247 (60.5)	0.009	1.63 (1.13-2.35)		
Smoking					
Yes	110 (58.8)		1		
No	214 (56.5)	0.15	1.57 (0.64-1.30)		
Drinking					
Yes	89 (60.5)		1		
No	235 (56.1)	0.08	1.84 (0.57-1.22)		
Exercise					
Yes	189 (55.8)				
No	135 (59.5)	0.76	1.08 (0.83-1.64)		
Time*					
≤ 1	213 (60.7)	0.00	1	0.06	1
1-6	55 (49.1)	0.03	0.63 (0.41-0.96)	0.02	0.60 (0.39-0.94)
> 6	56 (54.4)	0.25	0.77 (0.50-1.20)	0.29	0.78 (0.49-1.25)
Chronic diseases					
Yes	153 (55.6)		1		
No	171 (58.8)	0.45	1.14 (0.81-1.59)		
Sleep quality					
Good	196 (58.5)	0.76	0.86 (0.56-1.33)		
Fair	61 (55.0)	0.51	0.90 (0.59-1.37)		
Poor	67 (55.8)	0.61			
Anxiety					
Yes	25 (64.1)		1		
No	299 (56.7)	0.002	1.31 (0.69-2.68)		
Depression					
Yes	86 (60.1)				
No	238 (56.3)	0.01	1.28 (0.80-1.73)		

*Time from diagnosis of HIV to drug treatment (months).

effective with the earlier initiation of HAART. However, at least 38% of the participants in the current study started treatment one month after diagnosis. HAART was often initiated late. The time from diagnosis of HIV to initiation of HAART needs to be reduced for people age 50 and over. Taking HIV medication every day can result in an undetectable viral load. People who have and who maintain an undetectable viral load (or who have viral suppression) can live a long and healthy life. They also have effectively no risk of transmitting HIV

to an HIV-negative sex partner.

3.3.2. Depressive symptoms

Table 4 shows the crude association between each background variable and depressive symptoms for the entire sample. These findings indicate the need to alleviate mental distress in older patients with HIV in eastern China, since approximately 25% of the participants in the current study exhibited possible depressive symptoms. Univariate analysis indicated that significant variables associated with symptoms of anxiety included age, marital status, level of education, travel time to medical appointments, employment status, smoking, drinking, exercise, chronic illness, and sleep quality. Multivariate analysis indicated that patients of advanced age, who traveled a long time to medical appointments, and who had poor sleep were more likely to have a depressed mood. In contrast, those who exercised regularly and had no chronic illnesses were less likely to be depressed.

Depression has serious negative consequences for older patients. It may increase comorbidities and the risk of suicide and reduce quality of life (*21*). In the

Table 4. Univariate and multivariate logistic regression analysis of depressive symptoms (*n* = 566)

	Total	Univariate *P*-value	OR (95%CI)	Multivariate *P*-value	OR (95%CI)
Age (years)					
< 60	26 (19.1)	0.00	1	0.08	1
60 ~	82 (25.3)	0.15	1.43 (0.87-2.35)	0.25	1.46 (0.76-2.80)
≥ 70	35 (33.0)	0.01	2.08 (1.16-3.76)	0.03	2.33 (1.08-5.02)
Gender					
Male	127 (25.6)				
Female	16 (22.9)	0.62	0.86 (0.48-1.56)		
BMI (Kg/m)					
< 18.5	119 (34.4)	0.66	1		
18.5-23.9	91 (25.1)	0.26	0.64 (0.30-1.38)		
24-28	35 (24)	0.23	0.60 (0.26-1.37)		
≥ 28	6 (23.1)	0.35	0.57 (0.18-1.84)		
Marital status					
Married	109 (26.1)	0.00	1		
Unmarried	5 (11.6)	0.10	0.52 (0.24-1.14)		
Divorced or widowed	29 (27.6)	0.72	1.09 (0.67-1.77)		
Level of education					
Primary school or lower	13 (25)	0.00	1		
Middle school or High school	124 (27.1)	0.75	1.11 (0.58-2.17)		
University or higher	6 (10.7)	0.06	0.36 (0.13-1.03)		
Employment status					
Working	14 (15.2)	0.02	1		
Retired	118 (26.5)	0.03	2.00 (1.09-3.68)		
Unemployed	11 (39.3)	0.008	3.61 (1.40-9.30)		
Travel time to medical appointments					
0.5 h	6 (10.3)	0.005	1	0.007	1
0.5-1 h	48 (21.6)	0.06	2.39 (0.97-5.90)	0.024	2.94 (1.15-7.52)
1-3 h	84 (31)	0.003	3.89 (1.61-9.42)	0.001	4.44 (1.78-11.1)
> 3 h	5 (33.3)	0.035	4.33 (1.11-16.99)	0.031	4.79 (1.15-19.9)
Route of infection					
Heterosexual transmission	41 (23.7)	0.14	1		
Homosexual transmission	39 (21.4)	0.61	0.88 (0.53-1.45)		
Unknown	63 (29.9)	0.18	1.37 (0.87-2.17)		
Smoking					
Yes	48 (25.1)		1		
No	96 (25.3)	0.002	0.33(0.68-1.51)		
Drinking					
Yes	27 (18.4)		1		
No	116 (27.7)	0.000	0.13 (1.06-2.72)		
Exercise					
Yes	64 (18.9)		1		1
No	79 (34.8)	0.000	0.10 (1.56-3.37)	0.000	0.45 (1.68-4.32)
Chronic diseases					
Yes	86 (31.3)		1		1
No	57 (19.6)	0.001	0.54 (0.36-0.79)	0.04	0.64 (0.41-0.98)
Sleep quality					
Good	68 (20.3)	0.00	1	0.001	1
Fair	24 (21.6)	0.77	1.08 (0.64-1.83)	0.80	1.08 (0.61-1.90)
Poor	51 (42.5)	0.00	2.90 (1.85-4.55)	0.000	2.56 (1.54-4.25)

current study, 35.6% of participants with depressive symptoms reported having poor sleep. In contrast, 16.5% of participants without depressive symptoms reported poor sleep. This finding is consistent with other studies which have found that poor sleep is a major factor for depression (*22-23*). Some studies have suggested a bidirectional relationship between sleep and depression, where treating depression may improve sleep and treating sleep disorders may reduce the incidence of depression (*22-24*). Therefore, sleep quality should be assessed and interventions should be promptly implemented for older patients with depressive symptoms. Daily exercise is important for the physical and mental health of patients with HIV. However, the current study found that 40.2% of participants had not engaged in any physical exercise in the last 6 months. Evidence suggests that aerobic and resistance exercise can improve cardiovascular health, enhance muscle strength, and improve quality of life for patients with HIV (*25*). Group exercise will also increase social participation and reduce HIV-related isolation (*26*). The current findings indicate that in addition to HIV itself, physical, psychological, and social factors can influence depression-related symptoms.

Given China's large population and its aging, as well as the special problems particular to older patients, greater attention should be paid to HIV prevention and health management. Several targeted strategies are hereby proposed: *i*) Creation of a community-based HIV prevention and education program for the older population. Local CDCs can work together with community health service centers to disseminate HIV-related information to the older population, with a focus on reasonable sexual desires of older males to reduce the potential for infection associated with sexual intercourse. *ii*) Careful follow-up and early initiation of HAART for older patients with HIV. Education about HAART is an important step to ensuring that treatment is effective and adherence to treatment by older patients. Moreover, early initiation of HAART helps to achieve viral suppression and reduce the risk of transmission. Careful attention and follow-up are indispensable, and especially for patients newly diagnosed with HIV. *iii*) Assessment of sleep disorders should be incorporated into routine care. The impact of sleep problems on depressive symptoms is particularly evident in older patients with HIV. Mental health and social support services can be provided to older patients with HIV and sleep disorders in conjunction with sleep clinics.

The current study had several limitations that should be acknowledged. First, information on exposure and outcomes was obtained through a self-reported questionnaire, which may constitute a reporting bias. However, this is likely to be an undifferentiated bias. Second, this study used several self-administered scales and revised some of the original scales. Although reliability tests were conducted for each scale, the reliability of these scales still needs to be validated.

In conclusion, the current findings provide a snapshot of the current status of HAART outcomes and psychological status among older patients newly diagnosed with HIV in eastern China. Out of a total of 566 patients age 50 and over who were diagnosed with HIV in eastern China, treatment was effective in 324 (57.2%). One hundred and forty-three patients (25.3%) exhibited depressive tendencies. Level of education and the time from diagnosis to treatment were associated with the effectiveness of treatment. Age, sleep quality, chronic illness, exercise, and travel time to medical appointments were associated with depressive symptoms. These findings suggest that the burden of HIV among the older population remains high in more economically developed areas. The urgent need for HIV education and screening programs, as well as follow-up visits and early initiation of treatment in older patients, is called for.

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Original Article

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Elderly patients with comorbid hypertension who prefer primary care have a lower rate of polypharmacy: A cross-sectional study in Shanghai, China

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SUMMARY In 2017, the World Health Organization highlighted polypharmacy as one of the key focus areas of the Global Patient Safety Challenge on Medication Safety. According to the experience of developed countries, the provision of primary pharmaceutical care plays a very important role in the intervention of polypharmacy in the elderly. It is necessary to assess the associations between elderly polypharmacy status and primary care in developing countries. The findings of this paper provide the prevalence of polypharmacy in patients with comorbid hypertension, and the factors associated with it. A total of 19,332 elderly patients with hypertension were completed, among which the mean (SD) number of diseases was 4.83 (1.99), the mean (SD) daily maximum number of drugs was 5.13 (2.89), and the rate of polypharmacy was 50.5%. Age, living areas, total number of visits, preference for medical institutions and the number of diseases were associated with polypharmacy. Among them, advanced age, greater number of visits and diseases are the risk factors of polypharmacy for elderly patients with comorbid hypertension. The rate of polypharmacy in patients who intend to seek treatment in community healthcare centers is low. A total of 9,603 pharmaceutical workers worked in Shanghai public hospitals in 2020, among them 52.0% worked in the central city area, and more than 70% worked in secondary and tertiary hospitals. There was a large mismatch between patients' medical preference and the number of pharmaceutical personnel. As a consequence, it is necessary to strengthen the development of community pharmaceutical care in primary medical institutions for elderly polypharmacy management.

Keywords polypharmacy, the elderly, comorbid hypertension, visit preference, primary pharmaceutical care

1. Introduction

In 2017, the World Health Organization (WHO) highlighted polypharmacy as one of the key focus areas of the Global Patient Safety Challenge on Medication Safety (*1-2*). Due to the decrease of physiological function, the elderly have a high prevalence of chronic diseases, often complicated with multiple underlying diseases, and need to take long-term medication, becoming the key population to deal with the challenge of polypharmacy. Studies have shown that, in the U.S., 20% of Medicare elderly patients have 5 or more chronic diseases, and 50% of them receive 5 or more medications (*3*). In a survey of 2,707 elderly European patients with an average age of 82.2 years, 51% used more than 6 drugs (*4*). According to a survey of 300 thousand Koreans aged 65 and above, 86.4% of them have multiple drug use problems (*5*). Concern regarding possible harm of excessive prescribed medication for comorbid elderly has evolved into a research field covering the term "polypharmacy".

According to the experience of developed countries (Table 1), the important carrier to control the issue of polypharmacy is the two-way referral-based family doctors/pharmacists team, which carries out a comprehensive and systematic assessment of medication status of elderly patients, typically used interventions to reduce overall numbers of drugs or avoid certain categories of drugs thought to be inappropriate, decreasing polypharmacy and improving patient outcomes (*6-9*). This suggests that the provision of primary pharmaceutical care in primary health care

Table 1. Models of polypharmacy management for elderly in typical countries in the world

Country	Model	Content	Reference
the U.S.	Medication Therapy Management Services (MTMS)	Mainly including five core elements: Medication Therapy Review (MTR), Personal Medication Record (PMR), Medication-related Action Plan (MAP), Intervention and/or Referral (I & R) and Documentation and Follow-up (DFU).	Through pharmacists, patients are managed in the whole process of medicine
the UK	NHS pharmaceutical care	Essential Service (Dispensing of drugs, Repeat dispensing, Waste management, Public health, Signposting, Support for self-care and Clinical governance), Advanced Service, Enhanced Service.	Community based, providing continuous services with diversified contents
Germany	Family pharmacy contract	In 2004, the Health Insurance Fund signed a full health contract involving general practitioners, family physicians and family pharmacists, providing health promotion, rational prescription, pharmaceutical care, *etc.*	With the support of laws and regulations, a standardized pharmacist responsibility system has been established to ensure the orderly implementation of pharmaceutical care

(PHC) plays a very important role in the intervention of polypharmacy in the elderly.

As one of the countries with the most serious degree of aging, China's 7th National Census shows that the population aged 60 and over totaled 264.02 million, of which about 69% were patients with chronic diseases (*10*). Some studies have found that about 58.24% of elderly out-patients in China have multiple drug use (*11*). A survey of 426 hospitalized elderly patients in China showed that the average number of drugs used in elderly patients was 8, and the highest up to 23 (*12*). A survey on the medication of elderly patients in the Chinese community showed that the average medication types of patients were 10.2±5.6 (*13*). At present, some small-section studies still find that the current situation of multiple drug use among the elderly in China is relatively serious, but there is lack of a large sample size and systematic research data.

Although China issued a number of policy documents at the national level, and began to pay close attention to the rational use of drugs in the elderly, there are still some key issues in elderly polypharmacy management, such as a high degree of specialization, weak consciousness of drug use among the elderly, insufficient quantity and quality of pharmaceutical personnel, low social recognition of pharmaceutical care (especially grass-roots pharmaceutical care), *etc.* (*14*). Given these situations, it is necessary to assess the association between elderly polypharmacy status and primary care. In other words, in the current situation, whether the provision of grass-roots drug services through primary health care still has a positive impact on elderly polypharmacy management.

Shanghai is a province with a mature implementation of the family doctor system in China, which established the "1+1+1" team combination signing service model in 2015, proposing to increase the number of pharmacists in the family doctor team (*15*). In addition, Shanghai has a relatively high economic level and a large amount of aging, so it is more representative for conducting a sampling study on elderly patients with hypertension, which rank first in common diseases, frequently-occurring diseases and endemic diseases.

The objective of this study was to characterize and determine the prevalence of polypharmacy in patients with comorbid hypertension, and to identify the factors associated with it. In addition, the possible association between polypharmacy and preference for primary care among elderly patients was discussed, through the comparison of patient data (Demand-side) and pharmacists data (Supply-side), in order to provide a reference for policy-making of polypharmacy management for the elderly in China.

2. Methods

2.1. Study design

This is a cross-sectional study conducted in Shanghai by the Department of Health Policy and Management, School of Public Health, Fudan University. Data on outcome measures were obtained from institutions by Shanghai Municipal Health Commission and Shanghai Medical Insurance Administration Center. This study was approved by the Ethics Committee of School of Public Health, Fudan University (International Registration Number: IRB00002408 & FWA00002399).

2.2. Setting and participants

2.2.1. Patients

As of December 31, 2019, the elderly population aged 65 and above in Shanghai was 3,616,600. According to the pre-survey, 2.52% of the elderly in a district of Shanghai (2,956 people surveyed) took more than 6 medicines every day, and the prevalence of hypertension was about 20% in Shanghai. Therefore, according to the sample size calculation formula $N = z2(\alpha/2)\,\pi\,(1-\pi)/\delta 2$, the appropriate sample size is about 20,000. Diagnosis data (outpatient and inpatient diagnosis and records) of 20,000 patients with hypertension in 2019

were randomly extracted from the patient visit database of the information center of Shanghai Municipal Health Commission, and the outpatient medication data of these patients were obtained from Shanghai Medical Insurance Administration Center. After matching, there were 19,332 patients with the effective diagnosis and medication data in 2019 (the effective rate was 96.67%).

2.2.2. Pharmacists in Shanghai

Under the coordination of the Pharmaceutical Administration Office of Shanghai Municipal Health Commission, the information of pharmacists in all public medical institutions (tertiary hospitals, secondary hospitals and community healthcare centers) in Shanghai was provided.

2.3. Definitions and variables

2.3.1. Definitions

Geriatric comorbidity: refers to the phenomenon that two or more chronic diseases coexist in the same elderly patient. This chronic disease refers not only to the common diseases of the elderly (such as hypertension, coronary heart disease, diabetes, *etc.*), but also to the elderly's special syndrome of seniors or old age problems (such as falls, weakness, sleep disorders, malnutrition, urinary incontinence, delirium, depression and drug addiction).

Polypharmacy: at present, there is no recognized definition of polypharmacy in the international academic community, and there are still differences in the number and degree of multiple drug uses (*16-17*). In this study, polypharmacy is defined as simultaneous use of 5 or more drugs (excluding Chinese traditional medicine) or treatment of patients beyond clinical need.

2.3.2. Data

The main contents of institutional data include: *i*) Demand-side: age, gender, living areas, number of visits, number of diseases, maximum number of drugs per day, *etc.*; *ii*) Supply-side: age, gender, working areas, working medical institutions, work experience, specialty, education, professional title, authorized strength, post category, *etc.* The main dependent variable was the rate of polypharmacy in patients.

2.4. Statistical analysis

IBM SPSS Statistics 23.0 was used for data analysis. The normality of data was tested. The mean ± standard deviation was used to describe data with a normal distribution, quartiles were used to describe data with a non-normal distribution, and the percentage (%) was used to describe numerical data. Logistic regression analysis was used to analyze influencing factors. A P value < 0.05 was considered significant.

3. Results

3.1. Basic characteristics and outcomes of elderly patients with comorbid hypertension

A total of 19,336 elderly patients with hypertension were sampled, among which only 4 patients had a single hypertension disease, and almost all the sampled patients were hypertensive comorbidities (in addition to hypertension, they also had other chronic diseases). Data gathering was completed for these 19,332 patients with comorbid hypertension. Demographic and medical history characteristics of the study participants are presented in Table 2.

Nineteen thousand three hundred and thirty-two patients (men 9,738 [50.4%]; women 9,594 [49.6%]) were enrolled in this study. The mean (SD) age was 75.86 (7.69) years. The degree of aging in Shanghai is relatively high, and the elderly who aged 80 and over with comorbid hypertension account for 31.9%. Seven thousand eight hundred and fourteen patients (40.4%) were living in central city areas.

In terms of the number of visits, the average number of visits in community healthcare centers is higher than that in secondary and tertiary medical institutions. The median number of annual total visits was 21, of which community healthcare centers were the most frequent. In terms of disease and drug use, the mean (SD) number of diseases was 4.83 (1.99), the mean (SD) daily maximum number of drugs was 5.13 (2.89), and the polypharmacy rate was 50.5%.

Table 2. Basic Characteristics and outcomes of elderly patients with comorbid hypertension

Characteristics	n = 1,9332
Age	
< 80	13,162 (68.1%)
≥ 80	6,170 (31.9%)
Mean age	75.86 (7.69)
Gender	
Male	9,738 (50.4%)
Female	9,594 (49.6%)
Living areas	
Central city	7,814 (40.4%)
Suburb	7,644 (39.5%)
Countryside	3,874 (20.0%)
The number of visits	
Community healthcare center	11.00 (5.00, 18.00)
Secondary hospital	4.00 (1.00, 10.00)
Tertiary hospitals	1.00 (0.00, 5.00)
Total number of visits	21.00 (13.00, 31.00)
The number of diseases	4.83 (1.99)
Maximum number of drugs per day	5.13 (2.89)
Outcomes	
Polypharmacy	9,769 (50.5%)

3.2 Predictors of polypharmacy among elderly patients with comorbid hypertension

A significant association was observed between polypharmacy and the following variables by multivariate analysis (Table 3): age ≥ 80, living areas, total number of visits, preference for medical institutions and the number of diseases. Among them, advanced age, greater number of visits and diseases are the risk factors of polypharmacy for elderly patients with comorbid hypertension. In addition, compared with patients living in central city areas, patients living in suburb areas and countryside areas are more likely to establish polypharmacy. Compared with patients who prefer to see a doctor in the community healthcare center, patients who prefer secondary and tertiary hospitals are more likely to establish polypharmacy.

Figure 1 intuitively supports and displays the distribution of polypharmacy in different ages and genders of elderly patients with comorbid hypertension living in different urban areas in Shanghai. Patients living in the countryside, those with a higher willingness

Table 3. Predictors of Polypharmacy among elderly patients with comorbid hypertension

Clinical Characteristic	Polypharmacy cases	Univariate *P*-value	OR (95%CI)	Multivariate *P*-value	OR (95%CI)
Age ≥ 80	3,648 (37.3%)	< 0.0001	1.66 (1.56-1.77)	< 0.0001	1.68 (1.57-1.79)
Male	4,905 (50.2%)	0.65	0.99 (0.93-1.04)	-	-
Living areas					
CentralCity	3,888 (39.8%)	-	-	-	-
Suburb	4,071 (41.7%)	< 0.0001	1.15 (1.08-1.23)	< 0.0001	1.35 (1.26-1.44)
Countryside	1,810 (18.5%)	< 0.0001	0.89 (0.82-0.96)	< 0.0001	1.14 (1.05-1.24)
Total number of visits	27.96 (16.43)	< 0.0001	1.04 (1.04-1.05)	< 0.0001	1.04 (1.04-1.05)
Preference for medical institutions					
Community healthcare center	1,418 (14.5%)	-	-	-	-
Secondary hospital	2,913 (29.8%)	< 0.0001	1.83 (1.71-1.96)	< 0.0001	2.06 (1.91-2.21)
Tertiary hospitals	5,438 (55.7%)	< 0.0001	1.84 (1.68-2.01)	< 0.0001	2.25 (2.04-2.48)
The number of diseases	5.06 (2.07)	< 0.0001	1.13 (1.11-1.15)	< 0.0001	1.09 (1.08-1.11)

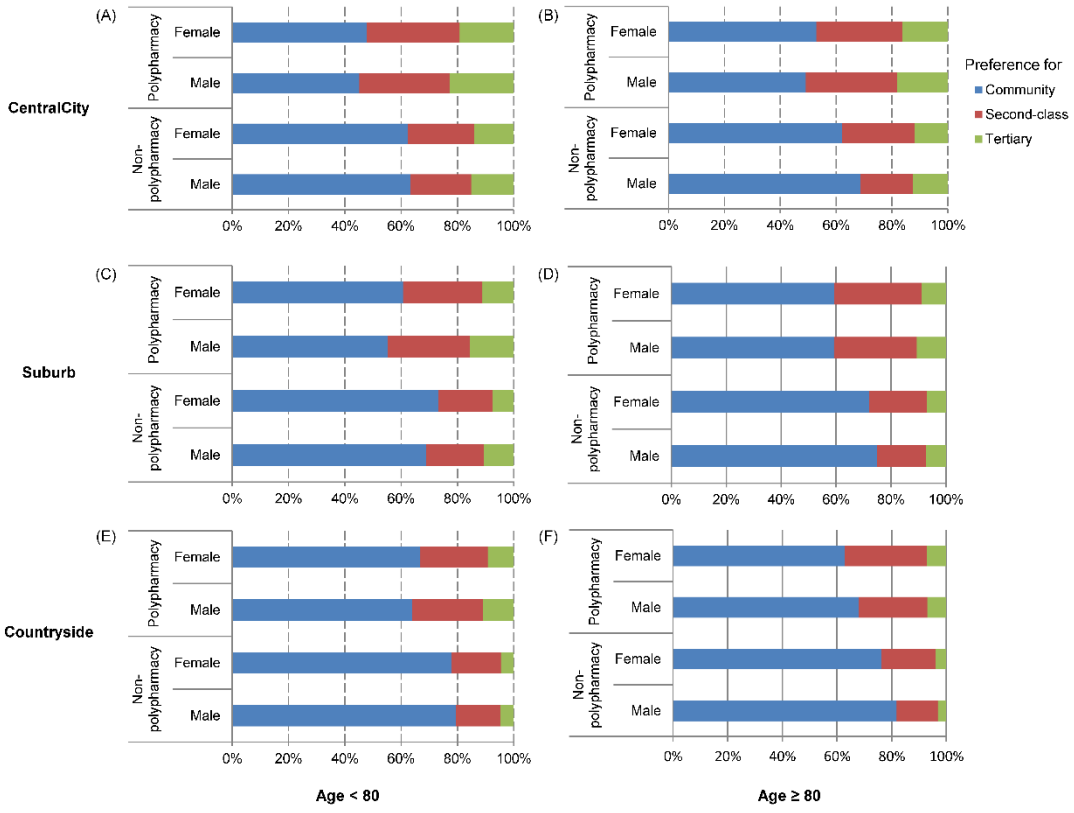

Figure 1. The distribution of polypharmacy among elderly patients with comorbid hypertension. The study visually shows the distribution of polypharmacy in different ages and genders of elderly patients with comorbid hypertension living in different urban areas. Gender had no significant effect on the distribution of polypharmacy. The rate of polypharmacy was higher in elderly patients. Patients living in the countryside, had a higher willingness to seek medical care in the community healthcare center, and a lower rate of polypharmacy than those who prefer secondary and tertiary hospitals.

to seek medical care in the community healthcare center, have a lower rate of polypharmacy than those who prefer secondary and tertiary hospitals. Overall, gender had no significant effect on the distribution of polypharmacy, and the rate of polypharmacy was higher in elderly patients aged over 80.

3.3. Associations between polypharmacy and preference for primary care

The demographic characteristic and working conditions of pharmacists in Shanghai public hospitals in 2020 are summarized in this study (Table 4). A total of 9,603 pharmaceutical workers were enrolled in this study, most of whom were female (72.2%). The mean (SD) age was 36.13 (8.42) years and the average (SD) working years was 12.79 (9.44) years. More pharmacists work in secondary and tertiary hospitals in the central city area, what the data result of this study shows 4,996 (52.0%) worked in the central city area, and more than 70% worked in secondary and tertiary hospitals. From the perspective of major, 79.4% of pharmacists majored in pharmacy, 17.6% in traditional Chinese pharmacology, and some other pharmacists majored in clinical medicine, nursing and management. From the perspective of the highest degree, 6,049 (63%) had Bachelor degree, which has doubled from the proportion of majors at the beginning of employment. In terms of post category, nearly half were dispensing pharmacists, while only 16.4% were clinical pharmacists. From the perspective of suppliers, the proportion of clinical pharmacists working in community healthcare centers is high.

Table 4. Basic Characteristics of pharmacists in Shanghai, China in 2020

Characteristics	*n* = 9,603
Working areas	
Central city	4,996 (52.0%)
Suburb	3,038 (31.6%)
Countryside	1,569 (16.3%)
Working medical institutions	
Community healthcare center	2,794 (29.1%)
Secondary hospital	3,411 (35.5%)
Tertiary hospital	3,398 (35.4%)
Gender	
Male	2,667 (27.8%)
Female	6,935 (72.2%)
Mean age	36.13 (8.42)
Work experience	12.79 (9.44)
Specialty	
Pharmacy	7,627 (79.4%)
Traditional Chinese pharmacology	1,688 (17.6%)
Medicine	16 (0.2%)
Nursing	17 (0.2%)
Management related	62 (0.6%)
Others	193 (2.0%)
Highest Education	
Junior college or below	2,602 (27.1%)
Bachelor degree	6,049 (63.0%)
Master degree	778 (8.1%)
Doctoral degree	174 (1.8%)
Education when entering the institution	
Junior college or below	6,150 (64.0%)
Bachelor degree	2,621 (27.3%)
Master degree	695 (7.2%)
Doctoral degree	137 (1.4%)
Professional title	
Primary	5,656 (58.9%)
Intermediate	3,272 (34.1%)
Advanced	384 (4.0%)
Unqualified	291 (3.0%)
Authorized strength	6,758 (70.4%)
Post category	
Dispensing pharmacist	4,402 (45.8%)
Prescription-checking pharmacist	2,813 (29.3%)
Clinical pharmacist	1,574 (16.4%)
Administrative management	83 (0.9%)
Others	731 (7.6%)

From the perspective of supply-demand matching (Figure 2): most patients' preferences do not match the number of pharmaceutical staff in institutions. Most patients prefer to see a doctor in tertiary medical institutions, especially in countryside areas. However, most pharmacists in the central city area are concentrated in tertiary hospitals; while in countryside areas, the majority of pharmacists work in community healthcare centers. This is related to the high degree of specialization of medical institutions, and also intuitively reflects the mismatch between patients' medical preference and the number of pharmaceutical personnel.

4. Discussion

With the improvement of living conditions and the progress of medical technology, China's population life expectancy is rising rapidly, and the process of population aging is accelerating, much faster than many low-income and high-income countries (*18-19*). One of the main problems related to population aging is the increasing burden of chronic diseases, with high incidence and coexistence of chronic diseases, which seriously affect the health status and quality of life of the elderly (*20*). The prolonged survival time of chronic diseases leads to increased risk of lifelong medication and multiple medications for many patients. In 2017, WHO proposed Medication Safety as the third Global Patient Safety Challenge. For governments of all countries, it is urgent to take measures to solve medication safety problems such as polypharmacy for the elderly and strengthen medication management for the elderly (*21-22*).

This study is the first large-scale survey on medication use of hypertensive comorbidities in China. More than half of the elderly patients with comorbid hypertension have polypharmacy in Shanghai, which as a city has a relatively high economic level and a deep degree of aging. Although this polypharmacy rate is similar to other studies, due to Chinese traditional medicine is excluded from the statistics of drug types in this study the results of the research data are actually far underestimated (*23-24*). In reality, the polypharmacy

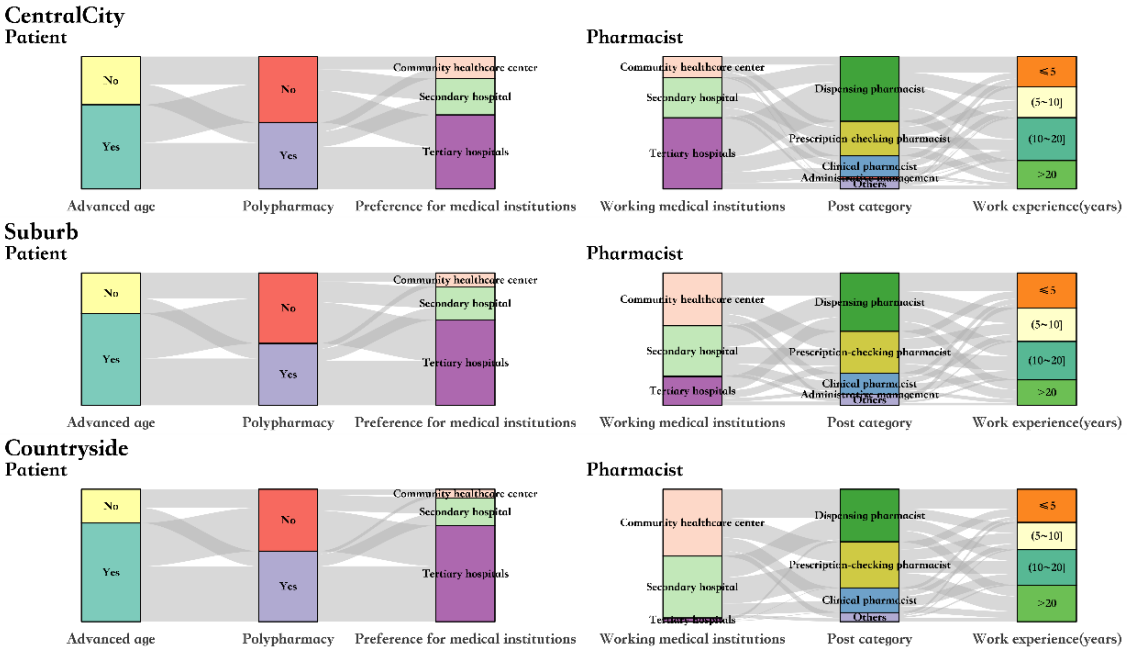

Figure 2. The distribution of the drug-related characteristics of elderly patients and pharmacists in a Sankey diagram. From the perspective of supply-demand matching, most patients' preferences do not match the number of pharmaceutical staff in institutions. Most patients prefer to see a doctor in tertiary medical institutions, especially in countryside areas. However, most pharmacists in the central city area are concentrated in tertiary hospitals; while in countryside areas, the majority of pharmacists work in community healthcare centers. This is related to the high degree of specialization of medical institutions, and also intuitively reflects the mismatch between patients' medical preference and the number of pharmaceutical personnel.

rate of elderly patients with comorbid hypertension in Shanghai is even more alarming. The data from this current study shows that in areas with a high degree of aging, the demand for medication management is much greater (*25-26*).

In order to improve the medication status of the elderly, influencing factors should be clarified first. Advanced age is a significant risk factor for multiple medications due to the decline in physical function associated with aging (*27*). It is worth noting that the variables of living in the central city areas, which was a protective factor in the univariate analysis, became a risk factor in the multivariate analysis. This is to consider that there is a potential protective factor, and most of the people with this factor are concentrated in the countryside area, so the countryside area will show a protective effect when there is only one factor. After the inclusion of multiple factors, this factor was isolated, and the protective effect of the countryside area was no longer significant. This suggests that we need to continue to expand subsequent studies and analyze the protective factors that can play a role in living in the countryside areas.

The rate of polypharmacy in patients intending to visit community healthcare centers was low ($P < 0.0001$). From the perspective of medical institutions' medical preference, compared with patients who prefer to seek treatment in community healthcare centers, patients who prefer to seek treatment in secondary and tertiary medical institutions are more likely to take multiple medications. This is similar to the experience of classic countries. Providing pharmaceutical care in primary medical institutions can better manage the whole process of drug use for patients, carry out drug integration and reduce polypharmacy. Certainly, the increase of the number of diseases and visits are risk factors, which may be due to the fact that patients have higher disease grades and need to visit medical institutions several times, or they have higher willingness to seek medical care. There may be an effect of the degree of disease, but the research group conducted interviews with some patients and found that advanced medical institutions can provide more drug choices for patients. This means that the degree of disease has a limited impact on the preference of medical institutions. In the case of a high degree of specialization in medical institutions, many patients choose to go to high-level hospitals even if they have common diseases.

Medical institutions equipped with sufficient professional pharmaceutical technicians is the basis of pharmaceutical care, however, in fact, most patients' preferences do not match the number of pharmaceutical staff in institutions in this current study. According to the *Provisions on Pharmaceutical Administration of Medical Institutions in China*, the number of pharmaceutical professional and technical personnel in medical institutions shall not be less than 8% of the number of health technical personnel in the institutions. However, the number of pharmaceutical personnel, especially clinical pharmacists, is seriously insufficient at present (*28*). As of 2018, the number of pharmacists (physicians) in China was only 460,000 (*29*), far less

than licensed (assistant) physicians (3.607 million) and registered nurses (physicians) (4.099 million). A survey of 415 tertiary medical institutions in China found that 50.1% of them had less than 5 clinical pharmacists (*30*). Another study of 39 medical institutions found that the average number of clinical pharmacists per 100 beds was only 0.43 (*31*). Especially in community healthcare centers and other basic institutions, the number of pharmaceutical staff is insufficient, and it is very common for doctors or nurses to exercise the duties of pharmacists part-time. The poor enthusiasm of pharmacists, high staff mobility and unstable teams are not conducive to the development of polypharmacy management (*32*). It is thus obvious that enhancing the quantity and quality of grassroots pharmaceutical staff is the key to reduce the multiple drug use of the elderly.

Polypharmacy is a complex phenomenon, which require to distinguish a drug use for real health needs from not necessary use. Polypharmacy involves multiple links and stakeholders, including doctors' prescription issuance and delivery, pharmacists' prescription review and dispensing, drug storage, medication compliance of the elderly, *etc.* Factors leading to polypharmacy exist in all links, which is a complex engineering system requiring multi-link cooperation and integration. The final support point for elderly health improvement is the family doctor team in community healthcare centers, including pharmaceutical staff. Chinese government vigorously promotes community pharmaceutical care, and this study also confirmed that the preference of community primary care has a positive effect on the polypharmacy management of elderly comorbidities. However, it is very difficult to implement community pharmaceutical care at the present stage, with deeply specialized medical institutions, in China. But if it is done, there is a long-term benefit, which is the efficiency of the process.

Based on this study, comprehensive measures to deal with the problem of polypharmacy were proposed: *i*) Increase the input of pharmaceutical care related resources, especially the input of talent training in pharmaceutical education transformation and upgrading; *ii*) Establish a pharmaceutical service price system to ensure reasonable remuneration for pharmacists; *iii*) Strengthen the development of community pharmaceutical care in primary medical institutions, such as home pharmaceutical care, medication management of elderly patients with chronic diseases, the use of Internet to empower polypharmacy management publicity, *etc.*

Several limitations of this study should be acknowledged. This study is a larger sample survey, which only collects the most basic demographic and medical history characteristics, and lacks the sociological characteristics of elderly patients. Therefore, the possible protective or risk factors cannot be explored in depth during the analysis of influencing factors. In addition, data on patients' medication used in the study were institutional data, rather than the actual medication use data of patients, which may constitute a bias. However, since the institutional data only included public medical institutions and did not include the data of private institutions such as private pharmacies, the impact of such deviation is limited.

5. Conclusion

In conclusion, the rate of polypharmacy among elderly patients with comorbid hypertension was 50.5% in Shanghai. Age, living areas, total number of visits, preference for medical institutions and the number of diseases were associated with polypharmacy. The rate of polypharmacy in elderly patients who intend to seek treatment in community healthcare centers is low. And this study also confirmed that the preference of community primary care has a positive effect on the polypharmacy management of elderly comorbidities in developing countries. As a consequence, obtaining better medication outcomes based on primary pharmaceutical care is necessary.

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