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Community-acquired pneumonia: Trends in and research on drug resistance and advances in new antibiotics

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SUMMARY Community-acquired pneumonia (CAP) refers to infectious inflammation of the lung parenchyma developing outside of a hospital. CAP has quite a high mortality and morbidity rate worldwide, and especially among elderly patients. The increasing burden of CAP is due to antibiotic resistance, the growth of the elderly population, and underlying comorbidities. *Streptococcus pneumoniae* remains the most common bacterial pathogen causing CAP, but multi-drug resistance bacteria and potential pathogens have increased the difficulty and challenges of managing CAP. Although preventive measures, diagnostic techniques, and treatment strategies are constantly advancing and improving, the susceptibility of multi-drug resistant pathogens, such as including Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, has not improved significantly in recent decades, thus highlighting the importance and necessity of developing new antibiotics for the treatment of CAP. New antimicrobials have been approved over the past few years that will expand treatment options for CAP, and especially for patients with potential comorbidities. This situation also offers the chance to reduce the abuse of antibiotics, their toxicities, and their adverse reactions and to provide effective personalized antibiotic treatment.

Keywords Community-acquired pneumonia (CAP), antimicrobial resistance, epidemiology, treatment guidelines

1. Introduction

Community-acquired pneumonia (CAP) refers to lower respiratory tract infections acquired outside of hospitals or local medical facilities. Clinical diagnosis is based on a set of signs and symptoms associated with lower respiratory tract infections, which may present as a fever, cough, expectoration, chest pain, dyspnea, and signs of infiltration of alveolar spaces (*1-3*). Worldwide, CAP is a major health issue and it imposes a massive clinical and economic burden. CAP results in a high rate of hospitalization, which is 63% in Dutch patients with CAP and 68% among U.S. adult patients with CAP (*4-6*). Despite progress in preventive measures, microbiological diagnostic tests, and antimicrobial therapy, the emergence of an increasing number of multidrug-resistant (MDR) bacteria, refractory microorganisms, and new pathogens has resulted in pneumonia still being the leading cause of mortality from infectious diseases worldwide (*7-9*). Another major reason for the continued increase in CAP is the growth of the elderly population and its underlying comorbidities (*10,11*). The current review will summarize advances in research and the medical status of CAP in terms of its epidemiology, global trends in antibiotic resistance, treatment guidelines and advances in research on new antibiotics.

2. Epidemiology of CAP

Lower respiratory tract infections, most of which are CAP, are one of the major health issues facing the world. Lower respiratory tract infections are the most common communicable disease that causes death (*12*), and the global death toll in 2019 was 2.6 million (*13*). Despite decades of efforts to control the morbidity and mortality of CAP through advances in preventive measures, diagnostic techniques, and treatment

strategies, CAP still imposes a very heavy burden, particularly in developing countries, and worldwide efforts are seeking to prevent CAP (*14*).

2.1. Etiology

The etiology of CAP varies in different countries and periods, but *Streptococcus pneumoniae* (*S. pneumoniae*) remains the most common bacterium responsible (*15-17*). Other pathogens include *Staphylococcus aureus* (*S. aureus*), *Haemophilus influenzae* (*H. influenzae*), and atypical pathogens such as *Chlamydia pneumoniae* (*C. pneumoniae*) and *Mycoplasma pneumoniae* (*M. pneumoniae*) (*18,19*). Although the proportion of CAP cases caused by Gram-negative pathogens such as *Klebsiella pneumoniae* (*K. pneumoniae*)is small, its related antimicrobial resistance causes difficulties with clinical treatment (*20*). In recent studies, atypical pathogens such as *C. pneumoniae* and *M. pneumoniae* have been detected at a higher rate than before (*21-23*), A statistical analysis of 4,300 patients from 35 countries in 2012 indicated that the rate of detection of pneumonia caused by atypical pathogens can be as high as 20% (*24*). A study has reported that the rate of infection with *M. pneumoniae* in Chinese adults exceeded that of *S. pneumoniae*, so *M. pneumoniae* is now the most common pathogen responsible for adult CAP (*25*). These findings suggest that these atypical bacteria may replace *S. pneumoniae* as the main bacteria causing CAP.

Although bacteria are the most common cause of CAP, CAP caused by respiratory viruses is increasingly detected as a result of advances in molecular diagnostic technology (*26,27*). As a single pathogen causing CAP, or a factor for co-infection, viruses both increase the risk of morbidity and antibiotic inefficacy (*26*). Importantly, recent studies have reported that two or more pathogens, usually a combination of bacteria and viruses, were found in more than one-third of CAP cases (*4,28)*. Over the last 20 years, there have been outbreaks of severe acute respiratory syndrome (SARS), influenza H1N1, influenza H7N9, and Middle East respiratory syndrome (MERS), as well as the current global epidemic of coronavirus disease 2019 (COVID-19). These facts continue to signal the severity of CAP caused by viruses. Monitoring current and emerging viruses that cause CAP and taking rapid and timely measures to combat them are necessary for public health.

2.2. Risk factors

The age over 65 years is one of the greatest risk factors for CAP. Elderly patients with CAP have a poor health status and more comorbidities, which means a higher rate and a longer duration of hospitalization (*11*). The morbidity of CAP increases with age in the United States, South Africa, and Europe (*4,29*). In Japan, the total number of adult CAP cases is approximately 1.88 million per year, 69.4% of which involve patients over the age of 65 (*30*). A study in the Netherlands indicated that about 45% of CAP cases involved people over the age of 65, and about 64% of CAP cases involved people over the age of 50 (*6*). In China, however, the highest morbidity of CAP is among children though the morbidity among the elderly is relatively high as well (Figure 1). The annual morbidity of CAP among children under the age of 5 in China is estimated to be 65.8% (*31*), which is much higher than that in other countries such as the United States (6.22%, age < 2 years) and the annual morbidity of CAP among adults over the age of 60 in China is 34.68% (*32*). Consistent with the overall etiology, *S. pneumoniae* is still the predominant pathogen causing CAP among the elderly (Figure 2), and the incidence of viral infection is higher than that of atypical bacteria in the elderly (*33*). The differences in morbidity and etiology of CAP in different countries may reflect the diversity in methodologies, systems of diagnosis and treatment, age groups, and population distribution (*22,34*).

Chronic comorbidities are another risk factor for CAP. Chronic obstructive pulmonary disease (COPD) is the highest risk for morbidity and hospitalization due to CAP, and the annual incidence of CAP in patients with COPD is 5,832 per 100,000 adults in the United States (*35*). Other chronic comorbidities associated with the increased morbidity of CAP include asthma, bronchiectasis, coronary heart disease, cardiac failure, chronic liver disease, diabetes mellitus, cerebrovascular diseases, immune deficiency, and malnutrition, all of which are related to poor outcomes of CAP (*36,37*). People with comorbidities have an increased risk of other complications and death while suffering from CAP (*38*). The morbidity of CAP with these comorbidities remains high across the globe. For example, a European study reported that up to 33% of patients with CAP had diabetes mellitus and up to 47% of patients with CAP had chronic heart disease (*39*). Elderly patients with CAP and patients with CAP and comorbidities are often treated with a combination of multiple antibiotics. Moreover, frequent exposure to healthcare settings and cumulative exposure to multiple antibiotics lead to a higher rate of infection with MDR pathogens in patients over the age of 65 (*33*). Adverse reactions to agents and interactions between agents impact therapeutic efficacy and antimicrobial resistance (*40*). The empirical treatment of CAP is becoming increasing challenging in patients over the age of 65 and patients with chronic comorbidities due to the limited treatment options.

2.3. Mortality

Elderly patients are the main population susceptible to CAP. The number of adult patients with CAP in the future will increase in conjunction with the increasing

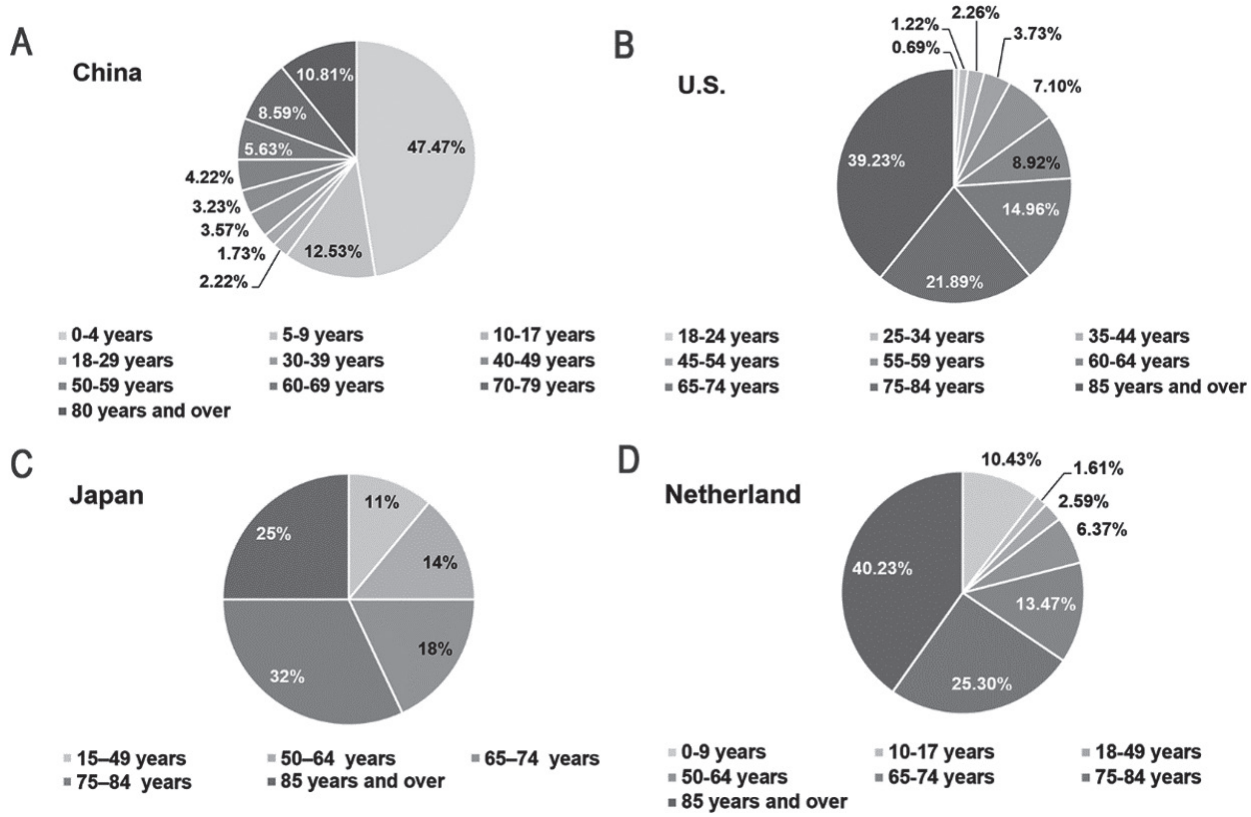

Figure 1. Proportion of CAP incidence in different age groups. The proportion of CAP incidence in China **(A)** (*31*) and the Netherlands **(D)** (*6*) for all age groups and the proportion of CAP incidence in the US **(B)** (*35*) and Japan **(C)** (*30*) in adults in different age groups.

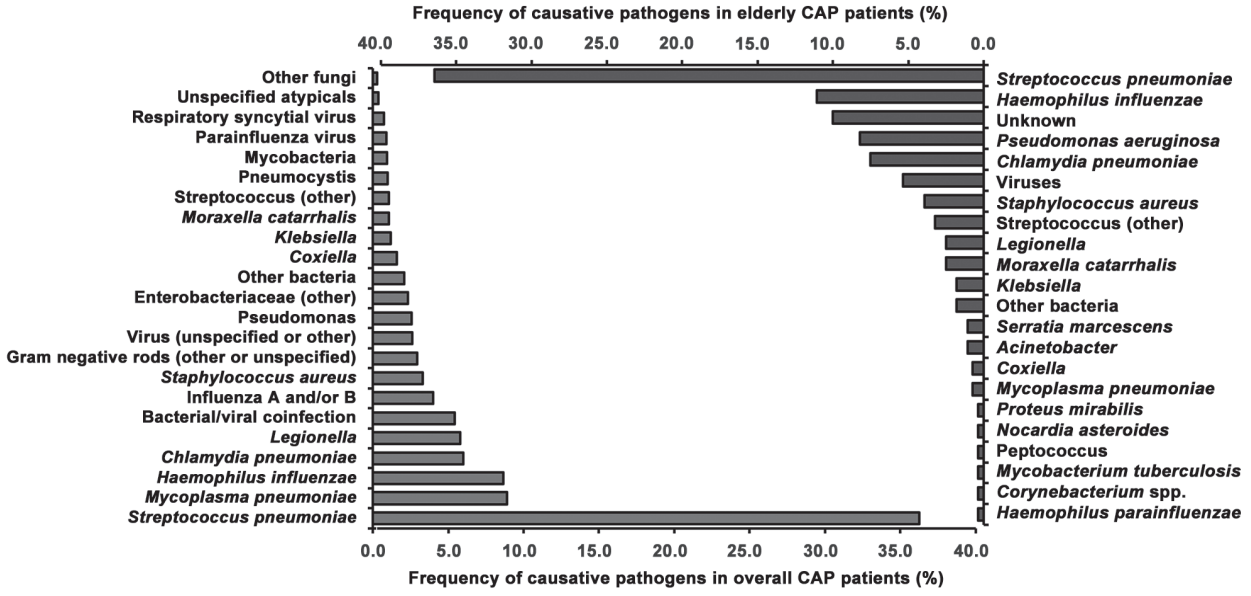

Figure 2. The characteristics of CAP etiology. Frequency of causative pathogens in patients with CAP as a whole (*19*) and in elderly patients with CAP (*93,94*).

proportion of the population over the age of 65, and the mortality of CAP is expected to fluctuate accordingly. The mortality rate of CAP varies greatly by country and demographic composition. The mortality rate in Europe ranges from less than 1% to 48% while that in the United Kingdom is 6% among patients under the age of 65 and 47% among patients over the age of 85 (*22*). In the United States, CAP is the sixth leading cause of death among the elderly (*41*), accounting for more than 50% of hospitalized patients over the age of 75 and resulting in a mortality rate of nearly 70% (*42*). An increasing rate of hospitalization and an increasing

mortality rate with age have also been noted in Asia (*42*). A Chinese study involving 5,828 adult patients with CAP indicated that the 30-day mortality rate of hospitalized patients with CAP was 4.2% and the in-hospital mortality rate increased significantly in the population over the age of 65 (*43*). The mortality rate of CAP has declined as the public healthcare system has improved and the use and availability of antibiotics, but the aging population and antibiotic resistance (detailed later) are still the main obstacles and challenges that need to be studied further.

3. Antimicrobial resistance

Antimicrobials frequently used to treat CAP include macrolides (*e.g.*, azithromycin), β-lactams (*e.g.*, amoxicillin/clavulanic acid), fluoroquinolones (*e.g.*, levofloxacin), and third-generation cephalosporins (*44*). Antibiotic treatment of CAP is usually empirical because it is generally impossible to ascertain the exact pathogenesis of CAP given the potential factors mentioned earlier. Although antibiotic treatment of CAP needs to target typical bacteria, there is no clinical consensus as to whether it needs to target atypical bacteria. A study has found that the adequacy of initial antimicrobial therapy is a critical factor affecting the course of treatment and the prognosis of pneumonia (*45*). However, increasing resistance to antibiotics remains a major issue for poor outcomes of CAP treatment (*46-48*). This is due to a growing number of MDR bacteria, intractable microorganisms, and the emergence of new pathogens (*49,50*).

Globally, *S. pneumoniae* remains the most common bacterium responsible for CAP. In approximately one-third of streptococcal pneumonia cases, bacteria are reported to be resistant to one or more antibiotics during clinical treatment (*51*). The frequency with which drug-resistant *S. pneumoniae* is isolated varies from regions. The frequency with which MDR and extensively resistant *S. pneumoniae* is isolated is highest in the Asia-Pacific region (39.2% and 10.9%, respectively) and lowest in Latin America (19.1% and 4.0%, respectively) (*52*). MDR *S. pneumoniae* has displayed resistance to macrolides (such as azithromycin and erythromycin), tetracycline, and penicillin (Table 1). The SENTRY Antimicrobial Surveillance Program reported that the global average susceptibility of MDR *S. pneumoniae* to azithromycin (> 4 mg/L) is 3.4%, its susceptibility to erythromycin (> 2 mg/L) is 1.9%, its susceptibility to tetracycline (> 4 mg/L) is 8.8%, and its susceptibility to penicillin (2 mg/L) is 15.7% (*52*). According to that Program, MDR *S. pneumoniae* is susceptible to vancomycin (≤ 1 mg/L, 100%), ceftaroline (0.12 mg/L, 99.9%), and levofloxacin (1 mg/L, 97.1%). The introduction of 7-valent and 13-valent pneumococcal conjugate vaccines (PCV-7 and PCV-13) has reduced the morbidity of macrolide-resistant invasive pneumococcal disease (*53,54*), but serotype substitution and the emergence of macrolide resistance are still major issues that urgently need to be addressed (*55,56)*.

Resistance to macrolides and other antibiotics is not limited to Pneumococcus, and other bacteria that cause CAP have also displayed drug resistance (*52,57*). The ESKAPE pathogens (*Enterococcus faecium*, *S. aureus*, *K. pneumoniae Acinetobacter baumannii*, *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Enterobacter* spp.)

Table 1. Antimicrobial susceptibility of several pathogenic bacteria causing CAP

Items	Susceptibility in %				
	Streptococcus pneumoniae (*52*)	MDR *Streptococcus pneumoniae* (*52*)	MSSA (*86,87*)	MRSA (*86,87*)	*Haemophilus influenzae* (*87,88*)
Ampicillin	100	100	100	100	
Amoxicillin-clavulanate	93.9	68.5			99.9
Azithromycin	63.1	3.4			99.5
Ceftaroline	99.9	99.7	97.4	88.6	99.8
Ceftriaxone	87.1	42.8	77.5		100
Ciprofloxacin			90	28	99.9
Clarithromycin					81.1
Clindamycin	83.1	24.3	26	70	
Doxycycline			99	96	
Erythromycin	63.1	1.9	74	18	
Gentamicin			98	89	
Levofloxacin	98.6	96.6	92.1	23.4	100
Meropenem	83.1	45.2			99.9
Penicillin	65.8	15.7	26		
Tetracycline	77.2	8.8	95	90	99.2
TMP-SMX	71.9	25.2	99	97	
Trimethoprim/sulfamethoxazole	68.5				78.6
Vancomycin	100	100	100	100	100

CAP, community-acquired pneumonia; MDR, multidrug-resistant; MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; TMP-SMX, trimethoprim-sulfamethoxazole.

cause a small proportion of CAP cases but have been highlighted as a faction of antibiotic-resistant bacteria that have become increasingly difficult to manage over the past few years (*49,57*). Studies have found that the ESKAPE pathogens have varying degrees of resistance to macrolides, fluoroquinolones, β-lactams, and third- and fourth-generation cephalosporins (*57,58*). Methicillin-Resistant *S. aureus* (MRSA), a typical ESKAPE pathogen, is highly resistant to macrolides and fluoroquinolones; its susceptibility to erythromycin is 18%, its susceptibility to ciprofloxacin is 28%, and its susceptibility to levofloxacin is 23.4%. The susceptibility of MRSA to antibiotics has improved over the past 20 years, *e.g.* its susceptibility to erythromycin has increased from 7% to 18%, but its susceptibility did not improve significantly from 2009 to 2016 (only increasing from 15% to 18%).

Atypical bacteria account for about 15% of CAP cases, so they are less common. However, atypical bacteria have received increasing attention in clinical practice because distinguishing pneumonia caused by atypical bacteria from pneumonia caused by typical bacteria based on clinical characteristics alone is difficult (*44*). Atypical pathogens as typified by *M. pneumoniae* have a high level of resistance to macrolides (*21*). β-lactam drugs as are recommended in guidelines are usually ineffective in treating pneumonia caused by atypical bacteria (*52,59*). Fluoroquinolones or tetracyclines should be considered as alternative therapy (*21*).

Increasing and intractable antibiotic resistance is mainly caused by bacterial gene mutations or the acquisition of drug resistance genes due to antibiotic overuse (*60*). Macrolides are one example. Macrolides are bacteriostats with bacteriostatic action as a result of binding with the 50S ribosomal subunit to inhibit protein synthesis (*61*). In *S. pneumoniae*, macrolide resistance is mainly due to the dimethylation of ribosomes by proteases encoded by erm(B) (*62*), the efflux of the efflux pump encoded by mef(E)/mel(msr (D)) (*63*), and ribosomal target site mutations (*64*). The agent efflux mechanism can lead to a low level of macrolide resistance, which is the most common mechanism of resistance in North America, and alteration of the ribosomes targeted by antimicrobials can induce a high level of resistance to macrolides, which is the mechanism of resistance commonly found in Europe (*65*). The distribution of macrolide resistance genotypes in China is mainly erm(B) (62.9%) and erm(B)+mef(E) (27.1%) (*66*). Antimicrobial resistance is a continuously developing global health threat, leading to alterations in the epidemiology of community-acquired bacterial pneumonia (CABP) (*4,35,67*), which makes empirical CAP therapy more challenging. Thus, understanding the genetic basis of different pathogens in the etiology of pneumonia is pivotal for the management and effective guidance of appropriate antimicrobial therapy.

4. Current treatment guidelines

Guidelines for the treatment and management of CAP have been issued in various countries and by various professional associations, so the recommended first-line treatment strategies vary by region (*68*). The Infectious Diseases Society of America/American Thoracic Society (IDSA/ATS) guidelines (*1*) are frequently cited. Guidelines from China (*69*), Europe (*70*), and the UK (*71*) are also widely used (Table 2). The guidelines for the treatment and management of CAP suggest that stratified empirical antibiotic treatment be based on the presence or absence of underlying disease and the severity of CAP. The CAP management guidelines updated by IDSA/ATS recommend amoxicillin or doxycycline as the first-line empirical treatment for drug-resistant *S. pneumoniae* in outpatients without comorbidities or risk factors. For patients with other chronic diseases such as COPD, diabetes mellitus, or liver disease, monotherapy with fluoroquinolones (levofloxacin or moxifloxacin) or combination therapy with β-lactams (such as amoxicillin) and macrolides is recommended. These treatment strategies also apply to patients with suppressed immunity/taking immunosuppressive drugs, patients who have received antibiotics within the past 3 months, or patients at risk of some other infection with drug-resistant *S. pneumoniae* (*1,2*).

Unlike in the West, the preferred therapeutic strategy for CAP in China involves cephalosporins. Chinese guidelines recommend aminopenicillin and first- or second-generation cephalosporins as the treatment of choice for outpatients without comorbidities. For outpatients who have comorbidities, a combination of penicillin and a β-lactamase inhibitor or monotherapy with second- or third-generation cephalosporins is recommended (*69*). For non-ICU inpatients, penicillin combined with a β-lactamase inhibitor or carbapenems (such as cephamycins, oxacephems, or ertapenem) is the treatment of choice. For hospitalized ICU patients with comorbidities such as severe COPD, penicillin combined with a β-lactamase-inhibitor is recommended as the preferred treatment (*69*).

The selection of empirical treatments requires a comprehensive assessment of the patient's condition, possible pathogens, and antibacterials. Thus, adverse reactions to antibiotics will limit the treatment options of clinicians. The use of macrolides is currently restricted due to adverse cardiac and gastrointestinal events, whereas fluoroquinolones remain a treatment option for CAP (*1,72*). Randomized controlled trials have indicated that the incidence of adverse events such as treatment failure and discontinuation of fluoroquinolones was relatively low compared to a combination of a β-lactam and macrolide (*73,74*). Therefore, new antibiotics for CAP treatment need to be developed to facilitate closely tailored and effective

Table 2. Empirical antibiotics suggested for CAP

Populations	US (IDSA/ATS) (*1*)		China (*69*)		Europe (*70*)		Britain (NICE/BTS) (*71*)	
	Preferred	Alternative	Preferred	Alternative	Preferred	Alternative	Preferred	Alternative
Outpatients without comorbidities; low severity	Amoxicillin	Doxycycline	Aminopenicillins, 1st or 2nd generation cephalosporins	Respiratory Fluoroquinolone[c], doxycycline and macrolide	Amoxicillin or tetracycline	Macrolide	Amoxicillin	Macrolide or tetracycline
Outpatients with comorbidities or a high rate of bacterial resistance	β-lactam with macrolide	Respiratory fluoroquinolone	Penicillins with β-lactamase inhibitor,	2nd or 3rd generation cephalosporins, Respiratory fluoroquinolone	Respiratory fluoroquinolone			
Inpatients not in the ICU; moderate severity	β-lactam[a] with macrolide	Respiratory fluoroquinolone	Penicillins with lactamase inhibitor, carbapenems[b]	Preferred drugs with macrolide, respiratory fluoroquinolone	Aminopenicillin with or without macrolide	Respiratory fluoroquinolone	Amoxicillin with macrolide	Respiratory fluoroquinolone
Inpatients in the ICU; high severity	β-lactam with macrolide	β-lactam with respiratory fluoroquinolone	Penicillins or 3rd generation cephalosporins with β-lactamase-inhibitor	Carbapenems with respiratory fluoroquinolone	3rd generation cephalosporin with macrolide	Respiratory fluoroquinolone with or without 3rd generation cephalosporin	β-lactamase stable β-lactams[d] with macrolide	Respiratory fluoroquinolone

CAP, community-acquired pneumonia; IDS: Infectious Diseases Society of America, ATS: American Thoracic Society, NICE: National Institute for Health and Care Excellence, BTS: British Thoracic Society, ICU: intensive care unit. [a] Preferred β-lactam drugs include cefotaxime, ceftriaxone, and ampicillin. [b] Preferred carbapenem drugs include cephamycins, oxacephems, and ertapenem. [c] Alternative respiratory fluoroquinolone drugs include levofloxacin, moxifloxacin, and gemifloxacin. [d] β-lactamase-stable β-lactams include co-amoxiclav, cefotaxime, ceftaroline fosamil, ceftriaxone, cefuroxime, and piperacillin-tazobactam.

treatment plans.

Recently, the concept of pneumonia, including CAP, has changed from just an acute lung disease to a multi-system disease with chronic adverse effects on health (*68*). The basis for the best therapeutic strategy against CAP has changed, diagnostic methods are being optimized, and pathogens are evolving. These events will determine the direction of future research.

5. New therapeutic agents

The constant emergence of antibiotic resistance is common in bacteria associated with CAP, and especially Staphylococcus and *S. pneumoniae*, making empirical treatment increasingly difficult (*75*). To improve the efficacy of initial empirical treatment of CAP, effective clinically antimicrobial therapy needs to combat a range of CAP etiologies, and particularly antibiotic-resistant pathogens (*49*). New antimicrobials offer an opportunity to improve the empirical treatment of CAP caused by drug-resistant pathogens (Table 3).

Lefamulin is a pleuromutilin antibiotic with antimicrobial activity against common pathogens that cause CABP (*76*). Lefamulin can be used to treat atypical pathogens, such as *M. pneumoniae*, *C. pneumoniae*, *H. influenzae*, and *Legionella*, as well as MDR *Neisseria gonorrhoeae* and *Mycoplasma genitalium* (*76*).

Omadacycline, a novel tetracycline, was recently approved for treatment of CABP and acute bacterial skin and skin structure infections (*77*). The therapeutic scope of omadacycline includes MRSA, various resistant *S. pneumoniae*, and a range of Gram-negative and atypical pathogens (*78*). Studies have confirmed that the efficacy of omadacycline is on par with that of moxifloxacin for the treatment of CABP in adults (*79*).

Ceftaroline, a fifth-generation cephalosporin, displays bactericidal action by interfering with the cell wall of bacteria and it displays bactericidal activity against most pathogens that cause CAP, including *S. pneumoniae* (*80*). The incidence of MRSA in outpatients with CAP is low but it can increase to more than 20% in inpatients with CAP (*81*). If an inpatient is suspected of having MRSA, ceftaroline may be the antibiotic of choice.

Delafloxacin, an anionic fluoroquinolone, has been approved for treatment of CABP caused by *S. pneumoniae*, *Escherichia coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *H. influenzae*, *Legionella pneumophila*, *Haemophilus parainfluenzae* (*H. parainfluenzae*), and *M. pneumoniae* (*82*). Delafloxacin is currently the only oral antibiotic with activity against MRSA and *P. aeruginosa* (*83*). Compared to moxifloxacin, delafloxacin has demonstrated excellent efficacy in patients with CAP and COPD or asthma and in patients with severe CAP (*65*). New anionic fluoroquinolones may be the best choice for elderly patients with comorbidities such as COPD or asthma.

Table 3. New antibiotics for community-acquired pneumonia (*75*)

Items	Antibiotic Classification	Mechanism	Route of Administration	Targeted Pathogens	Toxicity
Lefamulin (*89*)	pleuromutilin antibiotic	inhibits bacterial growth by binding to the peptidyl transferase center of the 50S ribosomal subunit	Intravenous and Oral	Haemophilus influenzae and Legionella, multidrug-resistant Neisseria gonorrhoeae, MRSA, MRSP, atypical pathogens	Diarrhea, vomiting
Omadacycline (*90*)	tetracycline	inhibits protein synthesis by binding to the 30S ribosomal subunit	Intravenous and Oral	MRSA, MRSP, Gram-negative and atypical pathogens	Nausea, headache
Ceftaroline (*91*)	fifth-generation cephalosporin	N-phosphonoamino water-soluble prodrug cephalosporin with broad-spectrum in-vitro antimicrobial activity	Intravenous	typical bacteria, MRSA, MRSP	Nausea
Delafloxacin (*92*)	anionic fluoroquinolones	targets both topoisomerase IV and DNA gyrase with a high level of affinity to inhibit bacterial DNA replication	Intravenous and Oral	MRSA, MRSP, S. pneumoniae, Staphylococcus aureus, Gram-negative and atypical pathogens	Diarrhea, nausea

MRSA: methicillin-resistant *Staphylococcus aureus*, MRSP: macrolide-resistant *Streptococcus pneumonia*.

6. Conclusion

Despite advances in antimicrobial therapy, CAP remains a major cause of mortality due to infectious diseases (*84*). The risk of CAP in patients with COPD is 6 to 8 times that in healthy individuals (*85*), and those patients also have increased morbidity and mortality (*37*). The proportion of the elderly and patients with comorbidities in the general population is increasing, and those groups are more likely to be hospitalized for CAP. Thus, the medical costs caused by CAP are expected to increase as well.

CAP is caused by a variety of typical and atypical pathogens, but *S. pneumoniae* remains the most common bacterium responsible. Due to its reduced sensitivity to macrolides, tetracyclines, and β-lactams that are frequently used, increasing attention has been paid to the efficacy of other antibiotics. New antibiotics that have recently been approved may represent more possibilities to expand the treatment options for CAP, and especially for patients with comorbidities. Several multi-center research and surveillance networks on pneumonia have been established worldwide, and they are coordinating large-scale longitudinal studies on the epidemiology, diagnosis, and treatment of pneumonia. In order to cope with the public health challenges posed by population trends and limited public healthcare resources, the CAP-China clinical research network (*31*) has been established to mitigate the research gap in surveillance, rapid diagnosis, and optimal treatment and to also ardently support the development of new antibiotics.

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References

1. Metlay JP, Waterer GW, Long AC, *et al*. Diagnosis and Treatment of Adults with Community-acquired Pneumonia. An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America. Am J Respir Crit Care Med. 2019; 200:e45-e67.
2. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, Dowell SF, File TM Jr, Musher DM, Niederman MS, Torres A, Whitney CG; Infectious Diseases Society of America; American Thoracic Society. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis. 2007; 44 Suppl 2:S27-72.
3. Wu J, Lu AD, Zhang LP, Zuo YX, Jia YP. Study of clinical outcome and prognosis in pediatric core binding factor-acute myeloid leukemia. Zhonghua Xue Ye Xue Za Zhi. 2019; 40:52-57. (in Chinese w/ English abstract)
4. Jain S, Self WH, Wunderink RG, *et al*. Community-acquired pneumonia requiring hospitalization among U.S. adults. N Engl J Med. 2015; 373:415-427.
5. Griffin MR, Zhu Y, Moore MR, Whitney CG, Grijalva CG. U.S. hospitalizations for pneumonia after a decade of pneumococcal vaccination. N Engl J Med. 2013; 369:155-163.
6. Rozenbaum MH, Mangen MJ, Huijts SM, van der Werf TS, Postma MJ. Incidence, direct costs and duration of hospitalization of patients hospitalized with community acquired pneumonia: A nationwide retrospective claims database analysis. Vaccine. 2015; 33:3193-3199.
7. Aston SJ, Wootton DG. Community-acquired pneumonia due to drug-resistant Enterobacteriaceae: A global perspective. Respirology. 2020; 25:468-469.
8. Murphy SL, Kochanek KD, Xu J, Arias E. Mortality in

the United States, 2014. NCHS Data Brief. 2015;1-8.
9. GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: A systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2018; 392:1789-1858.
10. Mangen MJ, Huijts SM, Bonten MJ, de Wit GA. The impact of community-acquired pneumonia on the health-related quality-of-life in elderly. BMC Infect Dis. 2017; 17:208.
11. Schöll N, Rohde GGU. Community-acquired pneumonia in the elderly. Pneumologie. 2019; 73:605-616. (in German)
12. Kolditz M, Ewig S. Community-acquired pneumonia in adults. Dtsch Arztebl Int. 2017; 114:838-848.
13. World Health Organization. Global Health Estimates 2020: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2019. Geneva; 2020. *https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates/ghe-leading-causes-of-death* (accessed December, 2020).
14. Qazi S, Aboubaker S, MacLean R, Fontaine O, Mantel C, Goodman T, Young M, Henderson P, Cherian T. Ending preventable child deaths from pneumonia and diarrhoea by 2025. Development of the integrated Global Action Plan for the Prevention and Control of Pneumonia and Diarrhoea. Arch Dis Child. 2015; 100 Suppl 1:S23-28.
15. Cillóniz C, Ewig S, Polverino E, Marcos MA, Esquinas C, Gabarrús A, Mensa J, Torres A. Microbial aetiology of community-acquired pneumonia and its relation to severity. Thorax. 2011; 66:340-346.
16. Simonetti AF, Garcia-Vidal C, Viasus D, García-Somoza D, Dorca J, Gudiol F, Carratalà J. Declining mortality among hospitalized patients with community-acquired pneumonia. Clin Microbiol Infect. 2016; 22:567.e561-567.
17. Rozenbaum MH, Pechlivanoglou P, van der Werf TS, Lo-Ten-Foe JR, Postma MJ, Hak E. The role of *Streptococcus pneumoniae* in community-acquired pneumonia among adults in Europe: A meta-analysis. Eur J Clin Microbiol Infect Dis. 2013; 32:305-316.
18. Sharma L, Losier A, Tolbert T, Dela Cruz CS, Marion CR. Atypical pneumonia: Updates on *Legionella*, Chlamydophila, and Mycoplasma pneumonia. Clin Chest Med. 2017; 38:45-58.
19. Shoar S, Musher DM. Etiology of community-acquired pneumonia in adults: A systematic review. Pneumonia (Nathan). 2020; 12:11.
20. Magiorakos AP, Srinivasan A, Carey RB, *et al*. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012; 18:268-281.
21. Yu Y, Fei A. Atypical pathogen infection in community-acquired pneumonia. Biosci Trends. 2016; 10:7-13.
22. Welte T, Torres A, Nathwani D. Clinical and economic burden of community-acquired pneumonia among adults in Europe. Thorax. 2012; 67:71-79.
23. Isturiz RE, Luna CM, Ramirez J. Clinical and economic burden of pneumonia among adults in Latin America. Int J Infect Dis. 2010; 14:e852-856.
24. Wiemken TL, Peyrani P, Ramirez JA. Global changes in the epidemiology of community-acquired pneumonia. Semin Respir Crit Care Med. 2012; 33:213-219.
25. Tao LL, Hu BJ, He LX, Wei L, Xie HM, Wang BQ, Li HY, Chen XH, Zhou CM, Deng WW. Etiology and antimicrobial resistance of community-acquired pneumonia in adult patients in China. Chin Med J (Engl). 2012; 125:2967-2972.
26. Burk M, El-Kersh K, Saad M, Wiemken T, Ramirez J, Cavallazzi R. Viral infection in community-acquired pneumonia: A systematic review and meta-analysis. Eur Respir Rev. 2016; 25:178-188.
27. Shang L, Xu J, Cao B. Viral pneumonia in China: From surveillance to response. Lancet Public Health. 2020; 5:e633-e634.
28. Holter JC, Müller F, Bjørang O, Samdal HH, Marthinsen JB, Jenum PA, Ueland T, Frøland SS, Aukrust P, Husebye E, Heggelund L. Etiology of community-acquired pneumonia and diagnostic yields of microbiological methods: a 3-year prospective study in Norway. BMC Infect Dis. 2015; 15:64.
29. Boyles TH, Brink A, Calligaro GL, Cohen C, Dheda K, Maartens G, Richards GA, van Zyl Smit R, Smith C, Wasserman S, Whitelaw AC, Feldman C. South African guideline for the management of community-acquired pneumonia in adults. J Thorac Dis. 2017; 9:1469-1502.
30. Morimoto K, Suzuki M, Ishifuji T, Yaegashi M, Asoh N, Hamashige N, Abe M, Aoshima M, Ariyoshi K. The burden and etiology of community-onset pneumonia in the aging Japanese population: A multicenter prospective study. PLoS One. 2015; 10:e0122247.
31. Sun Y, Li H, Pei Z, Wang S, Feng J, Xu L, Gao P, Cao B, Zhan S. Incidence of community-acquired pneumonia in urban China: A national population-based study. Vaccine. 2020; 38:8362-8370.
32. Jain S, Williams DJ, Arnold SR, *et al*. Community-acquired pneumonia requiring hospitalization among U.S. children. N Engl J Med. 2015; 372:835-845.
33. Faverio P, Aliberti S, Bellelli G, Suigo G, Lonni S, Pesci A, Restrepo MI. The management of community-acquired pneumonia in the elderly. Eur J Intern Med. 2014; 25:312-319.
34. Song JH, Huh K, Chung DR. Community-acquired pneumonia in the Asia-Pacific Region. Semin Respir Crit Care Med. 2016; 37:839-854.
35. Ramirez JA, Wiemken TL, Peyrani P, *et al*. Adults hospitalized with pneumonia in the United States: Incidence, epidemiology, and mortality. Clin Infect Dis. 2017; 65:1806-1812.
36. Almirall J, Serra-Prat M, Bolíbar I, Balasso V. Risk factors for community-Acquired pneumonia in adults: A systematic review of observational studies. Respiration. 2017; 94:299-311.
37. Torres A, Blasi F, Dartois N, Akova M. Which individuals are at increased risk of pneumococcal disease and why? Impact of COPD, asthma, smoking, diabetes, and/or chronic heart disease on community-acquired pneumonia and invasive pneumococcal disease. Thorax. 2015; 70:984-989.
38. LaPensee K, Mistry R, Lodise T. Budget impact of omadacycline for the treatment of patients with community-acquired bacterial pneumonia in the United States from the hospital perspective. Am Health Drug Benefits. 2019; 12:S1-s12.
39. Torres A, Peetermans WE, Viegi G, Blasi F. Risk factors for community-acquired pneumonia in adults in Europe: A literature review. Thorax. 2013; 68:1057-1065.
40. Faulkner CM, Cox HL, Williamson JC. Unique aspects of antimicrobial use in older adults. Clin Infect Dis. 2005;

40:997-1004.
41. Brar NK, Niederman MS. Management of community-acquired pneumonia: A review and update. Ther Adv Respir Dis. 2011; 5:61-78.
42. Buzzo AR, Roberts C, Mollinedo LG, Quevedo JM, Casas GL, Soldevilla JM. Morbidity and mortality of pneumonia in adults in six Latin American countries. Int J Infect Dis. 2013; 17:e673-677.
43. Chen L, Zhou F, Li H, *et al*. Disease characteristics and management of hospitalised adolescents and adults with community-acquired pneumonia in China: A retrospective multicentre survey. BMJ Open. 2018; 8:e018709.
44. Olson G, Davis AM. Diagnosis and treatment of adults With community-acquired pneumonia. JAMA. 2020; 323:885-886.
45. Cilloniz C, Martin-Loeches I, Garcia-Vidal C, San Jose A, Torres A. Microbial etiology of pneumonia: Epidemiology, diagnosis and resistance patterns. Int J Mol Sci. 2016; 17.
46. Cillóniz C, Dominedò C, Torres A. Multidrug resistant Gram-negative bacteria in community-acquired pneumonia. Crit Care. 2019; 23:79.
47. Cillóniz C, Gabarrús A, Ferrer M, Puig de la Bellacasa J, Rinaudo M, Mensa J, Niederman MS, Torres A. Community-acquired pneumonia due to multidrug- and non-multidrug-resistant *Pseudomonas aeruginosa*. Chest. 2016; 150:415-425.
48. Pakhale S, Mulpuru S, Verheij TJ, Kochen MM, Rohde GG, Bjerre LM. Antibiotics for community-acquired pneumonia in adult outpatients. Cochrane Database Syst Rev. 2014; 2014:Cd002109.
49. Liapikou A, Cilloniz C, Palomeque A, Torres T. Emerging antibiotics for community-acquired pneumonia. Expert Opin Emerg Drugs. 2019; 24:221-231.
50. Torres A, Chalmers JD, Dela Cruz CS, Dominedò C, Kollef M, Martin-Loeches I, Niederman M, Wunderink RG. Challenges in severe community-acquired pneumonia: A point-of-view review. Intensive Care Med. 2019; 45:159-171.
51. Schroeder MR, Stephens DS. Macrolide resistance in *Streptococcus pneumoniae*. Front Cell Infect Microbiol. 2016; 6:98.
52. Sader HS, Mendes RE, Le J, Denys G, Flamm RK, Jones RN. Antimicrobial susceptibility of *Streptococcus pneumoniae* from North America, Europe, Latin America, and the Asia-Pacific Region: Results From 20 Years of the SENTRY Antimicrobial Surveillance Program (1997-2016). Open Forum Infect Dis. 2019; 6:S14-s23.
53. Hawkins PA, Chochua S, Jackson D, Beall B, McGee L. Mobile elements and chromosomal changes associated with MLS resistance phenotypes of invasive pneumococci recovered in the United States. Microb Drug Resist. 2015; 21:121-129.
54. Desai AP, Sharma D, Crispell EK, Baughman W, Thomas S, Tunali A, Sherwood L, Zmitrovich A, Jerris R, Satola SW, Beall B, Moore MR, Jain S, Farley MM. Decline in pneumococcal nasopharyngeal carriage of vaccine serotypes after the introduction of the 13-valent pneumococcal conjugate vaccine in children in Atlanta, Georgia. Pediatr Infect Dis J. 2015; 34:1168-1174.
55. Wongsurakiat P, Chitwarakorn N. Severe community-acquired pneumonia in general medical wards: Outcomes and impact of initial antibiotic selection. BMC Pulm Med. 2019; 19:179.
56. Chi HC, Hsieh YC, Tsai MH, Lee CH, Kuo KC, Huang CT, Huang YC. Impact of pneumococcal conjugate vaccine in children on the serotypic epidemiology of adult invasive pneumococcal diseases in Taiwan. J Microbiol Immunol Infect. 2018; 51:332-336.
57. De Oliveira DMP, Forde BM, Kidd TJ, Harris PNA, Schembri MA, Beatson SA, Paterson DL, Walker MJ. Antimicrobial resistance in ESKAPE pathogens. Clin Microbiol Rev. 2020; 33.
58. Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: A review. Front Microbiol. 2019; 10:539.
59. Hogan DB. Did Osler suffer from "paranoia antitherapeuticum baltimorensis"? A comparative content analysis of The Principles and Practice of Medicine and Harrison's Principles of Internal Medicine, 11th edition. CMAJ. 1999; 161:842-845.
60. Munita JM, Arias CA. Mechanisms of antibiotic resistance. Microbiol Spectr. 2016; 4.
61. Kannan K, Mankin AS. Macrolide antibiotics in the ribosome exit tunnel: Species-specific binding and action. Ann N Y Acad Sci. 2011; 1241:33-47.
62. Brenciani A, Bacciaglia A, Vecchi M, Vitali LA, Varaldo PE, Giovanetti E. Genetic elements carrying erm(B) in *Streptococcus pyogenes* and association with tet(M) tetracycline resistance gene. Antimicrob Agents Chemother. 2007; 51:1209-1216.
63. Zhang Y, Tatsuno I, Okada R, Hata N, Matsumoto M, Isaka M, Isobe KI, Hasegawa T. Predominant role of msr(D) over mef(A) in macrolide resistance in *Streptococcus pyogenes*. Microbiology (Reading). 2016; 162:46-52.
64. Franceschi F, Kanyo Z, Sherer EC, Sutcliffe J. Macrolide resistance from the ribosome perspective. Curr Drug Targets Infect Disord. 2004; 4:177-191.
65. Sharma R, Sandrock CE, Meehan J, Theriault N. Community-acquired bacterial pneumonia-Changing epidemiology, resistance patterns, and newer antibiotics: Spotlight on delafloxacin. Clin Drug Investig. 2020; 40:947-960.
66. Geng Q, Zhang T, Ding Y, Tao Y, Lin Y, Wang Y, Black S, Zhao G. Molecular characterization and antimicrobial susceptibility of *Streptococcus pneumoniae* isolated from children hospitalized with respiratory infections in Suzhou, China. PLoS One. 2014; 9:e93752.
67. Musher DM, Thorner AR. Community-acquired pneumonia. N Engl J Med. 2014; 371:1619-1628.
68. Wunderink RG, Waterer G. Advances in the causes and management of community acquired pneumonia in adults. BMJ. 2017; 358:j2471.
69. Cao B, Huang Y, She DY, *et al*. Diagnosis and treatment of community-acquired pneumonia in adults: 2016 clinical practice guidelines by the Chinese Thoracic Society, Chinese Medical Association. Clin Respir J. 2018; 12:1320-1360.
70. Woodhead M, Blasi F, Ewig S, Garau J, Huchon G, Ieven M, Ortqvist A, Schaberg T, Torres A, van der Heijden G, Read R, Verheij TJ. Guidelines for the management of adult lower respiratory tract infections – Full version. Clin Microbiol Infect. 2011; 17 Suppl 6:E1-59.
71. National Clinical Guideline Centre. National Clinical Guideline C. National Institute for Health and Care Excellence: Clinical Guidelines. In: Pneumonia: Diagnosis and Management of Community- and Hospital-Acquired Pneumonia in Adults (National Institute for Health and Care Excellence (UK), 2014., London, 2014.

72. Lee JS, Giesler DL, Gellad WF, Fine MJ. Antibiotic therapy for adults hospitalized with community-acquired pneumonia: A Systematic Review. JAMA. 2016; 315:593-602.
73. Raz-Pasteur A, Shasha D, Paul M. Fluoroquinolones or macrolides alone versus combined with β-lactams for adults with community-acquired pneumonia: Systematic review and meta-analysis. Int J Antimicrob Agents. 2015; 46:242-248.
74. Ceccato A, Cilloniz C, Martin-Loeches I, Ranzani OT, Gabarrus A, Bueno L, Garcia-Vidal C, Ferrer M, Niederman MS, Torres A. Effect of combined β-lactam/macrolide therapy on mortality according to the microbial etiology and inflammatory status of patients with community-acquired pneumonia. Chest. 2019; 155:795-804.
75. Kollef MH, Betthauser KD. New antibiotics for community-acquired pneumonia. Curr Opin Infect Dis. 2019; 32:169-175.
76. Veve MP, Wagner JL. Lefamulin: Review of a promising novel pleuromutilin antibiotic. Pharmacotherapy. 2018; 38:935-946.
77. Karlowsky JA, Steenbergen J, Zhanel GG. Microbiology and preclinical review of omadacycline. Clin Infect Dis. 2019; 69:S6-s15.
78. Zhanel GG, Esquivel J, Zelenitsky S, *et al*. Omadacycline: A novel oral and intravenous aminomethylcycline antibiotic agent. Drugs. 2020; 80:285-313.
79. Stets R, Popescu M, Gonong JR, *et al*. Omadacycline for community-acquired bacterial pneumonia. N Engl J Med. 2019; 380:517-527.
80. Koulenti D, Xu E, Mok IYS, Song A, Karageorgopoulos DE, Armaganidis A, Lipman J, Tsiodras S. Novel antibiotics for multidrug-resistant Gram-positive microorganisms. Microorganisms. 2019; 7.
81. Lindsay JA. Hospital-associated MRSA and antibiotic resistance-what have we learned from genomics? Int J Med Microbiol. 2013; 303:318-323.
82. Markham A. Delafloxacin: First global approval. Drugs. 2017; 77:1481-1486.
83. Ocheretyaner ER, Park TE. Delafloxacin: A novel fluoroquinolone with activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. Expert Rev Anti Infect Ther. 2018; 16:523-530.
84. Murray CJ, Lopez AD. Measuring the global burden of disease. N Engl J Med. 2013; 369:448-457.
85. Pasquale CB, Vietri J, Choate R, McDaniel A, Sato R, Ford KD, Malanga E, Yawn BP. Patient-reported consequences of community-acquired pneumonia in patients with chronic obstructive pulmonary disease. Chronic Obstr Pulm Dis. 2019; 6:132-144.
86. Diekema DJ, Pfaller MA, Shortridge D, Zervos M, Jones RN. Twenty-year trends in antimicrobial susceptibilities among *Staphylococcus aureus* from the SENTRY Antimicrobial Surveillance Program. Open Forum Infect Dis. 2019; 6:S47-s53.
87. Sader HS, Flamm RK, Streit JM, Carvalhaes CG, Mendes RE. Antimicrobial activity of ceftaroline and comparator agents tested against organisms isolated from patients with community-acquired bacterial pneumonia in Europe, Asia, and Latin America. Int J Infect Dis. 2018; 77:82-86.
88. Gordon KA, Biedenbach DJ, Jones RN. Comparison of *Streptococcus pneumoniae* and *Haemophilus influenzae* susceptibilities from community-acquired respiratory tract infections and hospitalized patients with pneumonia: Five-year results for the SENTRY Antimicrobial Surveillance Program. Diagn Microbiol Infect Dis. 2003; 46:285-289.
89. Mendes RE, Farrell DJ, Flamm RK, Talbot GH, Ivezic-Schoenfeld Z, Paukner S, Sader HS. *In vitro* activity of lefamulin tested against *Streptococcus pneumoniae* with defined serotypes, including multidrug-resistant isolates causing lower respiratory tract infections in the United States. Antimicrob Agents Chemother. 2016; 60:4407-4411.
90. Pfaller MA, Rhomberg PR, Huband MD, Flamm RK. Activity of omadacycline tested against *Streptococcus pneumoniae* from a global surveillance program (2014). Diagn Microbiol Infect Dis. 2018; 90:143-147.
91. Carreno JJ, Lodise TP. Ceftaroline fosamil for the treatment of community-acquired pneumonia: From FOCUS to CAPTURE. Infect Dis Ther. 2014; 3:123-132.
92. Pfaller MA, Sader HS, Rhomberg PR, Flamm RK. *In vitro* activity of delafloxacin against contemporary bacterial pathogens from the United States and Europe, 2014. Antimicrob Agents Chemother. 2017; 61.
93. Vila-Corcoles A, Ochoa-Gondar O, Rodriguez-Blanco T, Raga-Luria X, Gomez-Bertomeu F. Epidemiology of community-acquired pneumonia in older adults: A population-based study. Respir Med. 2009; 103:309-316.
94. Saito A, Kohno S, Matsushima T, Watanabe A, Oizumi K, Yamaguchi K, Oda H. Prospective multicenter study of the causative organisms of community-acquired pneumonia in adults in Japan. J Infect Chemother. 2006; 12:63-69.

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Review

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Breast cancer and pregnancy: Why special considerations prior to treatment are needed in multidisciplinary care

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SUMMARY Breast cancer diagnosed during pregnancy poses ethical and professional challenges. Clinical management of that condition should ensure the safety of both the mother and fetus. Clinical trials on breast cancer exclude pregnant women, so sufficient evidence with which to formulate guidelines for the management of these patients is lacking. Failing to undergo a breast examination during pregnancy, breast symptoms explained by physiological changes such as pregnancy, and unnecessary abortions after the diagnosis of breast cancer lead to worse outcomes for these patients. Multidisciplinary teams including breast surgeons, obstetricians, radiologists, pathologists, and anesthesiologists need to make an early diagnosis and comprehensively evaluate patients in different gestational weeks and with different stages of breast cancer in order to optimize outcomes.

Keywords breast cancer diagnosed during pregnancy, multidisciplinary teams, special considerations

1. Introduction

Breast cancer (BC) is the most common carcinoma in women worldwide (*1-5*), and about 7% of the cases were diagnosed before the age of 40 years (*6,7*). Cancer diagnosed during pregnancy is rare, with an incidence of 1 case per 1,000 deliveries (*8*). Breast cancer developing during pregnancy is rare, but it is the most common cancer affecting pregnancy. The incidence of breast cancer during pregnancy has been increasing (*9*). The rate of breast cancer among pregnant women age < 45 years has varied from 2.6% to 6.9% (*10*). By comparison, the rate in those age < 35 years has been 15.6% for all breast cancer cases (*11*).

BC diagnosed during pregnancy presents a complex challenge to the patient and to clinicians deciding how to manage the condition. Although the expectations are that pregnant patients with breast cancer will be treated as effectively as nonpregnant patients with breast cancer, the selection and delivery of standard therapies must be modified to balance maternal benefit and fetal risk. Assessment of the risks and benefits and a multidisciplinary team (MDT) approach at specialized centers are crucial to managing this group of patients (*12-16*).

The aims of the current article are to highlight the special considerations for pregnant women prior to treatment of breast cancer (Table 1) and to review the evidence for management of pregnant patients with breast cancer.

2. Tumor biology

Although the clinical characteristics of breast cancer in pregnant women are similar to those in non-pregnant women, diagnosis may be delayed due to the differential diagnoses of physiologic alterations related to pregnancy (*17,18*). Breast cancer is not caused by pregnancy but can occur coincidentally with pregnancy. Pregnancy may have a profound effect on the biology of breast cancer. Most of the breast cancer developing during pregnancy is invasive ductal carcinoma (*19*). Invasive lobular carcinoma develops far less frequently (*20*). Studies have reported that triple-negative breast cancer (TNBC) is more prevalent during pregnancy (*21-23*). The ultimate prognosis for breast cancer during pregnancy is a subject of debate. Although some studies have noted no significant differences in the prognosis for breast cancer during pregnancy or otherwise (*24-26*), a recent meta-analysis noted a poor prognosis for breast cancer in pregnant women (*27*). Compared to sporadic breast cancer, breast cancer that develops during pregnancy has a higher histologic grade, a more aggressive profile, is in a more advanced stage at

Table 1. Special considerations when managing pregnant women with breast cancer

At diagnosis
- Calculate the gestational age and expected date of delivery
- Conduct a standard staging workup
- Complete a fetal examination before treatment

During pregnancy

Surgery
- Radical modified mastectomy or breast-conserving surgery is recommended for early-stage breast cancer

Systemic Treatment
- Chemotherapy is safe in the second and third trimesters
- AC (doxorubicin and cyclophosphamide) and EC (epirubicin and cyclophosphamide) are the most frequently used regimens

Delivery
- The mode of delivery is decided by obstetric indications
- Chemotherapy should not be administered after 35 weeks of gestation

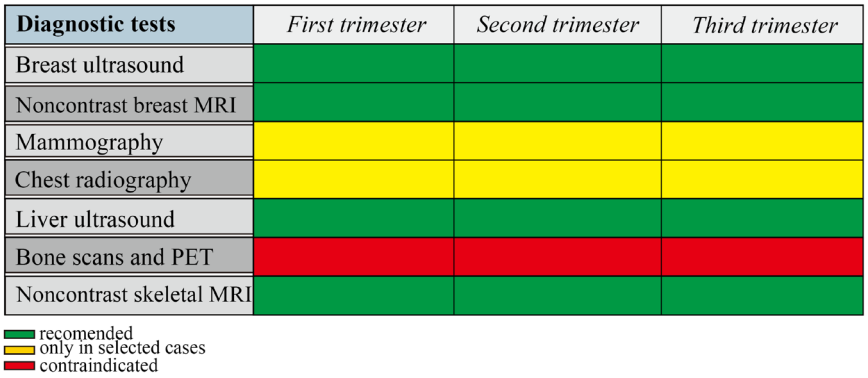

Figure 1. Management of tests to diagnose breast cancer during pregnancy per trimester. MRI magnetic resonance imaging, PET positron emission tomography. Green: recommended; Yellow: only in selected cases; Red: contraindicated.

diagnosis, is larger in size, has a higher frequency of nodal involvement, less frequently expresses estrogen receptors (ERs) and progesterone receptors (PRs), and is more likely to be inflammatory breast cancer (*28*).

The effects of pregnancy may alter certain patterns of gene expression in breast cancer cells compared to normal breast tissue (*29,30*). The aberrant expression of several oncogenes (*MYC*, *SRC*, and *FOS*), tumor suppressor genes (*TP53, PTEN*, and *CAV1*), apoptosis regulators (*PDCD4, BCL2*, and *BIRC5*), transcription regulators (*JUN, KLF1*, and *SP110*), genes involved in DNA repair mechanisms (*Sig20, BRCA1, BRCA2*, and *FEN1*), genes involved in cell proliferation (*AURKA* and *MKI67*), genes involved in the immune response (*PD1* and *PDL1*), and genes involved in other significant biological processes (protein modification or internal cell motility) has been noted in breast cancer diagnosed during pregnancy (*29*).

3. Diagnosis and staging

Diagnostic tests to diagnose breast cancer during pregnancy per trimester are summarized in Figure 1.

During pregnancy, the diagnosis of breast cancer is often delayed about 1 to 13 months due to enlargement of the breasts, colostrum secretion, and other changes (*31*). Although 80% of palpable breast masses found during pregnancy are benign, a mass diagnosed for > 2 weeks should be taken seriously (*32*).

The workup of a suspicious breast mass proceeds similarly during pregnancy or otherwise, but the standard staging workup should be predicated on fetal safety. A careful physical examination for suspected breast cancer during pregnancy that includes the breasts and regional lymph nodes is essential. A point worth noting is that breast ultrasonography should be the first imaging technique used to assess a breast mass during pregnancy because of its safety and high level of sensitivity. Although breast ultrasonography is less sensitive due to a higher density of the breast during pregnancy, mammography with appropriate abdominal shielding should be useful at evaluating the extent of disease (*33,34*). Gadolinium can cross the placental barrier and is considered potentially teratogenic (*35*), so magnetic resonance imaging (MRI) without gadolinium contrast can be used to further evaluate the breast during pregnancy (*33*).

A histopathologic examination of core needle biopsy specimens obtained under local anesthesia is the preferred method of sampling any clinically suspicious breast mass during pregnancy or otherwise (*13*). Suspected metastatic lymph nodes should also be evaluated with ultrasound and fine needle aspiration biopsy for cytologic confirmation (*36*). Pathologists should be informed of the patient's pregnancy because the presence of hyperplastic cells could simulate atypia,

leading to an increase in false-positive results (*37*).

Systemic staging studies are recommended for advanced cancers. Staging studies on patients during pregnancy must be performed only if the treatment options are going to be adjusted. If necessary, staging tests should include chest radiography with abdominal shielding, liver ultrasonography, and/or noncontrast skeletal MRI. The ESMO guidelines point out that bone scans and positron emission tomography (PET) should be avoided during pregnancy (*38*). Although a few studies have indicated that fluorodeoxyglucose (18F-FDG) PET and PET/magnetic resonance imaging (MRI) involve a low dose of fetal radiation exposure, there is not enough evidence to support the use of PET for breast cancer staging during pregnancy (*39,40*). Contrast-enhanced CT should be avoided during pregnancy.

A radiologist needs to be a key member of the medical team in order to calculate the total dose and review the indications as well as their risk-benefit ratios (*41*).

4. Pregnancy monitoring

Pregnant women with breast cancer should always be considered a high-risk group. Therefore, more careful and continuous monitoring with morphometric ultrasonography and umbilical artery Doppler assessment during gestation is mandatory. Calculation of gestational age and expected date of delivery are significant, which has an impact on breast cancer treatment planning (*42,43*). Allowing a pregnancy to reach full term (37 weeks) is strongly recommended. The gynecologist/obstetrician should be the part of the multidisciplinary team and determine the mode of delivery (*44*). Possible micrometastases in the placenta should be examined. In order to avoid hematological toxicity in the mother and fetus, the management of the last round of chemotherapy should be 3 weeks prior to the planned date of delivery (*3*). There is mounting evidence regarding the effects of breast cancer treatment on pregnancy outcomes. In general, therapy for breast cancer during pregnancy had no clear adverse effects on growth, cognitive function, and cardiac function in early childhood, suggesting that the diagnosis of cancer during pregnancy should not be an indication to abort the pregnancy. The only factor associated with a worse cognitive outcome was prematurity, irrespective of anticancer treatments (*45*).

5. Risk of anesthesia

Anesthesia considerations also include the safety of both the mother and the fetus. Changes in maternal anatomy and physiology during pregnancy increase the potential hazards for the mother and fetus undergoing anesthesia. Maternal changes include increased cardiac output (*46*), reduced functional residual capacity (*47*), dilation of the pyelocaliceal system (*48*), dilutional anemia (*49*), gastroesophageal reflux (*50*), and changes in glucose and adrenal metabolism (*51*). The risk of anesthesia-related morbidity and mortality during pregnancy mostly involves airway edema, restrictive lung physiology, and aspiration (*52*). A previous study suggested that adverse fetal outcomes after surgery may contribute to the mother's underlying condition rather than the effects of anesthesia (*53*). A point worth mentioning is that the patient should be positioned with a 15-30° left lateral tilt in order to reduce aortocaval compression and the incidence of supine hypotensive syndrome (*54*).

6. Treatment

Guidelines state that breast cancer during pregnancy should be treated in accordance with the management of breast tumors in non-pregnant women, including the local control of disease and the prevention of systemic metastases (*38,39*). To optimize the management of breast cancer during pregnancy, clinicopathological characteristics, gestational age at the diagnosis of breast cancer, expected date of delivery, and the patient's wishes should be considered. The goals of the multidisciplinary team are to cure the pregnant patient with breast cancer, to support the pregnancy, and to not harm the fetus (*12*).

6.1. Surgery during pregnancy

Surgery can be considered safe in all trimesters of pregnancy (*38*). The gestational age at diagnosis is an important factor in devising a surgical plan. Either a radical modified mastectomy (RMM) or breast-conserving surgery is a reasonable option for a pregnant woman with breast cancer. In the first trimester, however, radiotherapy following breast-conserving treatment may be delayed for the sake of fetal safety. Mastectomy should be recommended for patients who wish to continue the pregnancy (*55*). Breast-conserving surgery might be an option for early-stage BC in the second and third trimesters. Reconstructive surgery should be postponed until after birth, given concerns about normal changes in the breast after pregnancy and its unexpected cosmetic effects (*56*).

Although breast cancer during pregnancy has a high incidence of axillary metastases, sentinel lymph node biopsy should be suggested for patients with early-stage breast cancer. There is no level 1 evidence to support sentinel lymph node biopsy for breast cancer patients during pregnancy. The American Society of Clinical Oncology (ASCO) guidelines do not support this procedure (*57*). The National Comprehensive Cancer Network (NCCN) guidelines endorse the safety of this approach pursuant to the patient's wishes (*57,58*). Other guidelines advise sentinel lymph node biopsy when axillary ultrasound and a suspicious lymph node biopsy

are negative (*59*).

A sentinel lymph node biopsy should be performed using 99mTc-albumin nanocolloids (*39*). Blue dye and isosulfan blue should be avoided because of the risk of an allergic or anaphylactic maternal reaction, and methylene blue is contraindicated during the first trimester because it is teratogenic (*60*).

6.2. Systemic treatment during pregnancy

Generally, systemic treatments including chemotherapy, hormone therapy, targeted therapies, and immunotherapy are avoided in the first trimester because of the high risk of teratogenicity and abortion (Figure 2) (*39,61,62*). Available data on the teratogenic risks to pregnant women from all clinical trials are limited to case reports, animal studies, and studies with small samples. The major factors that should be taken into account before systemic therapy during pregnancy include physiologic changes during pregnancy, gestational age, placental passage, and the pharmacokinetic characteristics of the drug. Before any oncological treatment, a fetal ultrasound must be performed to exclude pre-existing abnormalities (*60*).

Anthracyclines are considered the treatment of choice because of the very low placental transfer (*63*). A previous study has reported that doxorubicin and epirubicin are not teratogenetic (*64*), but another study reported that they tend to cause prematurity and low birth weight (*65*). Retrospective and prospective studies have examined different schedules and chemotherapy combinations, such as 3-week cycles of FAC (5-fluororacil, doxorubicin, and cyclophosphamide), FEC (5-fluororacil, epirubicin, and cyclophosphamide), AC (doxorubicin and cyclophosphamide), EC (epirubicin and cyclophosphamide), or weekly epirubicin as monotherapy (*62,64-66*). The AC and EC regimens are most often used to treat breast cancer during pregnancy. A retrospective cohort study indicated that dose-dense chemotherapy was safe in 10 pregnant women with breast cancer (*67*). However, a dose-dense schedule may not be recommended (*68*). Although chemotherapy has considered safe and well-tolerated, the multidisciplinary team must monitor fetal safety and maternal blood pressure. Moreover, chemotherapy should not be administered after 35 weeks of gestation in order to prevent hematological complications during delivery (*69,70*).

The use of trastuzumab throughout pregnancy is contraindicated due to the high risk of oligohydramnios and/or anhydramnios (*39*). The *erbB2/neu* gene is related to fetal organogenesis (*71*). A systematic review and meta-analysis of the safety of trastuzumab during pregnancy concluded that more adverse events occur during the second/third trimester than during the first trimester (*72*). No data are available on pertuzumab and *T-DM1* administration in pregnant women, so both are contraindicated (*73*). A small molecule, lapatinib is presumed to be able to cross the placenta during all phases of pregnancy. The limited data on use of this tyrosine kinase inhibitor during pregnancy do not support its use in pregnant patients (*74,75*). Data on the use of *CDK4* and *CDK6* inhibitors throughout pregnancy are not yet available.

Endocrine therapy (tamoxifen and luteinizing hormone-releasing hormone analogues) is contraindicated for the treatment of breast cancer during pregnancy due to the high risk of birth defects (up to 17.6%) (*76*). Tamoxifen is teratogenic and increases the risk of breast cancer in offspring which has been verified in animal experiments (*77,78*). A systematic review has summarized major malformations (ambiguous genitalia, Pierre Robin sequence, and oculoauriculovertebral dysplasia) and minor malformations (preauricular skin tags and severe hypermetropia) in breast cancer patients exposed to tamoxifen during pregnancy (*79*). No data are available on human exposure to aromatase inhibitors during pregnancy, though there are data from animal models (*80*).

Radiation has dose- and gestational-week-dependent effects on the fetus (*81*). Due to its teratogenic effects, radiation therapy is not considered a safe treatment option (*82*). Radiotherapy could be performed in the first trimester and at the beginning of the second after a careful dose adjustment (exposure of 0.01 mGy is below the threshold dose) and proper abdominal shielding (*83*). The multidisciplinary team should balance the risks and benefits of administering radiotherapy, both for the mother and the fetus.

The *PD-1/PD-L1* pathway is involved in immune

Figure 2. Systemic treatment per trimester. Green: recommended; Yellow: only in selected cases; Red: contraindicated.

tolerance during pregnancy (*84*). In models involving pregnant animals, anti-*PD-1/PD-L1* treatment increased the risk of miscarriages, premature delivery, and birth mortality (*85*), so immunotherapy during pregnancy is contraindicated.

7. Conclusion

The management of breast cancer during pregnancy is a major ethical and professional challenge for both the patient and the multidisciplinary treatment team. Due to the special physiological stage that those patients are in, some special considerations should be made. The patient's medical history needs to be understood in detail, the patient needs to be evaluated as comprehensively as possible *via* limited additional examinations, and the entire pregnancy should be monitored in order to formulate a reasonable treatment plan, to ensure the safety of the fetus and the mother, and to ensure the effectiveness of cancer treatment.

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References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. CA Cancer J Clin. 2021; 71:7-33.
2. Soto-Trujillo D, Santos Aragon LN, Kimura Y. Pregnancy-associated breast cancer: What radiologists must know. Cureus. 2020; 12:e10343.
3. Bar-Joseph H, Peccatori FA, Goshen-Lago T, Cribiu FM, Scarfone G, Miller I, Nemerovsky L, Levi M, Shalgi R, Ben-Aharon I. Cancer during pregnancy: The role of vascular toxicity in chemotherapy-induced placental toxicity. Cancers (Basel). 2020; 12:1277.
4. Yang Z, Ji L, Jiang G, Liu R, Liu Z, Yang Y, Ma Q, Zhao H. FL118, a novel camptothecin analogue, suppressed migration and invasion of human breast cancer cells by inhibiting epithelial-mesenchymal transition *via* the Wnt/beta-catenin signaling pathway. Biosci Trends. 2018; 12:40-46.
5. Zhang N, Zhang Y, Lin J, Qiu X, Chen L, Pan X, Lu Y, Zhang J, Wang Y, Li D, Wang L. Low-density lipoprotein receptor deficiency impaired mice osteoblastogenesis in vitro. Biosci Trends. 2018; 11:658-666.
6. Thomas A, Rhoads A, Suhl J, Conway KM, Hundley WG, McNally LR, Oleson J, Melin SA, Lynch CF, Romitti PA. Incidence and survival by human epidermal growth factor receptor 2 status in young women with stage I-III breast cancer: SEER, 2010-2016. Clin Breast Cancer. 2020; 20:e410-e422.
7. Ghiasvand R, Adami HO, Harirchi I, Akrami R, Zendehdel K. Higher incidence of premenopausal breast cancer in less developed countries; myth or truth? BMC Cancer. 2014; 14:343.
8. Benoit L, Mir O, Vialard F, Berveiller P. Cancer during pregnancy: A review of preclinical and clinical transplacental transfer of anticancer agents. Cancers (Basel). 2021; 13:1238.
9. Dur ES, Irfan S, Islam ZS, Sheikh L. Impact of pregnancy on cancer survival: Experience at a tertiary care hospital. Pak J Med Sci. 2021; 37:335-338.
10. Ruiz R, Herrero C, Strasser-Weippl K, Touya D, St Louis J, Bukowski A, Goss PE. Epidemiology and pathophysiology of pregnancy-associated breast cancer: A review. Breast. 2017; 35:136-141.
11. Dodelzon K, Starikov A, Reichman M, Cheng E, Lu CM, Blackburn A, Reznik E, Kim J, Bose A, Thomas C, Askin G, Arleo EK. Breast cancer in women under age 40: A decade of trend analysis at a single institution. Clin Imaging. 2021; 78:165-170.
12. Poggio F, Tagliamento M, Pirrone C, Soldato D, Conte B, Molinelli C, Cosso M, Fregatti P, Del Mastro L, Lambertini M. Update on the management of breast cancer during pregnancy. Cancers (Basel). 2020; 12:3616.
13. Paris I, Di Giorgio D, Carbognin L, *et al.* Pregnancy-associated breast cancer: A multidisciplinary approach. Clin Breast Cancer. 2021; 21:e120-e127.
14. Wu Q, Wang X, Wu F, Peng D, Wu G, Yang L, Yuan L. Role of a multidisciplinary team (MDT) in the diagnosis, treatment, and outcomes of inflammatory bowel disease: A single Chinese center's experience. Biosci Trends. 2021; 15:171-179.
15. Zhang Z, Li Y, Li K, Zhai G, Dang X, Zhong C, Shi Z, Zou R, Wang L, Wei D, Tang B, Ge J. Value of multidisciplinary team (MDT) in minimally invasive treatment of complex intrahepatic bile duct stones. Biosci Trends. 2021; 15:161-170.
16. Qiu G, Xie K, Jin Z, Jiang C, Liu H, Wan H, Huang J. The multidisciplinary management of hepatocellular carcinoma with portal vein tumor thrombus. Biosci Trends. 2021; 15:148-154.
17. Al-Amri AM. Clinical presentation and causes of the delayed diagnosis of breast cancer in patients with pregnancy associated breast cancer. J Family Community Med. 2015; 22:96-100.
18. Jahanbin B, Soleimani V. Histology of pregnancy-associated breast cancer. Adv Exp Med Biol. 2020; 1252:81-86.
19. Schad A, Slostad J, Rao R. Gestational breast cancer: Current challenges in staging and treatment of breast cancer. BMJ Case Rep. 2020; 13:e235308.
20. Kim YG, Jeon YW, Ko BK, Sohn G, Kim EK, Moon

BI, Youn HJ, Kim HA, Korean Breast Cancer S. Clinicopathologic characteristics of pregnancy-associated breast cancer: Results of analysis of a nationwide breast cancer registry database. J Breast Cancer. 2017; 20:264-269.
21. Asztalos S, Pham TN, Gann PH, Hayes MK, Deaton R, Wiley EL, Emmadi R, Kajdacsy-Balla A, Banerji N, McDonald W, Khan SA, Tonetti DA. High incidence of triple negative breast cancers following pregnancy and an associated gene expression signature. Springerplus. 2015; 4:710.
22. Zhang R, Liu X, Huang W, Shao B, Yan Y, Liang X, Ran R, Song G, Di L, Jiang H, Li H. Clinicopathological features and prognosis of patients with pregnancy-associated breast cancer: A matched case control study. Asia Pac J Clin Oncol. 2021; 17:396-402.
23. Allouch S, Gupta I, Malik S, Al Farsi HF, Vranic S, Al Moustafa AE. Breast cancer during pregnancy: A marked propensity to triple-negative phenotype. Front Oncol. 2020; 10:580345.
24. Iqbal J, Amir E, Rochon PA, Giannakeas V, Sun P, Narod SA. Association of the timing of pregnancy with survival in women with breast cancer. JAMA Oncol. 2017; 3:659-665.
25. Ploquin A, Pistilli B, Tresch E, *et al.* 5-year overall survival after early breast cancer diagnosed during pregnancy: A retrospective case-control multicentre French study. Eur J Cancer. 2018; 95:30-37.
26. Choi M, Han J, Yang BR, Jang MJ, Kim M, Kim TY, Im SA, Lee HB, Moon HG, Han W, Noh DY, Lee KH. Prognostic impact of pregnancy in Korean patients with breast cancer. Oncologist. 2019; 24:e1268-e1276.
27. Shao C, Yu Z, Xiao J, Liu L, Hong F, Zhang Y, Jia H. Prognosis of pregnancy-associated breast cancer: A meta-analysis. BMC Cancer. 2020; 20:746.
28. Linhares S, Alrammah T, Alghamdi HA, Moller MG. Inflammatory breast cancer in pregnancy and lactation. Adv Exp Med Biol. 2020; 1252:143-151.
29. Korakiti AM, Moutafi M, Zografos E, Dimopoulos MA, Zagouri F. The genomic profile of pregnancy-associated breast cancer: A systematic review. Front Oncol. 2020; 10:1773.
30. Gutierrez-Diez PJ, Gomez-Pilar J, Hornero R, Martinez-Rodriguez J, Lopez-Marcos MA, Russo J. The role of gene to gene interaction in the breast's genomic signature of pregnancy. Sci Rep. 2021; 11:2643.
31. Woo JC, Yu T, Hurd TC. Breast cancer in pregnancy: A literature review. Arch Surg. 2003; 138:91-98; discussion 99.
32. Litton JK, Theriault RL, Gonzalez-Angulo AM. Breast cancer diagnosis during pregnancy. Womens Health (Lond). 2009; 5:243-249.
33. Wang PI, Chong ST, Kielar AZ, Kelly AM, Knoepp UD, Mazza MB, Goodsitt MM. Imaging of pregnant and lactating patients: Part 1, Evidence-based review and recommendations. AJR Am J Roentgenol. 2012; 198:778-784.
34. Mattsson S, Leide-Svegborn S, Andersson M. X-Ray and molecular imaging during pregnancy and breastfeeding-When should we be worried? Radiat Prot Dosimetry. 2021; ncab041.
35. Kumar R, De Jesus O. Radiation effects on the fetus. In: StatPearls (Treasure Island (FL), 2021.
36. Bajpai J, Simha V, Shylasree TS, *et al.* Pregnancy associated breast cancer (PABC): Report from a gestational cancer registry from a tertiary cancer care centre, India. Breast. 2021; 56:88-95.
37. Vinatier E, Merlot B, Poncelet E, Collinet P, Vinatier D. Breast cancer during pregnancy. Eur J Obstet Gynecol Reprod Biol. 2009; 147:9-14.
38. Peccatori FA, Azim HA, Jr., Orecchia R, Hoekstra HJ, Pavlidis N, Kesic V, Pentheroudakis G, Group EGW. Cancer, pregnancy and fertility: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2013; 24 Suppl 6:vi160-170.
39. Loibl S, Schmidt A, Gentilini O, *et al.* Breast cancer diagnosed during pregnancy: Adapting recent advances in breast cancer care for pregnant patients. JAMA Oncol. 2015; 1:1145-1153.
40. Zanotti-Fregonara P, Laforest R, Wallis JW. Fetal radiation dose from 18F-FDG in pregnant patients imaged with PET, PET/CT, and PET/MR. J Nucl Med. 2015; 56:1218-1222.
41. Cubillo A, Morales S, Goni E, Matute F, Munoz JL, Perez-Diaz D, de Santiago J, Rodriguez-Lescure A. Multidisciplinary consensus on cancer management during pregnancy. Clin Transl Oncol. 2021; 23:1054-1066.
42. Dong Y, Wang L, Lu Y, Fu Z, Du Y, Wang L. Factors affecting mode of delivery in women of advanced maternal age. Biosci Trends. 2021; 15:61-63.
43. Li C, Zhang N, Zhou J, Leung W, Gober HJ, Huang Z, Pan X, Chen L, Guan L, Wang L. Variations in the antithyroid antibody titre during pregnancy and after delivery. Risk Manag Healthc Policy. 2021; 14:847-859.
44. Azim HA, Jr., Del Mastro L, Scarfone G, Peccatori FA. Treatment of breast cancer during pregnancy: Regimen selection, pregnancy monitoring and more. Breast. 2011; 20:1-6.
45. Amant F, Vandenbroucke T, Verheecke M, *et al.* Pediatric outcome after maternal cancer diagnosed during pregnancy. N Engl J Med. 2015; 373:1824-1834.
46. Ouzounian JG, Elkayam U. Physiologic changes during normal pregnancy and delivery. Cardiol Clin. 2012; 30:317-329.
47. Hirnle L, Lysenko L, Gerber H, Lesnik P, Baranowska A, Rachwalik M, Leszczyszyn J, Strozecki L. Respiratory function in pregnant women. Adv Exp Med Biol. 2013; 788:153-160.
48. Ravindra GL, Madamangalam AS, Seetharamaiah S. Anaesthesia for non-obstetric surgery in obstetric patients. Indian J Anaesth. 2018; 62:710-716.
49. Sun D, McLeod A, Gandhi S, Malinowski AK, Shehata N. Anemia in pregnancy: A pragmatic approach. Obstet Gynecol Surv. 2017; 72:730-737.
50. Auron M, Duran Castillo MY, Garcia OFD. Perioperative management of pregnant women undergoing nonobstetric surgery. Cleve Clin J Med. 2020; 88:27-34.
51. Carlin A, Alfirevic Z. Physiological changes of pregnancy and monitoring. Best Pract Res Clin Obstet Gynaecol. 2008; 22:801-823.
52. Eskandari A, Alipour S. Aspects of anesthesia for breast surgery during pregnancy. Adv Exp Med Biol. 2020; 1252:107-114.
53. Mazze RI, Kallen B. Reproductive outcome after anesthesia and operation during pregnancy: A registry study of 5405 cases. Am J Obstet Gynecol. 1989; 161:1178-1185.
54. Moaveni DM, Birnbach DJ, Ranasinghe JS, Yasin SY. Fetal assessment for anesthesiologists: Are you evaluating

the other patient? Anesth Analg. 2013; 116:1278-1292.
55. Toesca A, Gentilini O, Peccatori F, Azim HA Jr, Amant F. Locoregional treatment of breast cancer during pregnancy. Gynecol Surg. 2014; 11:279-284.
56. Ji YI, Kim KT. Gynecologic malignancy in pregnancy. Obstet Gynecol Sci. 2013; 56:289-300.
57. Lyman GH, Somerfield MR, Bosserman LD, Perkins CL, Weaver DL, Giuliano AE. Sentinel lymph node biopsy for patients with early-stage breast cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol. 2017; 35:561-564.
58. Gradishar WJ, Anderson BO, Abraham J, *et al.* Breast Cancer, Version 3.2020, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw. 2020; 18:452-478.
59. Giammarile F, Alazraki N, Aarsvold JN, Audisio RA, Glass E, Grant SF, Kunikowska J, Leidenius M, Moncayo VM, Uren RF, Oyen WJ, Valdes Olmos RA, Vidal Sicart S. The EANM and SNMMI practice guideline for lymphoscintigraphy and sentinel node localization in breast cancer. Eur J Nucl Med Mol Imaging. 2013; 40:1932-1947.
60. Amant F, Loibl S, Neven P, Van Calsteren K. Breast cancer in pregnancy. Lancet. 2012; 379:570-579.
61. Paskulin GA, Gazzola Zen PR, de Camargo Pinto LL, Rosa R, Graziadio C. Combined chemotherapy and teratogenicity. Birth Defects Res A Clin Mol Teratol. 2005; 73:634-637.
62. Ring AE, Smith IE, Jones A, Shannon C, Galani E, Ellis PA. Chemotherapy for breast cancer during pregnancy: An 18-year experience from five London teaching hospitals. J Clin Oncol. 2005; 23:4192-4197.
63. Framarino-Dei-Malatesta M, Sammartino P, Napoli A. Does anthracycline-based chemotherapy in pregnant women with cancer offer safe cardiac and neurodevelopmental outcomes for the developing fetus? BMC Cancer. 2017; 17:777.
64. Loibl S, Han SN, von Minckwitz G, *et al.* Treatment of breast cancer during pregnancy: An observational study. Lancet Oncol. 2012; 13:887-896.
65. Cardonick E, Dougherty R, Grana G, Gilmandyar D, Ghaffar S, Usmani A. Breast cancer during pregnancy: Maternal and fetal outcomes. Cancer J. 2010; 16:76-82.
66. Hahn KM, Johnson PH, Gordon N, Kuerer H, Middleton L, Ramirez M, Yang W, Perkins G, Hortobagyi GN, Theriault RL. Treatment of pregnant breast cancer patients and outcomes of children exposed to chemotherapy in utero. Cancer. 2006; 107:1219-1226.
67. Cardonick E, Gilmandyar D, Somer RA. Maternal and neonatal outcomes of dose-dense chemotherapy for breast cancer in pregnancy. Obstet Gynecol. 2012; 120:1267-1272.
68. Peccatori FA, Lambertini M, Scarfone G, Del Pup L, Codacci-Pisanelli G. Biology, staging, and treatment of breast cancer during pregnancy: Reassessing the evidences. Cancer Biol Med. 2018; 15:6-13.
69. Alfasi A, Ben-Aharon I. Breast cancer during pregnancy-Current paradigms, paths to explore. Cancers (Basel). 2019; 11:1669.
70. Berveiller P, Mir O, Degrelle SA, Tsatsaris V, Selleret L, Guibourdenche J, Evain-Brion D, Fournier T, Gil S. Chemotherapy in pregnancy: Exploratory study of the effects of paclitaxel on the expression of placental drug transporters. Invest New Drugs. 2019; 37:1075-1085.
71. Lee KF, Simon H, Chen H, Bates B, Hung MC, Hauser C. Requirement for neuregulin receptor erbB2 in neural and cardiac development. Nature. 1995; 378:394-398.
72. Zagouri F, Sergentanis TN, Chrysikos D, Papadimitriou CA, Dimopoulos MA, Bartsch R. Trastuzumab administration during pregnancy: A systematic review and meta-analysis. Breast Cancer Res Treat. 2013; 137:349-357.
73. Shachar SS, Gallagher K, McGuire K, Zagar TM, Faso A, Muss HB, Sweeting R, Anders CK. Multidisciplinary management of breast cancer during pregnancy. Oncologist. 2017; 22:324-334.
74. Kelly H, Graham M, Humes E, Dorflinger LJ, Boggess KA, O'Neil BH, Harris J, Spector NL, Dees EC. Delivery of a healthy baby after first-trimester maternal exposure to lapatinib. Clin Breast Cancer. 2006; 7:339-341.
75. Lambertini M, Martel S, Campbell C, *et al.* Pregnancies during and after trastuzumab and/or lapatinib in patients with human epidermal growth factor receptor 2-positive early breast cancer: Analysis from the NeoALTTO (BIG 1-06) and ALTTO (BIG 2-06) trials. Cancer. 2019; 125:307-316.
76. Al Jishi T, Sergi C. Current perspective of diethylstilbestrol (DES) exposure in mothers and offspring. Reprod Toxicol. 2017; 71:71-77.
77. Barthelmes L, Gateley CA. Tamoxifen and pregnancy. Breast. 2004; 13:446-451.
78. Halakivi-Clarke L, Cho E, Onojafe I, Liao DJ, Clarke R. Maternal exposure to tamoxifen during pregnancy increases carcinogen-induced mammary tumorigenesis among female rat offspring. Clin Cancer Res. 2000; 6:305-308.
79. Buonomo B, Brunello A, Noli S, Miglietta L, Del Mastro L, Lambertini M, Peccatori FA. Tamoxifen exposure during pregnancy: A systematic review and three more cases. Breast Care (Basel). 2020; 15:148-156.
80. Tiboni GM. Aromatase inhibitors and teratogenesis. Fertil Steril. 2004; 81:1158-1159; author reply 1159.
81. Keyser EA, Staat BC, Fausett MB, Shields AD. Pregnancy-associated breast cancer. Rev Obstet Gynecol. 2012; 5:94-99.
82. Behrman RH, Homer MJ, Yang WT, Whitman GJ. Mammography and fetal dose. Radiology. 2007; 243:605; author reply 605-606.
83. Greskovich JF, Jr., Macklis RM. Radiation therapy in pregnancy: Risk calculation and risk minimization. Semin Oncol. 2000; 27:633-645.
84. Luppi P. How immune mechanisms are affected by pregnancy. Vaccine. 2003; 21:3352-3357.
85. Hepner A, Negrini D, Hase EA, Exman P, Testa L, Trinconi AF, Filassi JR, Francisco RPV, Zugaib M, O'Connor TL, Martin MG. Cancer during pregnancy: The oncologist overview. World J Oncol. 2019; 10:28-34.

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Review

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The positive role of traditional Chinese medicine as an adjunctive therapy for cancer

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SUMMARY Traditional Chinese medicine (TCM), especially Chinese herbal medicines and acupuncture, has been traditionally used to treat patients with cancers in China and other East Asian countries. Numerous studies have indicated that TCM not only alleviates the symptoms (*e.g.*, fatigue, chronic pain, anorexia/cachexia, and insomnia) of patients with cancer and improves their quality of life (QOL) but also diminishes adverse reactions and complications caused by chemotherapy, radiotherapy, or targeted-therapy. Therefore, Chinese herbal medicines and acupuncture and other alternative therapies need to be understood by TCM physicians and other health care providers. This review mainly summarizes the experimental results and conclusions from literature published since 2010, and a search of the literature as been performed in the PubMed, MEDLINE, Web of Science, Scopus, Springer, ScienceDirect, and China Hospital Knowledge Database (CHKD) databases. Some Chinese herbal medicines (*e.g.*, *Panax ginseng, Panax quinquefolius*, *Astragali radix*, Bu-zhong-yi-qi-tang (TJ-41), Liu-jun-zi-tang (TJ-43), Shi-quan-da-bu-tang (TJ-48), and Ban-xia-xie-xin-tang (TJ-14)) and some acupuncture points (*e.g.*, Zusanli (ST36), Zhongwan (CV12), Neiguan (PC6) and Baihui (GV20)) that are commonly used to treat cancer-related symptoms and/or to reduce the toxicity of chemotherapy, radiotherapy, or targeted-therapy are highlighted and summarized. Through a review of literature, we conclude that TCM can effectively alleviate adverse gastrointestinal reactions (including diarrhea, nausea, and vomiting) to these anti-cancer therapies, decrease the incidence of bone marrow suppression, alleviate cardiotoxicity, and protect against chemotherapy-induced peripheral neuropathy and radiation-induced pneumonitis. Moreover, TCM can alleviate epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI)-related acneiform eruptions, diarrhea, and other adverse reactions. The hope is that this review can contribute to an understanding of TCM as an adjuvant therapy for cancer and that it can provide useful information for the development of more effective anti-cancer therapies. However, more rigorously designed trials involving cancer treatment must be conducted in the future, including complete quality control and standardized models at the cellular, organic, animal and clinical levels, in order to study TCM in multiple forms and at multiple levels.

Keywords Traditional Chinese Medicine (TCM), Chinese herbal medicines, acupuncture, cancer-related symptoms, anti-cancer therapy-related adverse reactions and complications

1. Introduction

Cancer incidence and mortality are rapidly increasing worldwide. Cancer has become the second leading cause of death globally behind only ischemic heart disease, and it imposes serious economic burdens (*1*). Cancer will rank as a single leading barrier to increasing life expectancy in every country of the world. Based on the WHO Global Cancer Observatory (GLOBOCAN) 2018 registry, over the next four decades cancer deaths are expected to overcome those due to ischemic heart disease, with a 2.08-fold increase (versus a 1.76-fold increase in ischemic heart disease) by the year 2060 (*2*). Although a combination of screening, prevention, and successful treatment has resulted in a vast number of cancer survivors, in China alone there were 4,285,033 new cases and 2,865,174 deaths reported in 2018 according to the Global Cancer Observatory (*3*), and in the United States alone this amounts to nearly 16 million current cancer survivors, which is expected to increase to 20.3 million by 2026 (*4*). Thus, cancer prevention and treatment remain a major challenge for the world in the

coming years.

Currently, chemotherapy, radiotherapy, targeted-therapy, and immunotherapy are common anticancer therapies being used to treat patients with malignancies in the intermediate and advanced stages by controlling tumor growth, prolonging survival, and improving quality of life (QOL) to some extent (*5*). However, these therapies either alone or in combination have been found to have numerous limitations and drawbacks including myelosuppression, gastrointestinal reactions, cardiac damage, and liver and renal dysfunction, rashes, hand-foot syndrome, and local radiation damage (*6*). Not only can these toxicities severely affect patients' QOL, but some patients may discontinue treatment because they cannot tolerate these toxicities. With advances in medicine and updated knowledge, cancer therapy has entered a stage of diversified comprehensive treatment. Therefore, more effective or adjunctive therapies must soon be developed to treat cancer. Traditional Chinese medicine (TCM), especially Chinese herbal medicines and acupuncture, may represent a promising option.

TCM mainly includes herbal medicine, acupuncture, moxibustion, and massage. An important component of complementary and alternative medicine, TCM has evolved over thousands of years with its own unique system of theories, diagnostics, and therapies in Asian countries, and especially China. In recent decades, TCM has been increasingly used and has become well-known for its significant role in preventing and treating cancer. It is widely used by TCM physicians and other health care providers to alleviate the symptoms of patients with cancer and to control the adverse reactions and the toxicities of cancer therapies, thus improving patient QOL, preventing recurrence, and prolonging survival (*7*).

In reviews published over the past 10 years, the current authors have indicated that some Chinese herbal medicines used as adjuvant therapy in combination with chemotherapy, radiotherapy, or targeted-therapy are capable of enhancing the sensitivity of these anti-cancer therapeutics, improving an organism's immune system, and diminishing the adverse reactions and complications caused by chemotherapy, radiotherapy, or targeted-therapy. The current authors have also indicated that some Chinese herbal medicines as adjuvant therapy played an important role in alleviating the symptoms of patients with different stages of cancer lesions including those after surgery, radiotherapy or chemotherapy (*5,6,8*).

The current review will focus on the positive role of TCM as an adjunctive therapy for cancer. In this review, some individual Chinese herbal medicines (Table 1), Chinese herbal formulas (Table 2), and acupuncture points (Table 3) that are commonly used to treat cancer-related symptoms are discussed and trials of TCM therapies as adjuvant cancer therapies to reduce adverse reactions and complications during chemotherapy, radiotherapy, or targeted-therapy are also highlighted and summarized. This review mainly

Table 1. Individual Chinese herbal medicines as adjuvant therapy for cancer

Common name	Other names	Efficacy according to TCM theory	Major active ingredients	Biological activity	Clinical evidence of anticancer activity	*Ref.*
Panax ginseng	Ren-Shen in Chinese or Ginseng in Korea	As a tonic, prophylactic, and restorative agent with action to invigorate	Ginsenosides, essential oil, peptidoglycans, polysaccharides, nitrogen compounds, fatty acids, and phenolic compounds	Antitumor, antioxidant, immunomodulation, anti-ulcer, anti-adhesive, antioxidant, hepatoprotective, and hypoglycemic actions	Alleviates CRF without any discernible toxicity	*14-15*
Panax quinquefolius	Xi-Yang-Shen in Chinese or Wisconsin Ginseng in American	As a tonic with action to enhance *qi*, nourish *yin*, clear heat, and promote flow	Ginsenosides, and polysaccharides	Anti-aging, anti-cancer, anti-stress, anti-fatigue, immunostimulatory, and anxiolytic action	Has a potential clinical benefit in safely treating CRF	*17-18*
Astragali radix	Huang-Qi in Chinese	As a tonic with action to invigorate	Isoflavonoids, triterpenoid saponins, polysaccharides, amino butyric acids, and various trace elements	Antitumor, antioxidant, hepatoprotective, anti-diabetic, antimicrobial, antiviral, and immunomodulatory action	Alleviates CRF, adverse gastrointestinal reactions, and bone marrow suppression	*5,19-20*
Semen Ziziphi Spinosae	Suan Zao Ren in Chinese	Nourishing and calming the mind	Sanjoinine A, Jujuboside A, spinosin, and other flavonoids	Sedative and hypnotic action	As an effective replacement therapy for insomnia	*47-48*
Colla corii asini	E-Jiao in Chinese	As a tonic to enrich the blood	Peptides and proteins produced by partial hydrolysis of collagen	Anti-anemic and anti-aging action	Promotes the recovery of bone marrow hemopoietic function in cancer patients with myelosuppression	*56-57*
Rhizoma Corydalis	Yan Hu Suo	Activating the blood, invigorating the body, and relieving pain	Protoberberine alkaloids, aporphine alkaloids, opiate alkaloids, organic acids, steroids, and carbohydrates	Action to inhibit arrhythmia, myocardial infarction, coronary artery dilation, tumors, and thrombosis	Has the potential to mitigate antineoplastic drug-induced cardiotoxicity	*61-62*

Note: Abbreviations: traditional Chinese medicine (TCM); cancer-related fatigue (CRF).

Table 2. Some Chinese herbal formulas as adjuvant therapy for cancer

Common name	Other names	Composition	Biological activity	Clinical evidence of anticancer activity	*Ref.*
Bu-zhong-yi-qi-tang	Hochuekki-to or TJ-41 in Japanese; Bojungikki-Tang in Korean	Includes 7 herbs: *Pinelliae rhizoma, Scutellaria baicalensis, Zingiberis rhizoma, Zizyphi fructus, Coptidis rhizoma, Glycyrrhiza radix, Panax ginseng.*	Antitumor action, immunomodulation, and alleviation of fatigue	Alleviates CRF and improves QOL; reduces adverse reactions to radiotherapy or chemotherapy.	*5,21-23*
Shi-quan-da-bu-tang	Juzentaiho-to or TJ-48 in Japanese, Sipjeondaebo-tang in Korean	Includes 10 herbs: *Panax ginseng, Astragali radix, Angelicae radix, Rehmanniae radix, Atractylodis lanceae rhizoma, Cinnamomi cortex, Poria, Paeoniae radix, Ligustici rhizoma Glycyrrhizae radix.*	Antitumor, immunomodulation, and alleviation of fatigue	Reduces the incidence of adverse reactions to chemotherapy; alleviates cancer-related anorexia/ cachexia; prevents nutritional disorders, and increases physical fitness.	*26-28,40*
Liu-jun-zi-tang	Yukgunja-tang in Korean, Rikkunshito or TJ-43 in Japanese	Includes 6 herbs: *Ginseng radix,Poria cocos, Rhizoma atractylodis macrocephalae, Glycyrrhizae radix et rhizoma, Pinelliae rhizoma, Pericarpium citri,common ginger,* and *Jujube.*	Gastroprotective action	Effective at treating chemotherapy-induced dyspepsia and cancer cachexia-anorexia syndrome; alleviates the symptoms of postgastrectomy syndrome and improves the long-term QOL in patients with gastric cancer who have undergone proximal gastrectomy.	*6,41*
Sheng-jiang-xie-xin-tang	None	Includes 8 herbs: *Fresh Zingiberis rhizoma, Glycyrrhizae radix, Codonopsis radix, Zingiberis rhizoma, Astragali radix, Pinelliae rhizoma, Coptidis rhizome,* and *Jujubae fructus.*	Gastroprotective action	Reduces chemotherapy-induced hematological and gastrointestinal toxicities without affecting the clinical response to chemotherapy.	*53*
Huang-qi-gui-zhi-wu-wu-tang	AC591	Includes 5 herbs:*Hedysarum Multijugum Maxim, Cinnamomi Ramulus, Paeoniae radix alba, Zingiber officinale Roscoe,* and *Jujubae fructus.*	Alleviation of limb numbness and pain	Reduces chemotherapy-induced peripheral neuropathy.	*67-69*
Ban-xia-xie-xin-tang	Ban-xia-xie-xin-tang in Chinese or Hangeshashinto or TJ-14 in Japanese	Includes 5 herbs: *Coptis rhizoma, Panax ginseng, Glycyrrhizae radix, Jujube, Pinelliae rhizoma , ginger,* and *Scutellariae radix.*	Antioxidant action, anti-inflammatory, bactericidal, and analgesic action as well as action to promote healing	Alleviates chemotherapy-induced diarrhea and oral mucositis and radiation-induced enteritis; reduces the incidence of EGFR-TKI-induced skin rashes, paronychia, diarrhea, and oral mucositis.	*78-82*

Note: Abbreviations: cancer-related fatigue (CRF); quality of life (QOL); epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs);

Table 3. Clinical trials of acupuncture therapy as adjuvant therapy for cancer

Symptoms or adverse reactions	Sample	Acupuncture points	Outcome	*Ref.*
Chronic pain	*n* = 42	Acupuncture at Siguanxue (Taichong (LR3), Hegu (LI4), Neiguan (PC6), Zusanli (ST36), and Sanyinjiao (SP6).	Significantly reduced cancer pain.	*33*
Adverse gastrointestinal reactions	*n* = 150	Acupuncture at Zusanli (ST36), Zhongwan (CV12), and Neiguan (PC6)	Acupuncture combined with the slow intravenous injection of tropisetron hydrochloride was effective at preventing and treating vomiting induced by chemotherapy to treat lung cancer.	*51*
Adverse gastrointestinal reactions	*n* = 58	Moxibustion at Baihui (GV20) and Zhongwan (CV12)	Moxibustion combined with an 5-HT receptor antagonist markedly reduce the incidence and severityof nausea and vomiting caused by chemotherapy with cisplatin in patients with lung cancer.	*52*
Bone marrow suppression	A systematic review and meta-analysis	Acupuncture at Zusanli (T36), Neiguan (PC6), Geshu (BL17), Feishu (BL13), Guanyuan (RN4), and Shenshu (BL23)	Acupoint stimulation markedly reduced bone marrow suppression caused by conventional therapy, it increased hemoglobin levels and platelet counts in patients with lung cancer, and it decreased chemotherapy-induced nausea and vomiting.	*58*
CIPN	*n* = 6	Acupuncture at ST34 (Liang Qiu) as well as at EX-LE12 (Qi Duan) and EX-LE8 (Ba Feng)	Acupuncture alleviated CIPN.	*70*

Note: Abbreviations: chemotherapy-induced peripheral neuropathy (CIPN).

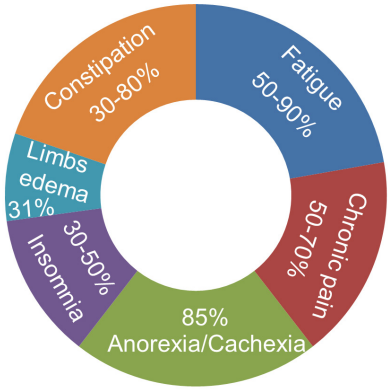

Figure 1. Common cancer-related symptoms and their incidence.

summarizes experimental results and findings from literature published since 2010. A search of the literature was conducted in the PubMed, MEDLINE, Web of Science, Scopus, Springer, ScienceDirect, and China Hospital Knowledge Database (CHKD) databases. The hope is that this contributes to an understanding of TCM as adjuvant therapy for cancer and that it provides useful information for development of more effective anti-cancer therapies.

2. TCM as adjuvant therapy for cancer-related symptoms

Patients with cancer experience multiple symptoms including fatigue, chronic pain, anorexia, insomnia, limbs edema, and constipation that seriously impair patients' daily functioning and their QOL. As shown in Figure 1, some studies of patients with cancer have reported that the prevalence of fatigue was 50% to 90%, that of chronic painwas 50% to70%, that of anorexia/cachexia was around 85%, that of insomnia was 30% to 50%, that of limb edema was 31%, and that of constipation was 30% to 80% (*9,10*). Despite the high prevalence of these symptoms in patients with cancer, conventional therapies are far from satisfactory. Some individual Chinese herbal medicines, Chinese herbal formulas, and acupuncture points have been found to be effective in alleviating the symptoms of patients with cancer. The current review provides a brief outline on the use of TCM to reduce some cancer-related symptoms (Table 4).

2.1. Fatigue

According to the National Comprehensive Cancer Network (NCCN), cancer-related fatigue (CRF) has been defined as a distressing, persistent, subjective sense of physical, emotional, and/or cognitive tiredness or exhaustion related to cancer or cancer treatment that is not proportional to recent activity and interferes with usual functioning (*11*). Regardless of the type of cancer and treatment modality, nearly all patients experience

Table 4. Clinical trials of traditional Chinese herbal medicines as adjuvant therapy to alleviate cancer-related symptoms

Symptoms	Chinese herbal medicine	Sample	Dosage	Outcome	Ref.
Fatigue	*Panax Ginseng*	$n = 30$	*Panax Ginseng* 800 mg taken daily for 29 days	High-dose *Panax Ginseng* was safe, it alleviated CRF, and it improved overall QOL, appetite, and sleep at night.	*14*
Fatigue	Korean red Ginseng	$n = 219$	Korean red Ginseng 2,000 mg/day for 16 weeks	Korean red Ginseng was safely combined with mFOLFOX-6 chemotherapy in patients with colorectal cancer and reduced CRF compared to a placebo.	*15*
Fatigue	American ginseng	$n = 364$	American Ginseng 2,000 mg/day for 8 weeks	American ginseng alleviated CRF without causing discernible toxicities.	*17*
Fatigue	American ginseng	$n = 28$	American Ginseng (2,000 mg/day) combined with methylphenidate (10-40 mg/day) for 30 days	The combination of methylphenidate and American ginseng caused no discernible toxicities and it displayed a potential clinical benefit in CRF.	*18*
Fatigue	TJ-41	$n = 40$	TJ-41 for 2 weeks	TJ-41 might have beneficial effects on CRF and QOL in patients with cancer.	*22*
Fatigue	TJ-48	$n = 45$	TJ-48 combined with chemotherapy	TJ-48 combined with chemotherapy might improve the progression-free survival of patients with postoperative recurrence of non-small cell lung cancer by preventing nutritional disorders and increasing physical fitness.	*28*
Chronic pain	Wen Jing Zhi Tong Fang	$n = 62$	Wen Jing Zhi Tong Fang combined with an appropriate analgesic	Relieved cancer-related pain with reduced doses of analgesics, fewer adverse reactions, and improved QOL.	*36*
Anorexia/cachexia	TJ-48	$n = 32$	TJ-48 3 g/day for 4 weeks	Improved QOL andalleviated anorexia in patients with cancer.	*40*
Anorexia/cachexia	TJ-43	$n = 40$	TJ-43 twice a day for 4 weeks (dose of 3.0 g taken a total of 56 times)	Increased the anorexia/cachexia subscale scores of patients with cancer.	*41*

Note: Bu-Zhong-Yi-Qi-Tang (TJ-41); Shi-quan-da-bu-tang (TJ-48); Liu-jun-zi-tang (TJ-43); cancer-related fatigue (CRF); quality of life (QOL).

Figure 2. Some individual Chinese herbal medicines that are commonly prescribed by traditional Chinese physicians for the treatment of cancer-related fatigue (CRF). **(A)** The *Panax Ginseng* plant; **(B)** White ginseng or raw ginseng for use in TCM; **(C)** Heat-processed ginseng or red ginseng for use in TCM; **(D)** The American ginseng plant; **(E)** American ginseng for use in TCM; **(F)** The *Astragali radix* plant; **(G)** *Astragali radix* for use in TCM. (Note: The pictures are from *http://sao.dajiazhongyi.com/* with modifications)

fatigue during cancer treatment and nearly a third report chronic fatigue that persists for years after treatment concludes. It significantly interferes with patients' daily activities and decreases their QOL. However, CRF is frequently under-recognized and under-treated, partly because of limited understanding of its pathophysiology and lack of effective interventions. Therefore, many patients with cancer use integrative therapies during and after cancer treatment, including treatments such as natural products (*e.g.* Chinese herbal medicines and supplements) and mind-body practices (*e.g.* yoga, mindfulness, and acupressure) (*12*). Based on the literature and the current authors' clinical experience, some individual Chinese herbal medicines (Figure 2) or formulas that are commonly prescribed by traditional Chinese physicians for the treatment of CRF are described here, along with clinical studies.

Asian Ginseng or *Panax ginseng* (Ren-Shen in Chinese or ginseng in Korea) is a well-known and popular Chinese herbal medicine that is believed to be the king of herbs in the Orient, and particularly in China, Korea, and Japan. It has been used for several thousand years with purported action as a tonic, prophylactic, and restorative agent for weakness and fatigue. Modern pharmacological studies have indicated that the main active components of *Panax ginseng* are ginsenosides, which have been found to have a variety of beneficial effects, including anti-inflammatory, antioxidant, and anticancer actions (*13*). An open-label preliminary study in which 30 patients with CRF took *Panax ginseng* 800 mg daily for 29 days was conducted to assess the safety of high-dose *Panax ginseng* for CRF (*14*). Results indicated that high-dose *Panax ginseng* was safe, it alleviated CRF, and it improved overall QOL, appetite, and sleep at night. In addition, a randomized, double blinded, placebo-controlled, parallel, multi-center trial in which patients with colorectal cancer who received mFOLFOX-6 were randomly assigned to take either Korean red ginseng 2,000 mg/day (n = 219) or a placebo (n = 219) for 16 weeks was conducted to evaluate whether Korean red ginseng could improve CRF compared to a placebo (*15*). Korean red ginseng is a processed form of Asian ginseng consumed as a powder after steaming and drying or concentrated and fermented after extraction with water or alcohol. The aforementioned study found that Korean red ginseng intake was more effective at alleviating fatigue compared to a placebo in patients with colorectal cancer receiving mFOLFOX-6 chemotherapy. Moreover, fatigue-related QOL and stress indices declined less from the baseline in the Korean red ginseng group than in the placebo group.

American ginseng or *Panax quinquefolius* (Xi-Yang-Shen in Chinese or Wisconsin ginseng in English) is also one of the most popular herbal medicines due to its purported actions against aging, cancer, stress, fatigue, and anxiety. It is native to eastern North America and widely inhabits several Canadian and US states. Along with ginsenosides, polysaccharides have been identified

as one of the major bioactive ingredients in American ginseng, and polysaccharides may possess paradoxical immunostimulatory and immunosuppressive properties (*16*). A multisite, double-blind randomized trial in which cancer survivors with fatigue (n = 364) took 2,000 mg of American ginseng vs. a placebo for 8 weeks was conducted to evaluate the efficacy of American ginseng on CRF (*17*). Results substantiated the benefit of American ginseng, 2,000 mg daily, in alleviating CRF over an 8-week period, and there were no discernible toxicities associated with the treatment. A retrospective review of medical records was conducted to evaluate the safety and effectiveness of combination therapy with methylphenidate (10-40 mg/day) and American ginseng (2,000 mg/day) (*18*). After about 30 days of follow-up, there was a significant reduction in the fatigue score in 60% of patients with no discernible associated toxicities, indicating that the combination of methylphenidate and American ginseng had a potential clinical benefit in treating CRF.

Astragali radix or *Astragalus* (Huang-Qi in Chinese) is a well-known herbal medicine with purported tonic properties that has been widely used to treat cancer and other immune disorders in China and Southeast Asia for thousands of years. Mounting evidence suggests that *Astragali radix* possesses diverse therapeutic activities including anti-cancer, anti-viral, anti-hyperglycemic, antioxidant, and immunomodulatory actions. It is usually prescribed to treat weakness, wounds, anemia, fever, multiple allergies, chronic fatigue, and loss of appetite, uterine bleeding, and uterine prolapse (*19*). Thus far, more than 100 compounds have been isolated and identified from *Astragali radix*; saponins, flavonoids, and polysaccharides are deemed to be the main bioactive constituents of *Astragali radix* that contribute to its anti-cancer and immunomodulatory actions (*20*). *Astragali radix* is one of the most frequently prescribed TCMs and a main component of formulas to treat chronic fatigue (*e.g.*, Bu-zhong-yi-qi-tang and Shi-quan-da-bu-tang). It has been widely used by TCM physicians as an adjuvant therapy to reduce symptoms and improve QOL and immunologic function in patients with various cancers including breast, gastric, liver, colon, and lung cancer (*5*). However, no randomized clinical trials have complied evidence of the efficacy of *Astragali radix* as an adjuvant therapy for the treatment of CRF except for those in Chinese databases. Therefore, well-designed clinical trials need to be conducted to provide more information for TCM physicians, researchers, and healthcare consumers.

Bu-zhong-yi-qi-tang (Hochuekki-to or TJ-41 in Japanese, or Bojungikki-Tang in Korean) is a long-standing formulation that has been widely used in China, Japan, and South Korea. It is purported to be a tonic for the treatment of weakness including fatigue, visceroptosis, gastrointestinal motility disorder, and rectal prolapse due to chronic diarrhea. Moreover, it has been described as an effective drug for the treatment of a spleen-*qi* deficiency in clinical TCM practice over the past few years. It contains 7 herbs including *Pinelliae rhizoma, Scutellaria baicalensis, Zingiberis rhizoma, Zizyphi fructus, Coptidis rhizoma, Glycyrrhizae radix,* and *Panax ginseng* (*6*). Recently, several pharmacological studies have indicated that Bu-zhong-yi-qi-tang has potent immunomodulatory, anticancer, and fatigue-reducing actions.

Li *et al.* conducted a study to investigate the frequency and forms of Chinese herbal medicine given to patients with lung cancer and the effect of Chinese herbal medicine on the probability of their survival in Taiwan (*21*). They indicated that the use of Chinese herbal medicine as an adjunctive therapy might reduce the mortality hazard ratio of patients with lung cancer, and Bu-zhong-yi-qi-tang was found to be the leading formula prescribed by traditional Chinese physicians for patients with lung cancer. A pilot randomized clinical trial suggested that Bu-zhong-yi-qi-tang might have beneficial effects on cancer-related fatigue and QOL in patients with cancer without any significant adverse effects (*22*). In comparison to the control group, patients administered Bu-zhong-yi-qi-tang for 2 weeks had decreased levels of fatigue improved scales for assessing overall general QOL. Minagawa *et al.* found that Bu-zhong-yi-qi-tang might be useful for management of general fatigue in patients with castration-resistant prostate cancer (CRPC) after the introduction of enzalutamide (*23*).

According to basic studies, the alleviation of CRF and anti-cancer action of Bu-zhong-yi-qi-tang might be attributed to activation of the immune system. It may modulate peripheral immunity and suppress the immune escape of tumors by increasing the infiltration of tumor lymphocytes, decreasing the expression of PD-1 in peripheral blood and reducing the infiltration of PD-1 and PD-L1 in tumors (*24*). In addition, Bu-zhong-yi-qi-tang alleviates neuroinflammation and oxidative stress in a mouse model of chronic fatigue syndrome by reducing the expression of IL-1β, IL-6, and IFN-γ in the hippocampus (*25*).

Shi-quan-da-bu-tang (Juzentaiho-to or TJ-48 in Japanese, or Sipjeondaebo-tang in Korean) is a well-known Chinese herbal formula purported to invigorate and to enhance health and immunity. There are 10 herbs in Shi-quan-da-bu-tang including *Ginseng radix, Astragaliradix, Angelicae radix, Rehmanniae radix, Atractylodis lanceae rhizoma, Cinnamomi cortex, Poriacocos, Paeoniaeradix, Ligustici rhizoma,* and *Glycyrrhizae radix* (*6*). It has been used for many years to treat various kinds of diseases such as anemia, rheumatoid arthritis, atopic dermatitis, chronic fatigue syndrome, and ulcerative colitis. Recently, Shi-quan-da-bu-tang has been found to have antitumor action by alleviating CRF and modulating immune responses in patients with cancer.

Because it purportedly treats the syndrome of a dual deficiency of *qi* and *blood* by balancing *Yin* and

Yang, Shi-quan-da-bu-tang is the third most commonly prescribed herbal medicine in South Korea. A pilot, randomized, double-blind, placebo-controlled, cross-over trial (registration number NCT02858856) has been conducted in South Korea by Cheon *et al.* since 2017 to evaluate the feasibility of Shi-quan-da-bu-tang for cancer-related fatigue, and it should provide meaningful data onthe treatment of CRF (*26*). As Shi-quan-da-bu-tang is commonly used by patients with lung cancer undergoing outpatient chemotherapy, patients being treated for non-small cell lung cancer (n = 16) completed a QOL questionnaire (*27*). Significant improvement inthe total QOL score was noted, mainly due to improvement in the patients' "physical condition." In addition, Kawai *et al.* indicated that Shi-quan-da-bu-tang combined with chemotherapy might reduce the incidence of adverse reactions, prevent nutritional disorders, and increase physical fitness, thereby improving the progression0free survival of patients with postoperative recurrence of non-small cell lung cancer (*28*).

2.2. Chronic Pain

Pain is the most common symptom of cancer at diagnosis and increases in prevalence throughout and beyond cancer treatment. Causes of cancer-related pain include the tumor itself or its metastases inflaming or eroding bone, viscera, or nerves, or pain related to tissue or nerve damage induced by cancer treatments such as surgery, chemotherapy, and radiation (*29*). Chronic pain can lead to a mood disturbance, dyspepsia, and poor QOL, in addition to the burden of a life-threatening disease. As indicated in current WHO guidelines, three-step analgesic ladder therapies are the standard of care for cancer pain. However, over half of all patients with cancer still suffer intolerable pain. Moreover, the inadequate management of chronic cancer-related pain has a significant harmful impact on the QOL for patients. Many patients suffer adverse effects from analgesic regimens, such as constipation, nausea, drowsiness, confusion, and hallucinations (*30*). Over the past few years, many clinical trials have suggested that TCM as adjunctive therapy increases the peripheral release of endogenous an algesica gents, reduces pain mediator secretion, and induces central nervous system (CNS) analgesia. Use of TCM to treat pain triggered by cancer is effective, economical, and causes fewer adverse reactions.

Acupuncture is a branch of TCM that modulates neurological processes to bring about an effect. Insertion of fine needles at acupuncture points can activate nerve fibers and peripheral afferent receptors, produce sensory interactions at various levels of the CNS, and release various transmitters, thus producing anti-inflammatory, neuroendocrine, and neuroimmune signals. Over the past few years, various acupuncture methods have been widely used in treating chronic cancer-related pain and adverse effects of cancer treatments. A recent survey found that 47.9% of patients with cancer were willing to undergo acupuncture if treatments were covered by insurance (*31*). A systematic review and meta-analysis including 14 randomized clinical trials with 920 patients indicated that acupuncture and/or acupressure was significantly associated with lower pain intensity in patients with cancer compared to a sham control, suggests a potential role for a combination of acupuncture and acupressure to help reduce opioid doses in patients with cancer (*32*). A single-blind, randomized controlled pilot trial involving 42 patients with moderate to severe cancer pain indicated that acupuncture at Si Guan Xue (Taichong (LR3) and Hegu (LI4)) and at commonly used acupoints including Neiguan (PC6), Zusanli (ST36), and Sanyinjiao (SP6) tended to be effective in reducing cancer pain (*33*). A recent meta-analysis has identified three well-designed randomized clinical trials that corroborate the use of acupuncture for aromatase inhibitor-associated arthralgia and musculoskeletal symptoms (*34*). Moreover, a systematic review indicated that interventions including acupuncture/acupressure, tai chi/qi gong, hypnosis, meditation, music therapy, yoga, massage, reflexology, and Reiki may alleviate cancer-related pain in patients with breast cancer (*35*).

Some studies have indicated that a warm compress of Chinese medicine on the back meridians relieves cancer pain, reduces doses of and adverse reactions to adjuvant analgesics, and improves QOL. Wen Jing Zhi Tong Fang is a Chinese herbal medicine first documented in the Qing Dynasty and consisting of *Evodia rutaecarpa, Semen sinapis, Ephedra sinica,* and As *arum sieboldii* . Its use on back meridians combined with a WHO 3-step analgesic ladder treatment was effective at relieving cancer-related pain (*36*).

In summary, TCM interventions appear to have beneficial effects on cancer-related pain. However, studies on TCM have several limitations such as indeterminate results, small sample sizes, and limited examination of outcomes. Therefore, further studies with a rigorous design and larger sample size need to be conducted to re-evaluate the effectiveness of TCM in treating cancer related pain.

2.3. Cancer-related anorexia/cachexia

Cancer-related anorexia/cachexia is defined as a metabolic, paraneo plastic syndrome characterized by decreased food intake, involuntary weight loss, and loss of fat and muscle (*37*). It is one of the most prevalent and troublesome clinical problems experienced by patients with cancer during and after therapy, it can adversely influence the nutritional status of patients, negatively impact patients' QOL and increase the burden on healthcare resources. Metabolic abnormalities, inflammation, insulin resistance, and increased muscle protein breakdown are often associated with cachexia.

The management of cancer-related anorexia/cachexia is a complex challenge that should address the different causes underlying this clinical event. Among effective treatments, progestogens such as megestrol and medroxyproge sterone are currently considered to be the best available treatment option. Some other drugs including thalidomide, cytokine inhibitors, steroids, nonsteroidal anti-inflammatory drugs, branched-chain amino acids, eicosapentaenoic acid, and anti-serotoninergic drugs have been proposed and used in clinical trials (*38*). Recent studies have indicated that integrated, multi-targeted approaches are more effective than single-agent approaches for the treatment of this syndrome. Some Chinese herbal medicines and herbal remedies including *Ginseng, Astragali radix*, Shi-quan-da-bu-tang (TJ-41), Bu-zhong-yi-qi-tang (TJ-48), and Huang-qin-tang (PHY906) and acupuncture therapy might be effective in improving QOL by treating anorexia in patients with cancer (*39*).

A pilot, randomized, double-blind, placebo-controlled trial (n = 32) by Cheon *et al.* found that Shi-quan-da-bu-tang had a potential benefit in terms of anorexia management for patients with cancer (*40*). Compared to the baseline, 4 weeks of Shi-quan-da-bu-tang treatment improved the QOL as assessed with the Functional Assessment of Anorexia/Cachexia Therapy (FAACT) and alleviated anorexia in patients with cancer. A randomized, controlled trial, pilot study (n = 40) was conducted by Ko *et al.* to estimate the efficacy and the safety of Yukgunja-tang (also known as Liu-jun-zi-tang in China and Rikkunshito (TJ-43) in Japanese), and to compile evidence for the use of herbal medicines in the management of cancer-related anorexia (*6,41*). Results indicated that the FAACT scores and the anorexia/cachexia subscale (ACS) scores differed significantly (P = 0.023) between the control and the treatment groups, but there were no significant differences in the scores on avisual analog scale (VAS) for appetite or in the levels of leptin, TNF-α, IL-6, and ghrelin, indicating the efficacy and safety of using Liu-jun-zi-tang as a treatment option for patients with cancer-related anorexia.

2.4. Insomnia

Sleep is essential for health while insomnia or poor sleep is consistently linked to the development of systemic disease, including depression, metabolic syndrome, and cognitive impairments. Recently compiled evidence has suggested the role of sleep in cancer initiation and progression, and especially in breast cancer (*42*). Insomnia is common among patients with cancer, occurring in approximately 30% to 50% of the cancer population, and manifesting as poor sleep, circadian misalignment, hypersonic, somnolence syndrome, hot flushes, and nightmares. These problems can lead to fatigue, mood disturbances, and contribute to immune-suppression, which can have a profound impact on QOL and perhaps affect the course of disease (*43*). Evidence suggests that management of insomnia through a combination of pharmacologic and non-pharmacologic means can have a positive impact not only on insomnia but also on related symptoms and, consequently, on overall health and QOL. Currently, treatment for insomnia includes cognitive behavioral therapy with sleep hygiene, bright-light therapy, exercise, yoga, melatonin, and hypnotic medications (*44*). However, these non-pharmacologic treatments still need to be further verified specifically in patients with cancer, while long-term use of benzodiazepine and non-benzodiazepine hypnotic agents is associated with some risks such as dizziness, headache, forgetfulness, bitter mouth, fatigue, withdrawal reaction, lethargy, hangover, and falls. Recently, TCM has begun to garner the attention of clinicians as an alternative for insomnia.

According to the theory of TCM, the heart ("Xin" in Chinese Pinyin) is considered to be closely related to the occurrence of insomnia. TCM Yang-xin-an-shen therapy, which purports to be a therapy to tranquilize the mind by nourishing the heart, is one of the crucial therapeutic principles for insomnia in TCM; TCM includes several Chinese herbal prescriptions that purportedly nourish the heart and tranquilize the mind (*45*). A comprehensive meta-analysis of 12 trials (1,549 participants) was conducted by Li *et al.* to evaluate the efficacy and safety of Yang-xin-an-shen therapy for insomnia (*46*). They found that Yang-xin-an-shen therapy was superior to a placebo in terms of polysomnography (PSG) parameters, the Pittsburgh Sleep Quality Index (PSQI) scale, TCM curative efficacy, and PSQI curative efficacy. Moreover, 42 Chinese herbal medicines were used to treat insomnia in that meta-analysis. *Semen Ziziphi Spinosae* (Suan Zao Ren) was used most frequently, followed by *Polygalae radix* (Yuan Zhi), *Caulis Polygoni Multiflori* (Ye Jiao Teng), *Glycyrrhizae radix* (Gan Cao), *Poriacocos* (Fu Ling), *Angelica sinensis* (Dang Gui), *Platycladi semen* (Bai Zi Ren), *Rehmannia glutinosa* (Di Huang), *Schisandra chinensis* (Wu Wei Zi), and *Radix Salviae Miltiorrhizae* (Dan Shen).

Suan Zao Ren, Ye Jiao Teng, Yuan Zhi, Fu Ling, Gan Cao, Dang Gui, Bai Zi Ren, Di Huang, and Wu Wei Zi have frequently been used to treat insomnia in TCM. Suan Zao Ren and Ye Jiao Teng are categorized as Anshen herbals in TCM and are designated as sovereign or minister herbals. Suan Zao Ren, a well-known Chinese herbal medicine, has been used to treat insomnia for thousands of years. It contains complex mixtures of phytochemicals including sanjoinine A, Jujuboside A, spinosin, and other flavonoids that have sedative and hypnotic actions primarily mediated by the GABAergic and serotonergic system (*47*). Zhou *et al.* collected and analyzed high-quality randomized clinical trials on the treatment of insomnia with formulations containing Suan Zao Ren and found that they were an effective replacement therapy for insomnia (*48*).

3. TCM to treat adverse reactions and complications of chemotherapy or radiotherapy

Chemotherapy and radiotherapy are major conventional cancer therapies and greatly promote the survival of patients; however, these treatments typically affect multiple organ systems including the gastrointestinal tract, heart, liver, kidneys, marrow, skin, peripheral nerves, and blood vessels. Adverse reactions may be acute (occurring within few weeks after therapy), intermediate, or late (occurring months or years after therapy). Nausea, vomiting, constipation, diarrhea, hair loss, cardiac injury, bone marrow suppression, liver and kidney dysfunction, and peripheral neuropathy symptoms are common adverse reactions and complications during chemotherapy, whereas radio therapy though administered locally can produce systemic adverse reactions and complications such as radiotherapy pneumonitis, pharyngitis, esophagitis, laryngitis, persistent dysphagia, fatigue, hepatotoxicity, infertility, and cognitive deficits (*5,49*). These complications and adverse reactions inconvenience and cause discomfort to patients and they may also limit or prevent delivery of therapy at its optimal dose and time, potentially causing fatalities. Febrile neutropenia is a life-threatening condition that requires immediate attention, and especially in patients with chemotherapy-related neutropenia, while cardiovascular disease is the most common potentially life-threatening later on (*5,50*). Thus, more effective therapies to help prevent and control complications and adverse reactions to conventional cancer therapies must soon be developed. Some TCMs have been found to be adjuncts of cancer therapies. TCM combined with chemotherapy or radiotherapy can improve clinical efficacy and the Karnofsky Performance Scale (KPS) score, as well as improve patients' QOL and reduce adverse reactions caused by cancer therapies. The current review will now briefly describe clinical studies outlining the use of TCM to reduce some complications and adverse reactions associated with conventional cancer therapies (Table 5).

3.1. Adverse gastrointestinal reactions

Adverse gastrointestinal reactions including loss of appetite, diarrhea, nausea, vomiting, and constipation, are the most common symptoms occurring in patients with cancer receiving chemotherapy or radiotherapy. However, there is still no effective treatment to alleviate these symptoms in patients with cancer. Many clinical trials have recently suggested that some Chinese herbal medicines and acupuncture and other alternative therapies may be effective at treating adverse gastrointestinal reactions.

A study by Wang *et al.* indicated that acupuncture at Zusanli (ST36), Zhongwan (CV12), and Neiguan (PC6) combined with the slow intravenous injection of

Table 5. Clinical trials of Chinese herbal medicines as adjuvant therapy to alleviate adverse reactions to and complications of cancer therapies

Symptoms	Chinese herbal medicines	Sample	Exposure	Outcome	Ref.
Adverse gastrointestinal reactions	Sheng-jiang-xie-xin-tang	$n = 115$	Sheng-jiang-xie-xin-tang administered from 1 day prior to chemotherapy to 6 daysafter chemotherapy	Significantly reduced irinotecan-induced hematological and gastrointestinal toxicities in patients with the UGT1A1*28 or *6 polymorphism.	*56*
Bone marrow suppression	Fufang E-Jiao Jiang	$n = 64$	Fufang E-Jiao Jiang + chemotherapy + rhIL-11 + rhG-CSF	Relieved myelosuppression caused by chemotherapy and increased white cell and platelet counts.	*56*
Cardiotoxicity	Zhi-Gan-Cao-Tang	A case report	Zhi-Gan-Cao-Tang combined with anthracycline compounds	Chest X-ray: substantial alleviation of pulmonary edema and cardiomegaly.	*64*
CIPN	AC591	$n = 72$	AC591 combined with chemotherapy	Prevented oxaliplatin-induced neuropathy without reducing its antitumor activity.	*69*
Radiation pneumonitis	Aidi injection	$n = 1,192$	Aidi injection combined with radiotherapy	Alleviated radiation pneumonitis, radiation esophagitis, and myelosuppression caused by radiotherapy.	*75*
Adverse reactions to EGFR-TKIs	TJ-14	$n = 29$	TJ-14 + minocycline + afatinib in patients with non-small cell lung cancer	Effectively reduced the incidence of an afatinib-induced skin rash, paronychia, diarrhea, and oral mucositis.	*82*

Note: Abbreviations: chemotherapy-induced peripheral neuropathy (CIPN); Huangqi Guizhi Wuwu decoction (AC591); epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs); Ban-xia-xie-xin-tang (TJ-14).

tropisetron hydrochloride prevented and treated vomiting induced by chemotherapy for lung cancer, with a significantly improved digestive reaction score and KPS score (*51*). A study by Li *et al.* indicated that moxibustion at Baihui (GV20) and Zhongwan (CV12) combined with a 5-HT receptor antagonist was better than a 5-HT receptor antagonist alone at markedly reducing the incidence and severity of nausea and vomiting caused by chemotherapy with cisplatin in patients with lung cancer without causing obvious adverse reactions (*52*).

Sheng-jiang-xie-xin-tang is a classical traditional Chinese herbal formulation documented in "ShangHan Lun" for treating digestive system diseases. Sheng-jiang-xie-xin-tang includes 8 herbs: Sheng Jiang (fresh *Zingiberis Rhizoma*), Gan Cao (*Glycyrrhizae radix*), Dang Shen (*Codonopsis radix*), Gan Jiang (*ZingiberisRhizoma*), Huang Qi (*Astragali radix*), Ban Xia (*Pinelliae rhizoma*), Huang Lian (*Coptidis Rhizoma*), and Da Zao (*Jujubae fructus*). Deng *et al.* indicated that Sheng-jiang-xie-xin-tang significantly reduced irinotecan-induced hematological and gastrointestinal toxicities in patients with the UGT1A1*28 or *6 polymorphism (high risk group) without affecting the clinical response tochemotherapy (*53*).

Xiang-sha-liu-jun-zi-tang was created by Yunbo Ke, a doctor of Chinese medicine in the Qing Dynasty. Xiang-sha-liu-jun-zi-tang is a classic formula of TCM. Modern studies have indicated that Xiang-sha-liu-jun-zi-tang can be used to treat nausea, vomiting, abdominal distension, and diarrhea. Xiao *et al.* devised a protocol for systematic review and meta-analysis to determine the effectiveness of Xiang-sha-liu-jun-zi-tang in the treatment of chemotherapy-induced nausea and vomiting (CINV) (*54*). To investigate the effects of TCM on CINV, Lv *et al.* conducted a review including 92 clinical trials and 3,778 patients with different typesof cancer, chemotherapy regimens, prescriptions, durations of treatment, and combinations with Western medicines (*55*). They found that the most frequently used herbs were *Pinelliae rhizoma* (Ban Xia), *Glycyrrhizae radix* (Gan Cao), *Poriacocos* (Fu Ling), and *Atractylodis Macrocephalae rhizoma* (Bai Zhu)to purportedly regulate the flow of *qi* and remove phlegm and dampness in the stomach and spleen. Although all of the reported trials are randomized open trials, the curative effects range from 55.81% to 100%.

3.2. Bone marrow suppression

Bone marrow suppression is a reduction in the activity of bone marrow, resulting in decreased numbers of red blood cells, platelets, and white blood cells. One of the most common reasons for a patient to have this condition is chemotherapy or radio therapy to treat cancer. When the bone marrow is functioning below normal levels, the patient is at risk and needs to be monitored very closely. In some cases, hospitalization is recommended for people with bone marrow suppression until their bone marrow is functioning normally. Over the past few years, some Chinese herbal medicines, herbal remedies and acupuncture and other alternative therapies have been reported to have beneficial effects on chemotherapy or radiotherapy-related bone marrow suppression.

Colla corii asini (or E-Jiao in Chinese), donkey hide gelatin prepared by stewing and concentrating *Equus asinus* L. donkey hide, is a health food and TCM widely used for rejuvenation and to treat anemia for more than 2,000 years in China (*5*). Many studies have indicated that E-Jiao and its preparations such as Fufang E-Jiao Jiang may effectively promote the recovery of bone marrow hemopoietic function in cancer patients with myelosuppression. Fufang E-Jiao Jiang in combination with conventional interleukin-11 (rhIL-11)and recombinant human granulocyte colony stimulating factor (rhG-CSF) in patients with cancer significantly alleviated the myelosuppression caused by a GP (Gemcitabine + DDP) chemotherapy regimen and increased the white cell and platelet counts compared to rhIL-11 and rhG-CSF alone (*56*). Moreover, Fufang E-Jiao Jiang was found to clearly promote the recovery of bone marrow hemopoietic function in a mouse model of myelosuppression (*57*). This action may be attributed to (*i*) improvement in the bone marrow hematopoietic microenvironment; (*ii*) facilitation the proliferation and preventingthe apoptosisof blood and bone marrow nucleated cells (BMNC); and (*iii*) stimulation of the expression of IL-1β, IL-3, IL-6, SCF, and GM-CSF and inhibition of the expression of TGF-β.

A systematic review and meta-analysis by Chen *et al.* indicated that acupoint stimulation has immunomodulatory action in patients with lung cancer, as evinced by a significant increase in IL-2, CD3+ and CD4+ T cells, and NK cells but not CD8+ T cells (*58*). Further analysis also revealed that acupoint stimulation markedly reduces the bone marrow suppression induced by conventional therapy, it increases hemoglobin levels and platelet counts in patients with lung cancer, and it decreases chemotherapy-induced nausea and vomiting. Zusanli (T36), Neiguan (PC6), Geshu (BL17), Feishu (BL13), Guanyuan (RN4), and Shenshu (BL23) were most frequently used acupoints.

3.3. Cardiotoxicity

Cardiotoxicity is a significant complication of various cancer treatments that can negatively impact QOL and prognosis in cancer survivors. Several anticancer agents, including anthracyclines, trastuzumab, alkylating agents, and antimetabolites that have been in use for decades as well as recently introduced anticancer therapies such as tyrosine kinase inhibitors, angiogenesis inhibitors, checkpoint inhibitors, and proteasome inhibitors are associated with significant cardiotoxicity (*59*). These treatments can cause multiple forms of cardiotoxicity

including arrhythmia, pericardial disease, valvular dysfunction, and myocardial ischemia. In order to optimize outcomes for patients with cancer and cardiovascular disease existing prior to cancer treatment or developing as a consequence of that treatment, a new discipline called "cardio-oncology" has evolved over the past few years (*60*). Close monitoring for cardiotoxicity and enhanced cardiac management are recommended for patients who receive potentially cardiotoxic therapies, and especially for patients with high-risk factors for serious cardiotoxicity. Through the use of certain β-blockers, ACEI inhibitors, angiotensin receptor blockers, and other drugs for symptomatic treatment, adverse reactions to radiotherapy and chemotherapy can be partially alleviated. In addition, over the past few years, some Chinese herbal medicines have been reported to have beneficial effects on cancer treatment-induced cardiotoxicity.

Dan *et al.* conducted a study to explore the material basis and the rationale of TCM to treat antineoplastic drug-induced cardiotoxicity based on network pharmacology and data mining (*61*). In that study, five core herbs including *Corydalis rhizoma* (Yan Hu Suo), *Uncaria rhynchophylla* (Gou Teng), *Phellodendri cortex* (Huang Bai), *Forsythia fructus* (Lian Qiao), and *Glycyrrhizae radix* (Gan Cao) were identified in the target-compound-herb network, and these herbs might have the potential to mitigate anti-neoplastic drug-induced cardiotoxicity. Corydalisrhizoma is the herb with the most therapeutic potential, and a modern pharmacological study has indicated that it acts against arrhythmia, myocardial infarction, coronary artery dilation, tumors, and thrombosis (*62*).

Cui *et al.* performed a network pharmacology analysis and compiled experimental evidence to investigate potential protection from cisplatin-induced cardiotoxicity provided by Tongmai Yangxin pills (*63*). They indicated that Tongmai Yangxin pills regulate the cardiomyocyte free radical balance and reduce apoptosis *via* the Nrf2/HO-1 pathway and the p38-MAPK pathway, meaning that the medicine might be used to treat platinum chemotherapy-induced cardiac injury. Tongmai Yangxin pills, a traditional Chinese formulation, have been widely used to treat coronary heart disease for several decades. Tongmai Yangxin pills, which consist of the classic formula "Zhi-gan-cao-tang", and "Sheng-mai-san," and the active ingredients of the sovereign drugs are mostly flavonoids, saponins, and lignans that have anti-oxidative stress, anti-inflammatory, and anti-tumor actions.

Zhi-gan-cao-tang is an herbal formula documented in "Shang-Han-Lun" to purportedly supplement Yang-Qi, nourish the Ying-blood, and strengthen the heart spirit to relieve heart failure-related symptoms. Zhi-gan-cao-tang is reported to be the Chinese herbal formula most frequently prescribed by TCM practitioners to treat heart failure. In the case of an 18-year-old adolescent male with refractory acute lymphoblastic leukemia (ALL), anthracycline-induced cardiotoxicity gradually resolved following the administration of modified Zhi-gan-cao-tang (*64*). After 2 months of treatment with Zhi-gan-cao-tang, a follow-up chest X-ray revealed substantial alleviation of pulmonary edema and cardiomegaly.

Sheng-mai-san is a well-known TCM formula to treat coronary heart disease with a 3,000-year-old history. Sheng-mai-san consists of three herbs: *Ginseng radix* (Ren Shen), *Ophiopogonis radix* (Mai Dong) and *Schisandrae fructus* (Wu Wei Zi). It has been developed into several TCMs to meet the demands of different patients including a Sheng-mai Oral Solution (Sheng-mai-yin), Sheng-mai Capsules, and a Shen-mai injection (*65*). These TCMs are widely used to treat heart failure, myocardial infarction, cardiogenic shock, and cardiotoxicity in China. A series of animal studies have reported that the Sheng-mai preparation may increase glutathione peroxidase (GSH-Px) activity, superoxide dismutase (SOD) activity, and Ca^{2+}-ATP enzyme activity and enhance the ultra structure of myocardial tissue in rats with doxorubicin-induced cardiac injury (*66*). However, few clinical trials have assessed the effectiveness and safety of the Sheng-mai preparation at treating anti-neoplastic drug-induced cardiotoxicity. Further rigorously designed large-scale trials are warranted to verify the merits of the Sheng-mai preparation.

3.4. Peripheral neuropathy

Chemotherapy-induced peripheral neuropathy (CIPN) is a common significant and debilitating adverse reaction to the administration of neurotoxic chemotherapeutic agents. These pharmacy-chemotherapeutics can include taxanes, vinca alkaloids, and platinum analogues. Approximately 30% to 40% of patients receiving neurotoxic chemotherapy will develop CIPN (*67*). Moderate to severe CIPN significantly decreases the QOL and physical abilities of patients with cancer. However, the pathogenesis of CIPN in not fully understood, and there are no drugs that are effective at preventing CIPN. Recently, many clinical trials have suggested that some Chinese herbal medicines and acupuncture and other alternative therapies may be effective at treating CIPN.

Huang-qi-gui-zhi-wu-wu-tang is an herbal formula documented in the "Synopsis of the Golden Chamber" for alleviating limb numbness and pain. It consists of five herbs: *Hedysarum Multijugum Maxim* (Huang Qi), *Cinnamomi Ramulus* (Gui Zhi), *Paeoniae radix alba* (Bai Shao), *Zingiber officinale Roscoe* (Sheng Jiang), and *Jujubae fructus* (Da Zao). Currently, Huang-qi-gui-zhi-wu-wu-tang is mainlyused to treat hand-foot syndrome, CIPN, diabetic peripheral neuropathy, and rheumatoid arthritis. Gu *et al.* used the network pharmacology approach to investigate the potential pathogenesis of

CIPN and the therapeutic mechanisms by which Huang-qi-gui-zhi-wu-wu-tang treated CIPN (*68*). They indicated that the main pathologies of CIPN might involve the inflammatory response and nerve injury and that Huang-qi-gui-zhi-wu-wu-tang played a therapeutic role in CIPN by regulating the inflammatory response and repairing nerve injury, thus verifying the reliability and efficacy of this herbal formula.

AC591 is a standardized extract of Huang-qi-gui-zhi-wu-wu-tang. Pretreatment with AC591 may reduce oxaliplatin-induced cold hyperalgesia, mechanical allodynia, and morphological damage to the dorsal root ganglion. Microarray analysis indicated the neuroprotective action of AC591 depended on the modulation of multiple molecular targets and pathways involved in the down-regulation of inflammation and immune response. In summary, AC591 may prevent oxaliplatin-induced neurotoxicity without reducing its antitumor activity, and it might be a promising adjuvant to alleviate sensory symptoms in clinical practice (*69*).

A pilot study was conducted by Schroeder *et al.* to evaluate the therapeutic effect of acupuncture on CIPN as measured by changes in nerve conduction studies (NCSes) in patients treated with acupuncture (*70*). Each patient received a standard 10-week treatment at ST34 (Liang Qiu) as well as at EX-LE12 (Qi Duan) and EX-LE8 (Ba Feng). Results suggested that acupuncture had a positive effect on CIPN as measured by objective parameters (NCSes); a therapeutic intervention with acupuncture over a period of 10 weeks alleviated the symptoms of CIPN and also induced a normalization of histological morphology. Acupuncture might increase the blood flow in the limbs. Increased blood flow to the vasa nervorum and dependent capillary beds supplying the neurons may contribute to nerve repair with measurable improvement of axons or myelin sheaths (*71*).

3.5. Radiation pneumonitis

Radiation pneumonitis is a well-known complication of thoracic radiation for patients with breast, lung, thymic, and esophageal malignancies and mediastinal lymphomas (*72*). It typically presents 1-6 months after radiation therapy. The clinical features usually include a mild dry cough, a mild fever, and mild dyspnea, but in some cases, severe respiratory failure appears and leads to death. The incidence of moderate to severe radiotherapy pneumonitis is 10-20%. When radiation pneumonitisis left untreated for a long time, it may develop into pulmonary fibrosis, which has a high rate of mortality. Despite great efforts to develop agents to reduce the severity and incidence of pulmonary toxicities caused by radiotherapy, no effective agents currently exist. General supportive management, mobilization of airway secretions, anti-inflammatory therapy, and management of acute exacerbation are the treatment options. Over the past few years, some TCMs have been reported to have beneficial effects on radiotherapy-related radiation pneumonitis.

To evaluate the efficacy and safety of herbal medicines as adjunctive therapy for the prevention of radiation pneumonitis in patients with lung cancer undergoing radiotherapy, a systematic review involving 22 randomized clinical trials and 1,819 participants was conducted by Kim *et al.* (*73*). They indicated that administration of herbal medicines during radiotherapy may prevent or minimize the risk of radiotherapy pneumonia. Because radiation therapy is considered to be a heat toxin pathogen according to TCM theory, prescriptions generally focus on removing heat. Thus, the herbal formulas used with radiotherapy mainly consist of herbs that purportedly nourish *Yin* such as *Ophiopogonis radix* (Mai Dong) and *Adenophorae radix* (Sha Shen), coupled with *qi*-tonifying herbs such as *Astragali radix* (Huang Qi). To evaluate the benefits of Chinese herbal prescriptions containing *Astragali radix* in combination with radiotherapy for non-small cell lung cancer, a meta-analysis of 29 studies and 2,547 individuals was conducted by He *et al.* (*74*). They indicated that Chinese herbal prescriptions containing *Astragali radix* increased the effectiveness andreduced the toxicity of radiotherapy.

An Aidi injection (Z52020236, China Food and Drug Administration (CFDA)) is an adjuvant chemotherapy drug commonly used in China consisting of extracts from *Astragali radix*, *Eleutherococcus senticosus*, *Panax ginseng*, and *Cantharidin*. It appears to have antitumor and immunoregulatory activity and it attenuates acute or subacute toxicity induced by chemotherapy (*75*). An Aidi injection plus radiotherapy may significantly improve the clinical efficacy of chemotherapy and the QOL of patients with lung cancer. Moreover, it may alleviate myelosuppression, radiation pneumonitis, and radiation esophagitis.

In summary, there is some encouraging evidence that administration of herbal medicines in combination with radiotherapy may benefit patients with thoracic cancer by preventing or minimizing radiation pneumonitis. However, due to the poor methodological quality of the available studies, a definitive conclusion cannot be reached. Rigorously designed large-scale trials are warranted to verify the merits of this approach.

4. TCM for adverse effects of targeted drugs

When targeted drugs and especially EGFR-TKIs (*e.g.* erlotinib gefitinib, icotinib, osimertinib, and dacomitinib) are widely used, the adverse effects of thosetreatments including acneiform eruptions, paronychia, xerosis, mucositis, and alopecia are thought to be less severe but can still be significant (*76*). Not only can these toxicities severely affect patients' QOL, but in some specific instances they can be associated with increased sensitivity to therapy. The incidence of acneiform eruptions is about 47-100% (grade 3/4 1-10%) in patients

receiving EGFR-TKIs. They usually appear within 1 to 3 weeks after EGFR-TKI administration and peak within3 to 5 weeks.

According to TCM theory, EGFR-TKI-associated adverse effects are categorized as "drug toxicities," and their pathogenesis purportedly involves wind, dampness, and heat invading the lungs due to a deficiency. Thus, the basic principle of treatment is dispelling wind and dampness, promoting eruptions and itching, clearing heat-toxins and cooling the blood, nourishing yin and blood, and moistening dryness. Some herb combinations are commonly prescribed by TCM practitioners for skin toxicity (*76*). *Schizonepetae herba* (Jing Jie) and *Lonicerae flos* (Fang Feng) are combined to dispel wind. *Schizonepetae herba* (Jin Yin Hua) and *Forsythiae fructus* (Lian Qiao) are combined to clear heat toxins. *Moutan cortex radicis* (Mu Dan Pi) and *Paeoniae radix rubra* (Chi Shao) are combined to clear heat and cool the blood. *Taraxaci herba* (Pu Gong Ying) is widely used to remove toxins to reduce swelling in the eventof a secondary infection. *Dictamni cortex* (Bai Xian Pi), and *Sophorae flavescentis radix* (Ku Shen) are used to promote diuresis and itching if pruritus is a cardinalsymptom. Deng *et al.* conducted a meta-analysis including 22 studies involving16 prescriptions and 50 herbal medicines to evaluate the effect of TCM on EGFR-TKI-induced rashes, and results suggested that TCM may significantly relieve EGFRI-induced rashes and symptoms and improve patients' QOL (*77*). Herbs with purported cold properties and a bitter flavor that clear heat were used most frequently; *Lonicera Japonica flos* was used in 68.42% of studies on rashes and 66.67% of studies on hand-foot skin reactions while *Sophorae flavescentis radix* was used in 52.63% of studies on rashes. However, few studies outside of Asia have examined the effects of these Chinese herbal medicines on adverse effects of targeted drugs. Moreover, due to the poor methodological quality of the available studies, a definitive conclusion cannot be reached.

TJ-14 (Hangeshashinto in Japanese or Ban-xia-xie-xin-tang in Chinese), a famous herbalformula documented in "Shang-Han-Lun," has been used empirically to treat various gastrointestinal disorders such as dyspepsia, gastroenteritis, gastrasthenia, and oral mucositis for thousands of years in China, Japan and other East Asia countries. It consists of seven herbal extracts including *Coptis rhizoma* (Huang Lian), *Panax ginseng* (Ren Sen), *Glycyrrhizae radix* (Gan Cao), *Jujubae fructus* (Da Zao), *Pinelliae rhizoma* (Ban Xia), *Zingiber officinale* (Ginger) (Sheng Jiang), and *Scutellariae radix* (Huang Qin). Some studies have indicated that TJ-14 might possess antioxidant, anti-inflammatory, bactericidal, and analgesic actions and promote healing (*78*). Over the past few years, TJ-14 has been reported to attenuate adverse reactions to cancer treatment. It is effective against chemotherapy-induced diarrhea and oral mucositis and radiation-induced enteritis because it suppresses PGE2 or COX-2 (*79-81*). Moreover, Ichiki *et al.* conducted a single-arm phase II study to evaluate the prophylactic effects of TJ-14 plus minocycline on afatinib-induced diarrhea and skin rashes in patients with non-small cell lung cancer (*82*). They found that TJ-14 plus minocycline effectively reduced the incidence of afatinib-induced skin rashes, paronychia, diarrhea, and oral mucositis. However, a higher level of evidence from trials with a large sample size is required to verify the efficacy of TJ-14 in treating adverse reactions to EGFR-TKIs.

5. Conclusion

In conclusion, TCM, especially Chinese herbal medicines and acupuncture as adjunctive therapies, has played a positive role in the treatment of various types of cancers. TCM not only alleviates the symptoms of patients with cancer and improves their QOL, but it also diminishes the adverse reactions to and complications caused by chemotherapy, radiotherapy, or targeted-therapy. It can effectively alleviate adverse gastrointestinal reactions to these anti-cancer therapies including diarrhea, nausea, and vomiting, decrease the incidence of bone marrow suppression, alleviate cardiotoxicity, and protect against chemotherapy-induced peripheral neuropathy and radiation-induced pneumonitis. Moreover, TCM can alleviate EGFR-TKI-related acneiform eruptions, diarrhea, and other adverse reactions. In summary, this review should contribute to an understanding of TCM as adjuvant therapy for cancer and provide useful information for the development of more effective anti-cancer therapies.

Although TCM is currently receiving increasing attention worldwide as an alternative and complementary treatment for cancer therapy, published studies have several limitations that should be taken seriously by TCM practitioners: (*i*) The complexity of TCM theory and prescriptions hampers a full and complete study of TCM with a high level of repeatability and definite findings; (*ii*) Most of the current studies have mainly focused on efficacy instead of the systematic and in-depth pharmacological actions of medicines; (*iii*) Clinical studies of TCM lack large-samples, involvement of multiple centers, randomized controls, and a comparison of efficacy, resulting in questions about their scientific validity; and (*iv*) The clinical effects of herbs are influenced by many factors including species, cultivation, time of harvest, and processing, that can markedly increase uncertainty in active ingredient content (*83*). In addition, many people currently believe that Chinese herbal medicines are safe because they come from natural products (*84*). Therefore, TCM practitioners have a duty to advise patients on the toxicity and safety of Chinese herbal medicines. In summary, more rigorously designed trials involving cancer treatment must be conducted in the future, including complete quality

control and standardized models at the cellular, organic, animal, and clinical levels, in order to study TCM in multiple forms and at multiple levels.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68:394-424.
2. World Health Organization. Projections of mortality and causes of death, 2016 to 2060. *https://www.who.int/healthinfo/global_burden_disease/projections/en/* (accessed March 16, 2021).
3. Cao M, Li H, Sun D, Chen W. Cancer burden of major cancers in China: A need for sustainable actions. Cancer Commun (Lond). 2020; 40:205-210.
4. Hulvat MC. Cancer Incidence and Trends. Surg Clin North Am. 2020; 100:469-481.
5. Wang Z, Qi F, Cui Y, Zhao L, Sun X, Tang W, Cai P. An update on Chinese herbal medicines as adjuvant treatment of anticancer therapeutics. Biosci Trends. 2018; 12:220-239.
6. Qi F, Zhao L, Zhou A, Zhang B, Li A, Wang Z, Han J. The advantages of using traditional Chinese medicine as an adjunctive therapy in the whole course of cancer treatment instead of only terminal stage of cancer. Biosci Trends. 2015; 9:16-34.
7. Wang Y, Zhang Q, Chen Y, Liang CL, Liu H, Qiu F, Dai Z. Antitumor effects of immunity-enhancing traditional Chinese medicine. Biomed Pharmacother. 2020; 121:109570.
8. Qi F, Li A, Inagaki Y, Gao J, Li J, Kokudo N, Li XK, TangW. Chinese herbal medicines as adjuvant treatment duringchemo- or radio-therapy for cancer. BiosciTrends. 2010; 4:297-307.
9. Mohandas H, Jaganathan SK, Mani MP, Ayyar M, Rohini Thevi GV. Cancer-related fatigue treatment: An overview. J Cancer Res Ther. 2017; 13:916-929.
10. Chung VCH, Wu X, Lu P, Hui EP, Zhang Y, Zhang AL, Lau AYL, Zhao J, Fan M, Ziea ETC, Ng BFL, Wong SYS, Wu JCY. Chinese Herbal Medicine for Symptom Management in Cancer Palliative Care: Systematic Review and Meta-analysis. Medicine (Baltimore). 2016; 95:e2793.
11. Berger AM, Mooney K, Alvarez-Perez A, *et al.* Cancer-Related Fatigue, Version 2.2015. J Natl Compr Canc Netw. 2015; 13:1012-1039.
12. Arring NM, Barton DL, Brooks T, Zick SM. Integrative Therapies for Cancer-Related Fatigue. Cancer J. 2019; 25:349-356.
13. Kiefer D, Pantuso T. *Panax ginseng*. Am Fam Physician. 2003; 68:1539-1542.
14. Yennurajalingam S, Reddy A, Tannir NM, Chisholm GB, Lee RT, Lopez G, Escalante CP, Manzullo EF, Frisbee Hume S, Williams JL, Cohen L, Bruera E. High-Dose Asian Ginseng (*Panax Ginseng*) for Cancer-Related Fatigue: A Preliminary Report. Integr Cancer Ther. 2015; 14:419-427.
15. Kim JW, Han SW, Cho JY, *et al.* Korean red ginseng for cancer-related fatigue in colorectal cancer patients with chemotherapy: A randomised phase III trial. Eur J Cancer. 2020; 130:51-62.
16. Ghosh R, Bryant DL, Farone AL. *Panax quinquefolius* (North American Ginseng) Polysaccharides as Immunomodulators: Current Research Status and Future Directions. Molecules. 2020; 25:5854.
17. Barton DL, Liu H, Dakhil SR, Linquist B, Sloan JA, Nichols CR, McGinn TW, Stella PJ, Seeger GR, Sood A, Loprinzi CL. Wisconsin Ginseng (*Panax quinquefolius*) to improve cancer-related fatigue: a randomized, double-blind trial, N07C2. J Natl Cancer Inst. 2013; 105:1230-1238.
18. Chang YD, Smith J, Portman D, Kim R, Oberoi-Jassal R, Rajasekhara S, Davis M. Single Institute Experience With Methylphenidate and American Ginseng in Cancer-Related Fatigue. Am J Hosp Palliat Care. 2018; 35:144-150.
19. Fu J, Wang Z, Huang L, Zheng S, Wang D, Chen S, Zhang H, Yang S. Review of the botanical characteristics, phytochemistry, and pharmacology of Astragalus membranaceus (Huangqi). Phytother Res. 2014; 28:1275-1283.
20. Chen Z, Liu L, Gao C, Chen W, Vong CT, Yao P, Yang Y, Li X, Tang X, Wang S, Wang Y. *Astragali Radix* (Huangqi): A promising edible immunomodulatory herbal medicine. J Ethnopharmacol. 2020; 258:112895.
21. Li TM, Yu YH, Tsai FJ, *et al.* Characteristics of Chinese herbal medicine usage and its effect on survival of lung cancer patients in Taiwan. J Ethnopharmacol. 2018; 213:92-100.
22. Jeong JS, Ryu BH, Kim JS, Park JW, Choi WC, Yoon SW. Bojungikki-tang for cancer-related fatigue: a pilot randomized clinical trial. Integr Cancer Ther. 2010; 9:331-338.
23. Minagawa T, Domen T, Suzuki T, Ueno M, Nagai T, Ogawa T, Kiyokawa H, Ishizuka O. Effectiveness of hochuekkito (Japanese Herbal Medicine) for general fatigue after introduction of enzalutamide in three cases of gastration-resistant prostate cancer. Nihon Hinyokika Gakkai Zasshi. 2019; 110:86-91. (In Japanese)
24. Xu R, Wu J, Zhang X, Zou X, Li C, Wang H, Yuan M, Chen M, Sun Q, Liu S. Modified Bu-zhong-yi-qi decoction synergies with 5 fluorouracile to inhibits gastric cancer progress *via* PD-1/PD- L1-dependent T cell immunization. Pharmacol Res. 2020; 152:104623.
25. He Q, Sawada M, Yamasaki N, Akazawa S, Furuta H, Uenishi H, Meng X, Nakahashi T, Ishigaki Y, Moriya J. Neuroinflammation, Oxidative Stress, and Neurogenesis in a Mouse Model of Chronic Fatigue Syndrome, and the Treatment with Kampo Medicine. Biol Pharm Bull. 2020; 43:110-115.
26. Cheon C, Kang S, Ko Y, Kim M, Jang BH, Shin YC, Ko SG. Sipjeondaebo-tang in patients with breast cancer with fatigue: a protocol for a pilot, randomised, double-blind, placebo-controlled, cross-over trial. BMJ Open. 2018; 8:e021242.

27. Ishiura Y, Shiba Y, Terasaki Y, Hayase H, Hamada M, Izawa K, Sugimoto A, Hirokami K, Segawa M, Kasahara K, Fujimura M. Effect of Japanese Traditional Medicine, TJ-48, on the Quality of Life of Patients with Non-Small Cell Lung Cancer Receiving Outpatient Chemotherapy. Gan To Kagaku Ryoho. 2016; 43:331-334. (in Japanese)
28. Kawai H, Saito Y. Combination of Juzentaihoto and chemotherapy improves the prognosis of patients with postoperative recurrence of non-small cell lung cancer. Mol Clin Oncol. 2020; 13:13.
29. Bennett MI, Kaasa S, Barke A, Korwisi B, Rief W, Treede RD; IASP Taskforce for the Classification of Chronic Pain. The IASP classification of chronic pain for ICD-11: chronic cancer-related pain. Pain. 2019; 160:38-44.
30. Fallon M, Giusti R, Aielli F, Hoskin P, Rolke R, Sharma M, Ripamonti CI; ESMO Guidelines Committee. Management of cancer pain in adult patients: ESMO Clinical Practice Guidelines. Ann Oncol. 2018; 29 (Suppl 4):iv166-iv191.
31. Liou KT, Hung TKW, Meghani SH, Epstein AS, Li QS, Romero SAD, Cohen RB, Mao JJ. What if Acupuncture Were Covered by Insurance for Pain Management? A Cross-Sectional Study of Cancer Patients at One Academic Center and 11 Community Hospitals. Pain Med. 2019; 20:2060-2068.
32. He Y, Guo X, May BH, Zhang AL, Liu Y, Lu C, Mao JJ, Xue CC, Zhang H. Clinical Evidence for Association of Acupuncture and Acupressure With Improved Cancer Pain: A Systematic Review and Meta-Analysis. JAMA Oncol. 2020; 6:271-278.
33. Lam TY, Lu LM, Ling WM, Lin LZ. A pilot randomized controlled trial of acupuncture at the Si Guan Xue for cancer pain. BMC Complement Altern Med. 2017; 17:335.
34. Chiu HY, Hsieh YJ, Tsai PS. Systematic review and meta-analysis of acupuncture to reduce cancer-related pain. Eur J Cancer Care (Engl). 2017; 26.
35. Behzadmehr R, Dastyar N, Moghadam MP, Abavisani M, Moradi M. Effect of complementary and alternative medicine interventions on cancer related pain among breast cancer patients: A systematic review. Complement Ther Med. 2020; 49:102318
36. Cai P, Li L, Hong H, Zhang L, He C, Chai X, Liu B, Chen Z. A Chinese medicine warm compress (Wen Jing Zhi Tong Fang), combined with WHO 3-step analgesic ladder treatment for cancer pain relief: A comparative randomized trial. Medicine (Baltimore). 2018; 97:e9965.
37. Johannes CM, Musser ML. Anorexia and the Cancer Patient. Vet Clin North Am Small Anim Pract. 2019; 49:837-854.
38. Madeddu C, Maccio A, Mantovani G. Multitargeted treatment of cancer cachexia. Crit Rev Oncog. 2012; 17:305-314.
39. Cheng KC, Li YX, Cheng JT. The use of herbal medicine in cancer-related anorexia/cachexia treatment around the world. Curr Pharm Des. 2012; 18:4819-26.
40. Cheon C, Yoo JE, Yoo HS, Cho CK, Kang S, Kim M, Jang BH, Shin YC, Ko SG. Efficacy and Safety of Sipjeondaebo-Tang for Anorexia in Patients with Cancer: A Pilot, Randomized, Double-Blind, Placebo-Controlled Trial. Evid Based Complement Alternat Med. 2017; 2017:8780325.
41. Ko MH, Song SY, Ha SJ, Lee JY, Yoon SW, Park JH, Park SJ, Yoo HS. Efficacy and Safety of Yukgunja-Tang for Patients with Cancer-related Anorexia: A Randomized, Controlled Trial, Pilot Study. Integr Cancer Ther. 2021; 20:15347354211019107.
42. Walker WH 2nd, Borniger JC. Molecular Mechanisms of Cancer-Induced Sleep Disruption. Int J Mol Sci. 2019; 20:2780.
43. O'Donnell JF. Insomnia in cancer patients. Clin Cornerstone. 2004; 6 Suppl 1D:S6-S14.
44. Davis MP, Goforth HW. Long-term and short-term effects of insomnia in cancer and effective interventions. Cancer J. 2014; 20:330-344.
45. O'Brien K, Weber D. Insomnia in Chinese medicine: the heart of the matter. J Altern Complement Med. 2016; 22:684-694.
46. Li F, Xu B, Shi H, Zhang T, Song Z, Chen Y, Liu L, Wang P. Efficacy and safety of TCM Yangxin Anshen Therapy for insomnia: A systematic review and meta-analysis. Medicine (Baltimore). 2020; 99:e19330.
47. Shergis JL, Ni X, Sarris J, Zhang AL, Guo X, Xue CC, Lu C, Hugel H. Ziziphus spinosa seeds for insomnia: A review of chemistry and psychopharmacology. Phytomedicine. 2017; 34:38-43.
48. Zhou QH, Zhou XL, Xu MB, Jin TY, Rong PQ, Zheng GQ, Lin Y. Suanzaoren Formulae for Insomnia: Updated Clinical Evidence and Possible Mechanisms. Front Pharmacol. 2018; 9:76.
49. Metri K, Bhargav H, Chowdhury P, Koka PS. Ayurveda for chemo-radiotherapy induced side effects in cancer patients. J Stem Cells. 2013; 8:115-129.
50. Babiker HM, McBride A, Newton M, Boehmer LM, Drucker AG, Gowan M, Cassagnol M, Camenisch TD, Anwer F, Hollands JM. Cardiotoxic effects of chemotherapy: A review of both cytotoxic and molecular targeted oncology therapies and their effect on the cardiovascular system. Crit Rev Oncol Hematol. 2018; 126:186-200.
51. Wang YL, Li JX, Guo XQ, Fu RY, Guan XJ. Effect of acupuncture in different time on nausea and vomiting induced by chemotherapy of lung cancer. Zhongguo Zhen Jiu. 2019; 39:1269-1273. (in Chinese)
52. Li W, Li L. Effect of moxibustion on prevention and treatment of nausea and vomiting caused by cisplatin in lung cancer. Zhongguo Zhen Jiu. 2018; 38:695-699. (in Chinese)
53. Deng B, Jia L, Tan H, Lou Y, Li X, Li Y, Yu L. Effects of Shengjiangxiexin decoction on irinotecan-induced toxicity in patients with UGT1A1*28 and UGT1A1*6 polymorphisms. J Tradit Chin Med. 2017; 37:35-42.
54. Xiao H, Liu L, Ke S, Zhang Y, Zhang W, Xiong S, Zhang W, Ouyang J. Efficacy of Xiang-Sha-Liu-Jun-Zi on chemotherapy-induced nausea and vomiting: A protocol for systematic review and meta-analysis. Medicine (Baltimore). 2021; 100:e25848.
55. Lv C, Shi C, Li L, Wen X, Xian CJ. Chinese herbal medicines in the prevention and treatment of chemotherapy-induced nausea and vomiting. Curr Opin Support Palliat Care. 2018; 12:174-180.
56. Fu L, Fu H, Liu LQ, Huo ZJ, Yu YH, Yu JM. Efficacy of donkey-hide gelatin mixture for gemcitabine and cisplatin chemotherapy regimen induced myelosuppression. Chinese Clinical Oncology. 2014; 19:739-742. (in Chinese)
57. Liu M, Tan H, Zhang X, Liu Z, Cheng Y, Wang D, Wang F. Hematopoietic effects and mechanisms of Fufang e'jiao jiang on radiotherapy and chemotherapy-induced myelosuppressed mice. J Ethnopharmacol. 2014; 152:575-584.

58. Chen HY, Li SG, Cho WC, Zhang ZJ. The role of acupoint stimulation as an adjunct therapy for lung cancer: a systematic review and meta-analysis. BMC Complement Altern Med. 2013; 13:362.
59. Jain D, Aronow W. Cardiotoxicity of cancer chemotherapy in clinical practice. Hosp Pract (1995). 2019; 47:6-15.
60. Herrmann J, Lerman A. An update on cardio-oncology. Trends Cardiovasc Med. 2014; 24:285-95.
61. Dan W, Liu J, Guo X, Zhang B, Qu Y, He Q. Study on Medication Rules of Traditional Chinese Medicine against Antineoplastic Drug-Induced Cardiotoxicity Based on Network Pharmacology and Data Mining. Evid Based Complement Alternat Med. 2020; 2020:7498525.
62. Tian B, Tian M, Huang SM. Advances in phytochemical and modern pharmacological research of Rhizoma Corydalis. Pharm Biol. 2020; 58:265-275.
63. Cui Y, Li C, Zeng C, Li J, Zhu Z, Chen W, Huang A, Qi X. Tongmai Yangxin pills anti-oxidative stress alleviates cisplatin-induced cardiotoxicity: Network pharmacology analysis and experimental evidence. Biomed Pharmacother. 2018; 108:1081-1089.
64. Wu BY, Liu CT, Chen SY, Tsai MY. A case of chemotherapy-induced congestive heart failure successfully treated with Chinese herbal medicine. Complement Ther Med. 2015; 23:251-256.
65. Xu L, Shang Z, Tian Y, Xiong M, Nijat D, Wang Y, Qiao X, Ye M. Chemical Variations among Shengmaisan-Based TCM Patent Drugs by Ultra-High Performance Liquid Chromatography Coupled with Hybrid Quadrupole Orbitrap Mass Spectrometry. Molecules. 2021; 26:4000.
66. Ma S, Li X, Dong L, Zhu J, Zhang H, Jia Y. Protective effect of Sheng-Mai Yin, a traditional Chinese preparation, against doxorubicin-induced cardiac toxicity in rats. BMC Complement Altern Med. 2016; 16:61.
67. Staff NP, Grisold A, Grisold W, Windebank AJ. Chemotherapy-induced peripheral neuropathy: A current review. Ann Neurol. 2017; 81:772-781.
68. Gu JL, Wei GL, Ma YZ, Zhang JZ, Ji Y, Li LC, Yu JL, Hu CH, Huo JG. Exploring the Possible Mechanism and Drug Targets of Huang-Qi-Gui-Zhi-Wu-Wu Decoction for the Treatment of Chemotherapy-Induced Peripheral Neuropathy on Network Pharmacology. Evid Based Complement Alternat Med. 2020; 2020:2363262.
69. Cheng X, Huo J, Wang D, Cai X, Sun X, Lu W, Yang Y, Hu C, Wang X, Cao P. Herbal Medicine AC591 Prevents Oxaliplatin-Induced Peripheral Neuropathy in Animal Model and Cancer Patients. Front Pharmacol. 2017; 8:344.
70. Schroeder S, Meyer-Hamme G, Epplée S. Acupuncture for chemotherapy-induced peripheral neuropathy (CIPN): a pilot study using neurography. Acupunct Med. 2012; 30:4-7.
71. Litscher G, Wang L, Huber E, Nilsson G. Changed skin blood perfusion in the fingertip following acupuncture needle introduction as evaluated by laser Doppler perfusion imaging. Lasers Med Sci. 2002; 17:19-25.
72. Bledsoe TJ, Nath SK, Decker RH. Radiation Pneumonitis. Clin Chest Med. 2017; 38:201-208.
73. Kim KI, Jun JH, Baek H, Kim JH, Lee BJ, Jung HJ. Oral administration of herbal medicines for radiation pneumonitis in lung cancer patients: A systematic review and meta-analysis. PLoS One. 2018; 13:e0198015.
74. He H, Zhou X, Wang Q, Zhao Y. Does the couse of astragalus-containing chinese herbal prescriptions and radiotherapy benefit to non-small-cell lung cancer treatment: a meta-analysis of randomized trials. Evid Based Complement Alternat Med. 2013; 2013:426207.
75. Xiao Z, Liang R, Wang CQ, Xu S, Li N, He Y, Tang F, Chen L, Ma H. Can Aidi injection alleviate the toxicity and improve the clinical efficacy of radiotherapy in lung cancer?: A meta-analysis of 16 randomized controlled trials following the PRISMA guidelines. Medicine (Baltimore). 2016; 95:e4517.
76. Peng Y, Li Q, Zhang J, Shen W, Zhang X, Sun C, Cui H. Update review of skin adverse events during treatment of lung cancer and colorectal carcinoma with epidermal growth receptor factor inhibitors. Biosci Trends. 2019; 12:537-552.
77. Deng B, Jia LQ, Cui HJ. Effects of traditional Chinese medicine on epidermal growth factor receptor inhibitors induced rash: A meta-analysis. Journal of China-Japan Friend ship Hospital. 2016; 30:30-35. (in Chinese)
78. Ozawa N, Onda T, Hayashi K, Honda H, Shibahara T. Effects of Topical Hangeshashinto (TJ-14) on Chemotherapy-Induced Oral Mucositis. Cancer Manag Res. 2020; 12:1069-1078.
79. Murai T, Matsuo M, Tanaka H, Manabe Y, Takaoka T, Hachiya K, Yamaguchi T, Otsuka S, Shibamoto Y. Efficacy of herbal medicine TJ-14 for acute radiation-induced enteritis: a multi-institutional prospective Phase II trial. J Radiat Res. 2020; 61:140-145.
80. Nishikawa K, Aoyama T, Oba MS, *et al.* The clinical impact of Hangeshashinto (TJ-14) in the treatment of chemotherapy-induced oral mucositis in gastric cancer and colorectal cancer: Analyses of pooled data from two phase II randomized clinical trials (HANGESHA-G and HANGESHA-C). J Cancer. 2018; 9:1725-1730.
81. Urushiyama H, Jo T, Yasunaga H, Michihata N, Yamana H, Matsui H, Hasegawa W, Hiraishi Y, Mitani A, Fushimi K, Nagase T, Yamauchi Y. Effect of Hangeshashin-To (Japanese Herbal Medicine Tj-14) on Tolerability of Irinotecan: Propensity Score and Instrumental Variable Analyses. J Clin Med. 2018; 7:246.
82. Ichiki M, Wataya H, Yamada K, Tsuruta N, Takeoka H, Okayama Y, Sasaki J, Hoshino T. Preventive effect of kampo medicine (hangeshashin-to, TJ-14) plus minocycline against afatinib-induced diarrhea and skin rash in patients with non-small cell lung cancer. Onco Targets Ther. 2017; 10:5107-5113.
83. Yan Z, Lai Z, Lin J. Anticancer Properties of Traditional Chinese Medicine. Comb Chem High Throughput Screen. 2017; 20:423-429.
84. Cai P, Qiu H, Qi F, Zhang X. The toxicity and safety of traditional Chinese medicines: Please treat with rationality. Biosci Trends. 2019; 13:367-373.

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Review

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Formulation and interpretation of the Chinese Guidelines for Surgical Treatment of Obesity and Type 2 Diabetes Mellitus

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SUMMARY Obesity and related metabolic diseases have become one of the world's most serious public health problems. Bariatric surgery has gone through a long and difficult development process, from being rejected to gradually recognized, then widely accepted, and finally becoming the "gold standard" for the treatment of morbid obesity with metabolic diseases. Procedures have constantly been improving and evolving as the concept of bariatric surgery has been reappraised. The comparison and selection of different procedures, the emergence of new technologies and treatment methods, and the in-depth study of the mechanism of metabolic weight loss surgery are effectively promoting the rapid development of bariatric surgery. This article looks at both the 2014 and 2019 editions of the Guidelines for Diagnosis and Treatment of Obesity and Type 2 Diabetes Mellitus from the Chinese Society of Metabolic and Bariatric Surgery (CSMBS), its review the development of bariatric surgery, and it describes surgical indications and contraindications, the mechanism of weight loss, and tailored selection of the surgical procedure in order to serve as a reference.

Keywords Chinese Society of Metabolic and Bariatric Surgery (CSMBS), obesity, bariatric surgery, Chinese guidelines

1. Introduction

The Chinese Society for Metabolic & Bariatric Surgery (CSMBS) organized domestic metabolic and bariatric experts in 2014 and formulated its first guidelines – the Chinese Guidelines for Surgical Treatment of Obesity and Type 2 Diabetes Mellitus (2014 edition). Specified and standardized by the Guidelines, bariatric surgery in China has made great progress, particularly after the Chinese Medical Association created its Division of Thyroid and Metabolic Surgery in 2017 (*1*). Clinical study centers have been established successively in various regions of the country, and multi-center cooperation has been promoted to constantly accumulate multi-center hard clinical data. The number of bariatric surgeries performed has increased from 4000 cases in 2014 to more than 12,000 cases, but there were no obvious differences in the procedures compared to Europe and the US (*2*). In 2017, the American and European guidelines for metabolic and bariatric surgery were correspondingly updated; procedures such as adjustable gastric banding (AGB) are now gone from the pages of history. In 2019, the CSMBS formulated its second guidelines, the Chinese Guidelines for Surgical Treatment of Obesity and Type 2 Diabetes Mellitus (2019 edition) (Figure 1), to better reflect developments in bariatric surgery.

2. Surgical Indications and Contraindications

Surgical indications for patients with simple obesity: if BMI ≥ 37.5, bariatric surgery is highly recommended; if 32.5 ≤ BMI < 37.5, bariatric surgery is recommended; if 27.5 ≥ BMI < 32.5, obesity cannot be readily controlled with lifestyle changes and medical treatment, and the candidate has at least 2 components of metabolic syndrome or complications of obesity, surgery may be considered pursuant to a comprehensive assessment. For males with a waist circumference ≥ 90 cm, and females with a waist circumference ≥ 85 cm, if an imaging study suggests central obesity, the level of recommendation may be increased. The recommended age is 16-65 years.

Surgical indications for patients with type 2 diabetes mellitus: (1) If a patient with type 2 diabetes mellitus still secretes insulin to an extent. (2) If BMI ≥ 32.5, bariatric surgery is strongly recommended; if 27.5 ≤ BMI < 32.5, bariatric surgery is recommended; if 25 ≤ BMI < 27.5, obesity cannot be readily controlled with lifestyle changes and medical treatment, and the candidate has at least 2 components of metabolic syndrome or

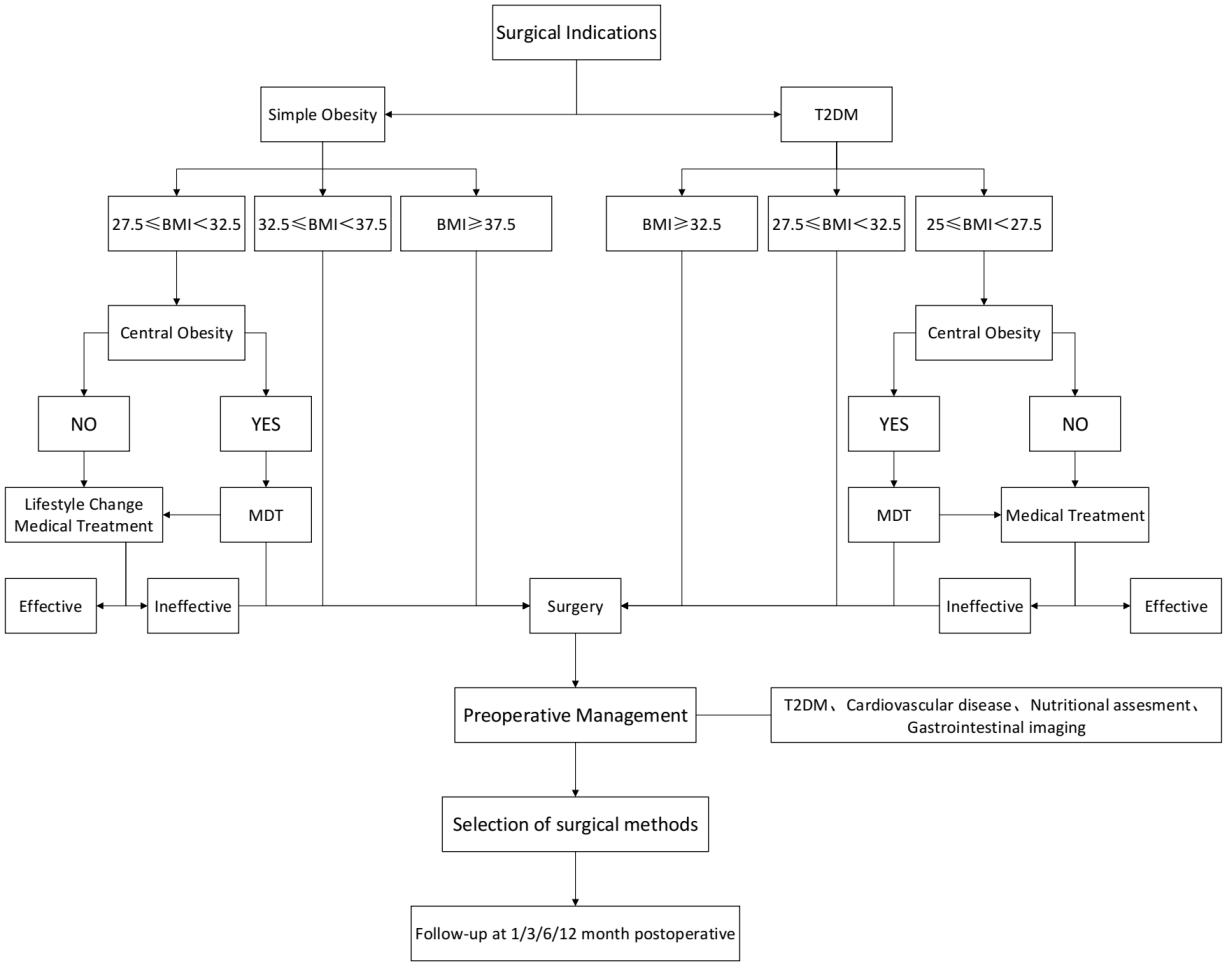

Figure1. The Chinese algorithm for treatment of obesity and metabolic disease.

complications of obesity, surgery may be considered pursuant to a comprehensive assessment. (3) For patients with 25 ≤ BMI < 27.5 (males with a waist circumference ≥ 90 cm, and females with a waist circumference ≥ 85 cm), if an imaging study suggests central obesity, the level of recommendation may be increased. (4) The suggested age for surgery is 16-65 years. For patients < 16 years of age, a multidisciplinary discussion involving a nutritionist and pediatrician should be conducted to comprehensive assess the feasibility and risks, surgery should be performed with informed consent, and surgery should not be heavily promoted. For patients > 65 years of age, their health status, concomitant diseases, and treatment profile should be seriously considered, a multidisciplinary discussion should be conducted to evaluate the patient's cardiopulmonary function and tolerance to surgery, and then surgery should be performed with informed consent.

Compared to the 2014 Guidelines, the 2019 Guidelines are more proactive in recommending surgery for patients with diabetes mellitus and a BMI ranging from 27.5-32.5. Two comorbidities of obesity were required in the 2014 edition of the guidelines but not in the 2019 edition. According to the 2014 Guidelines, bariatric surgery has demonstrated effectiveness in treating type 2 diabetes mellitus associated with obesity and is therefore also called metabolic surgery. Surgery is superior to diet therapy or drug therapy in treating type 2 diabetes mellitus and may be effective long-term. In 1991, the National Institutes of Health Consensus Development Panel recommended that nonsurgical treatment such as dietary and lifestyle changes and exercise should first be considered for patients with severe obesity and that surgery be considered for those with type 2 diabetes mellitus and a BMI ≥ 35 (class II). Although surgery is effective in treating patients with type 2 diabetes mellitus and a BMI ≥ 35, a large proportion of patients with type 2 diabetes mellitus have a BMI < 35 (class I), they are excluded as surgical candidates, and remission is difficult to achieve with medication or lifestyle changes alone. Surgery is an option for patients with type 2 diabetes mellitus and a BMI < 35, and studies have indicated that the remission of type 2 diabetes mellitus achieved by metabolic surgery is independent of weight loss and that the type 2 diabetes mellitus response rate after metabolic surgery was not statistically associated with the preoperative BMI. Surgery also resulted in remission of diabetes

in patients who are slightly obese (low BMI, class I). Since, European and American guidelines on metabolic surgery have changed the indications from severe obesity (BMI ≥ 40) or they recommend that this approach be considered for patients with diabetes mellitus and a BMI ≥ 30 (or ≥ 25.7 for Asians) (*3*). In 2014, the International Federation for the Surgery of Obesity and Metabolic Disorders (IFSO) issued a statement that surgery should be considered for patients with class I obesity and serious complications after the failure of proper nonsurgical treatment and that it should be based not only on the BMI but also on comorbidities. In 2017, the second Diabetes Surgery Summit issued a statement: Metabolic surgery to treat type 2 diabetes mellitus is recommended for patients with class III obesity (body mass index, BMI ≥ 40) regardless of glycemic control and for patients with class II obesity (BMI 35.0-39.9) with inadequately controlled hyperglycemia despite lifestyle changes and optimal medical therapy. Metabolic surgery should also be considered to treat type 2 diabetes mellitus in patients with class I obesity (BMI 30.0-34.9) and inadequately controlled hyperglycemia despite optimal medical treatment with oral or injectable medications (including insulin) (*4*). In 2018, the American Society for Metabolic & Bariatric Surgery (ASMBS) highly recommended surgery for patients with diabetes mellitus and class I obesity (BMI 30-35 kg/m^2), and the currently recommended age ranges from 18 to 65 years (*5*). In 2018, the Korean Society for the Study of Obesity (KSSO) Guidelines stated that metabolic surgery is indicated for obese patients with a BMI ≥ 35 (Class II) and for patients with diabetes mellitus and a BMI ≥ 30 (Class I) who have comorbidities (*6*). In light of the particular characteristics of Asians, the 2019 Chinese Guidelines recommend surgery for patients with type 2 diabetes mellitus and a BMI ≥ 27.5.

The 2019 Guidelines indicate that obese patients with diabetes who are < 16 or > 65 years of age may undergo surgery if they provide informed consent. At present, the main concerns regarding metabolic surgery in adolescents are surgical complications, uncertainty about long-term outcomes, and probable and possible ethical considerations. However, some of the existing literature has reported that metabolic surgery in adolescents was not associated with a significantly higher complication rate than that in adults and that it achieved a satisfactory outcome in terms of weight loss. Although adolescent patients who are obese are less likely to have diabetes, those with diabetes would face earlier failure of drug treatment and require insulin earlier. In 2018, the Pediatric Metabolic and Bariatric Surgery Guidelines of the ASMBS stated that bariatric surgery can be considered for adolescents with a BMI > 40 or a BMI > 35 and complications (*7*). A point worth noting is that bariatric surgery is not currently considered to be frontline treatment for adolescent obesity and surgery should be considered only after nonsurgical approaches fail to achieve weight control. For obese patients > 65 years of age, the overall complication rate after bariatric surgery does not significantly from that in adults, and laparoscopic sleeve gastrectomy (LSG) is relatively much safer than Roux-en-Y gastric bypass (RYGB). According to the Chinese guidelines, randomized controlled trials with a larger sample size and a longer follow-up period need to demonstrate the safety and efficacy of bariatric surgery in obese patients < 16 or > 65 years of age. Bariatric surgery should be performed on adolescent or elderly patients who are obese with their informed consent.

3. Mechanism of Weight Loss

Although bariatric surgery is effective at alleviating type 2 diabetes mellitus, the mechanism by which it does so is not completely clear at the moment. Initially, Kalam *et al.* speculated that energy intake control may play an important role in regulating blood glucose, and in addition, changes in bile acid metabolism, GI tract nutrient sensing and glucose utilization, incretins or anti-incretin(s), and intestinal microbiome may all participate in blood glucose regulation after bariatric surgery (*8*). The more likely conjecture is that multiple mechanisms work simultaneously to generate liver glycogen and to promote the uptake of blood glucose in tissues, lead to greater insulin sensitivity, improved β-cell function, and according regulation of blood glucose.

Previous studies contended that the mechanism by which bariatric/metabolic surgery treats type 2 diabetes mellitus is through a combination of the "foregut hypothesis" and "hindgut hypothesis" (*9*). The "foregut hypothesis" posits that the duodenum and proximal jejunum secrete special hormones by excluding the duodenum-jejunum bypass, whilst the "hindgut hypothesis" posits that surgery affects the secretion of glucagon-like peptide-1 (GLP-1) by distal ileal L cells, thus improving blood glucose metabolism. As further studies have been conducted, however, both the "foregut hypothesis" and "hindgut hypothesis" have failed to explain all of the clinical phenomena. Therefore, this mechanism is still a topic of interest for clinical and basic research on bariatric surgery (*10,11*). Any breakthrough may generate a new target for the treatment of obesity and metabolic disorders and facilitate the evidence-based development of metabolic surgery.

In addition, a recent study found that neurocircuits located within a brain-centered glucoregulatory system work cooperatively with pancreatic islets to promote glucose homeostasis, and the authors put forward the concept of the "gut-brain-liver axis" for blood glucose regulation (*12*). After eating, enteral nutrition will induce complex neural and hormonal changes. Neural signs submitted upwards from the gut to the brain, together with peptide hormones produced in the gut, act on the brain and regulate blood glucose through the negative

feedback pathway, which is mainly achieved by affecting the generation of liver glycogen. This could be another potential mechanism by which metabolic surgery treats diabetes mellitus.

At present, the gut microbiota has also attracted attention due to its role in the control of obesity. Studies have indicated that the gut microbiota is closely associated with obesity and that in the obese population; gut microbes colonize in an unhealthy manner to uptake and store more energy as fat, and there is less species richness of the gut microbiota than that in the healthy population (*13*). The species richness of the gut microbiota increases in patients after metabolic surgery, and the change in the gut microbiota is considered to be closely associated with insulin resistance, which may be a potential mechanism by which metabolic surgery treats diabetes mellitus. A study has indicated that after RYGB surgery (*14*) the numbers of Prevotellaceae, Archea, Firmicutes, and Bacteroidetes decreased while there was an increase in the Bacteroidetes:Prevotella ratio and the number of γ-proteobacteria. This may occur due to changes in the composition of the diet or changes in bile acid metabolism. However, Murphy *et al*. found that differences in the postoperative diet contributed to the different changes in the gut microbiota (*15*). Interestingly, Depommier *et al*. found that the supplementation of beneficial bacteria such as Akkermansia muciniphila effectively reduced body weight, increased insulin sensitivity, and alleviated insulin resistance (*16*). Ascertaining the postoperative changes in the gut microbiota and developing gut microbiota supplements accordingly could provide insight into the nonsurgical treatment of metabolic syndromes.

4. Tailored Selection of the Procedure

Compared to the 2014 Chinese guidelines on bariatric surgery, the 2019 guidelines removed AGB. The long-term weight loss outcomes of AGB were unsatisfactory. Band slippage and esophageal dilatation, fistulae, and infections were common reasons for the removal of gastric banding. By 2020, AGB accounted for about 3% of all surgical procedures worldwide (*17*) and 0% in China, indicating its disappearance from the pages of history. In light of the satisfactory outcomes achieved by LSG in terms of weight loss and remission of diabetes mellitus, the frequency of LSG has increased in recent years. According to the Fourth IFSO Global Registry Report 2018 (*18*), LSG has surpassed RYGB (46% *vs.* 38.2%) on a global scale and it has become the most commonly adopted surgical procedure for metabolic syndrome, accounting for 67% of all such procedures in China. Therefore, the Chinese Guidelines have listed LSG as the procedure of choice. According to the Guidelines, currently recommended surgical procedures include LSG, LRYGB, BPD/DS, OAGB, and SG + JJB, SG + DJB.

LSG is mainly indicated for patients with moderate to severe simple obesity and those with minor symptoms of metabolic syndrome. Since LSG may aggravate GERD and GERD-induced Barrett esophagus, moderate to severe GERD is a relative contraindication. Now, there is an expert consensus on reinforcing sutures to reduce gastric stump bleeding.

Compared to LSG, LGB is more advantageous in terms of postoperative long-term weight control and remission of diabetes mellitus, so gastric bypass may be a better option for patients with severe metabolic symptoms. At present, LGB has been performed less frequently each year. Since LSG can achieve the same outcomes in terms of weight loss and metabolic remission with fewer complications, LGB may be gradually replaced by LSG. LGB could be used as salvage surgery in the event of LSG failure, and it will still account for a certain proportion of surgeries on a long-term basis. LGB is indicated for patients with moderate to severe GERD or those with severe metabolic syndrome. Because gastroscopy is difficult to perform after LGB surgery, this procedure should be considered for patients with gastric precancerous disease and a family history of gastric cancer.

BPD/BS has a higher complication and mortality rate, its proportion has continued to decrease, and it only accounted for 0.5% of metabolic surgeries in 2020 (*19*). BPD/DS is mainly used in patients in whom sleeve gastrectomy fails to achieve a satisfactory outcome in terms of weight loss and therefore should be selected with caution.

Dr. Robert Rutledge performed a duodenal exclusion with an anastomosis in 2001, which he termed the "mini gastric bypass." In 2018, the procedure was re-named the one anastomosis gastric bypass (OAGB) by the IFSO. A possible risk of OAGB is bile regurgitation, which may induce gastritis and esophagitis and possibly induce subsequent gastric cancer and esophageal cancer, but such speculations have not yet been corroborated by existing studies. OAGB achieves satisfactory outcomes in terms of long-term weight loss and diabetes remission, so it is being performed more frequently (*20*). In the Asian-Pacific region, OAGB is performed more often than gastric bypass. Its complications mainly include afferent loop obstruction, anastomotic bleeding, an anastomotic leak, anastomotic stenosis, and wound infection. Clinical studies still need to be conducted to evaluate the procedure and its long-term nutritional implications.

Other procedures include SG+JJB and SG+DJB. At present, more clinical studies need to be conducted and more results of long-term follow-up need to be compiled to confirm long-term weight loss by and complications due to different procedures.

Metabolic surgery is now accepted for the treatment of type 2 diabetes mellitus. However, there is still no unified standard for personalized treatment based

Table 1. DiaRem scoring system

Factor	Score
Age	
< 40	1
40-49	1
50-59	2
≥ 60	3
HbA1c (%)	
< 6.5	0
6.5-6.9	2
7.0-8.9	4
≥ 9.0	6
Other diabetic drugs	
No sulfonylureas or insu-lin-sensitizing agents other than metformin	0
Sulfonylureas and insu-lin-sensitizing agents other than metformin	3
Treatment with insulin	
No	0
Yes	10
Total Score	0-22

Table 2. ABCD scoring system

Factor	Score
Age	
< 40	0
≥ 40	1
BMI	
< 27	0
27-34.9	1
35-41.9	2
≥ 42	3
C-peptide (ng/mL)	
< 2	0
2-2.9	1
3-4.9	2
≥ 5	3
Duration of DM (years)	
> 8	0
4-8	1
1-3.9	2
< 1	3
Total Score	0-10

on the patient's condition. Today, three preoperative scoring methods are available: the ABCD score (*21*), the DiaRem score (*22*), and the IMS score (*23*). In the current literature, no studies have clearly demonstrated which scoring method is more accurate, and there is no significant difference in the preoperative prediction of the rate of diabetes mellitus remission among the three scoring methods.

According to DiaRem scoring, early remission was achieved in 88% (95% CI 83-92%) of patients with a score of 0-2 points, 64% (58-71%) of those with a score of 3-7 points, 23% (13-33%) of those with a score of 8-12 points, 11% (6-16%) of those with a score of 13-17 points, and 2% (0-5%) of those with a score of 18-22 points (Table 1). The DiaRem score was used to predict the rate of diabetes rate at 1 year post-RYGB, but it was not accurate for long-term predictions such as 5 years post-operatively or for other procedures (*24*).

The predictors of the ABCD score are age, BMI, C-peptide level, and duration of diabetes mellitus, and the predicted rate of diabetes remission ranges from

Table 3. DRS scoring system

Factor	Score
Age	
30-60	1
< 30 or > 60	2
BMI	
< 27	1
> 27	2
Duration of T2DM (years)	
< 10	1
> 10	2
Microvascular complications	
No	1
Yes	2
Macrovascular complications	
No	1
Yes	2
Pre-operative insulin use	
No	1
Yes	2
Stimulated C-peptide (ng/mL)	
≥ 4	1
< 4	2
Total score	7-14

33-100% (0-10 points), that is, the rate of remission increases by 6.7% per point (Table 2). ABCD is a model established based on the Asian population is probably more suitable for use in the Chinese population. Although the ABCD score does not recommend surgery based on specific scores, a statistical analysis suggested that LSG may be more suitable for patients with an ABCD score higher than 7 and that RYGB may be more suitable for those with an ABCD score lower than 7 (*25*).

The IMS score consists of four predictors: preoperative duration of type 2 diabetes mellitus, preoperative number of diabetes medications, insulin use, and glycemic control (HbA1C < 7%). In mild type 2 diabetes mellitus (IMS score ≤ 25), both procedures significantly alleviated type 2 diabetes mellitus (Table 3). In severe type 2 diabetes mellitus (IMS score > 95), when clinical features suggest limited functional β-cell reserve, both procedures were similarly ineffective at diabetes remission. There was an intermediate group, however, in which RYGB was significantly more effective than SG, but this is likely related to its more pronounced neurohormonal effects. The IMS score can not only be used to predict the remission of diabetes mellitus after LSG and RYGB, it can also be used to preoperatively guide the selection of a procedure. If the IMS score ≤ 25, both sleeve gastrectomy and gastric bypass both result in a satisfactory outcome in terms of remission. If the IMS score > 25, gastric bypass should be selected. If the IMS score > 95, neither procedure will result in a satisfactory outcome in terms of weight loss. A point worth mentioning is that procedures are recommended based on the preoperative IMS score (*26*).

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References

1. Chan DL, Tam PT, Kan IY, Wong SK, Ng EK. Bariatric surgery in vegetarians: Asia-Pacific Metabolic and Bariatric Surgery Society (APMBSS) survey of Asian surgeon experience. Asian J Surg. 2021; 44:303-306.
2. Yang K, Zhou Y, Wang M, Shen M, Zhang X, Wang Y. Status of the field of bariatric surgery: A national survey of China in 2018. Obes Surg. 2019; 29:1911-1921.
3. Kim JH, Pyo JS, Cho WJ, Kim SY. The effects of bariatric surgery on type 2 diabetes in Asian populations: A meta-analysis of randomized controlled trials. Obes Surg. 2020; 30:910-923.
4. Welbourn R, Hollyman M, Kinsman R, Dixon J, Liem R, Ottosson J, Ramos A, Vage V, Al-Sabah S, Brown W, Cohen R, Walton P, Himpens J. Bariatric surgery worldwide: Baseline demographic description and one-year outcomes from the Fourth IFSO Global Registry Report 2018. Obes Surg. 2019; 29:782-795.
5. Aminian A, Chang J, Brethauer SA, Kim JJ, American Society for Metabolic and Bariatric Surgery Clinical Issues Committee. ASMBS updated position statement on bariatric surgery in class I obesity (BMI 30-35 kg/m^2). Surg Obes Relat Dis. 2018; 14:1071-1087.
6. Seo MH, Lee WY, Kim SS, *et al*. 2018 Korean Society for the Study of Obesity Guideline for the Management of Obesity in Korea. J Obes Metab Syndr. 2019; 28:40-45.
7. Till H, Mann O, Singer G, Weihrauch-Bluher S. Update on metabolic bariatric surgery for morbidly obese adolescents. Children (Basel). 2021; 8:372.
8. Kalam F, Kroeger CM, Trepanowski JF, Gabel K, Song JH, Cienfuegos S, Varady KA. Beverage intake during alternate-day fasting: Relationship to energy intake and body weight. Nutr Health. 2019; 25:167-171.
9. Zhu J, Gupta R, Safwa M. The mechanism of metabolic surgery: Gastric center hypothesis. Obes Surg. 2016; 26:1639-1641.
10. Korner J, Cline GW, Slifstein M, Barba P, Rayat GR, Febres G, Leibel RL, Maffei A, Harris PE. A role for foregut tyrosine metabolism in glucose tolerance. Mol Metab. 2019; 23:37-50.
11. Ebert KM, Arnold WG, Ebert PR, Merritt DJ. Hindgut microbiota reflects different digestive strategies in dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae). Appl Environ Microbiol. 2020; 87:e02100-20.
12. Wang SZ, Yu YJ, Adeli K. Role of gut microbiota in neuroendocrine regulation of carbohydrate and lipid metabolism via the microbiota-gut-brain-liver Axis. Microorganisms. 2020; 8:527.
13. Abenavoli L, Scarpellini E, Colica C, Boccuto L, Salehi B, Sharifi-Rad J, Aiello V, Romano B, De Lorenzo A, Izzo AA, Capasso R. Gut microbiota and obesity: A role for probiotics. Nutrients. 2019; 11:2690.
14. Aron-Wisnewsky J, Prifti E, Belda E, *et al*. Major microbiota dysbiosis in severe obesity: Fate after bariatric surgery. Gut. 2019; 68:70-82.
15. Murphy R, Tsai P, Jullig M, Liu A, Plank L, Booth M. Differential changes in gut microbiota after gastric bypass and sleeve gastrectomy bariatric surgery vary according to diabetes remission. Obes Surg. 2017; 27:917-925.
16. Depommier C, Everard A, Druart C, *et al*. Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: A proof-of-concept exploratory study. Nat Med. 2019; 25:1096-1103.
17. Spaniolas K, Yang J, Zhu C, Maria A, Bates AT, Docimo S, Talamini M, Pryor AD. Conversion of adjustable gastric banding to stapling bariatric procedures: Single- or two-stage approach. Ann Surg. 2021; 273:542-547.
18. Welbourn R, Hollyman M, Kinsman R, *et al*. Bariatric-metabolic surgery utilisation in patients with and without diabetes: Data from the IFSO Global Registry 2015-2018. Obes Surg. 2021; 31:2391-2400.
19. Alejo Ramos M, Ballesteros Pomar MD, Urioste Fondo AM, Gonzalez Herraez L, Gonzalez de Francisco T, Sierra Vega M, Cano Rodriguez IM. Bone metabolism and fracture risk after biliopancreatic diversion. Endocrinol Diabetes Nutr (Engl Ed). 2021; 68:144-152.
20. Jain M, Tantia O, Goyal G, Chaudhuri T, Khanna S, Poddar A, Majumdar K, Gupta S. LSG vs MGB-OAGB: 5-year follow-up data and comparative outcome of the two procedures over long term-Results of a randomised control trial. Obes Surg. 2021; 31:1223-1232.
21. Umemura A, Sasaki A, Nitta H, Nikai H, Baba S, Takahara T, Hasegawa Y, Katagiri H, Kanno S, Ishigaki Y. Prognostic factors and a new preliminary scoring system for remission of type 2 diabetes mellitus after laparoscopic sleeve gastrectomy. Surg Today. 2020; 50:1056-1064.
22. Chowbey P, Kelkar R, Soni V, Khullar R, Sharma A, Baijal M. Role of DiaRem score in preoperative prediction of type 2 diabetes mellitus remission after laparoscopic Roux-en-Y gastric bypass: Indian perspective. Obes Surg. 2021; 31:1265-1270.
23. Ohta M, Seki Y, Ohyama T, *et al*. Prediction of long-term diabetes remission after metabolic surgery in obese East Asian patients: A comparison between ABCD and IMS scores. Obes Surg. 2021; 31:1485-1495.
24. Mizera M, Wysocki M, Bartosiak K, Franczak P, Hady HR, Kalinowski P, Mysliwiec P, Orlowski M, Paluszkiewicz R, Piecuch J, Szeliga J, Waledziak M, Major P, Pedziwiatr M. Type 2 diabetes remission 5 years after laparoscopic sleeve gastrectomy: Multicenter cohort study. Obes Surg. 2021; 31:980-986.
25. Shen SC, Lee WJ, Kasama K, Seki Y, Su YH, Wong SK, Huang YM, Wang W. Efficacy of different procedures of metabolic surgery for type 2 diabetes in Asia: A multinational and multicenter exploratory study. Obes Surg. 2021; 31:2153-2160.
26. Aminian A, Andalib A. Individualized metabolic surgery (IMS) score. Surg Obes Relat Dis. 2018; 14:1921-1922.

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Review

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Current status of gastroesophageal reflux disease after sleeve gastrectomy: Still a long way to go

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SUMMARY Obesity is a public health concern that is becoming increasingly more serious around the world. Bariatric surgery has become more prevalent due to the obesity epidemic worldwide. Sleeve gastrectomy (SG) is one of the most popular procedures which is safe and efficient. Despite all its favorable features, however, there is an increasing evidence from the literature that the long-term incidence of gastroesophageal reflux disease (GERD) is likely to represent the Achilles' heel of this procedure. Management of severe reflux after SG usually requires revisional surgery. The relationship between SG and GERD needs to be better ascertained in order to prevent related complications, such as esophageal adenocarcinoma. This review attempts to elucidate the effect of SG on GERD and the postoperative management of reflux disease according to recent literature in the hope of drawing the attention of clinicians to postoperative gastroesophageal reflux and guiding the optimal management strategy associated with this "troublesome complication".

Keywords bariatric surgery, morbid obesity, sleeve gastrectomy (SG), gastroesophageal reflux disease (GERD)

1. Introduction

Obesity is becoming a worldwide health threat, and it is presently the most common and costly nutritional problem, with the prevalence of obesity and metabolic syndrome increasing to epidemic levels over the last few decades. Mean worldwide body mass index (BMI) has been steadily increasing since 1975, and current trends predict that 20% of the global population will be classified as obese (BMI ≥ 30 kg/m^2) by 2030 (*1,2*). Bariatric surgery has been associated with reduced overall mortality rates in obese patients and leads to remission of associated metabolic disorders (*3*). Sleeve gastrectomy (SG) is currently the most common bariatric procedure performed worldwide because of its advantages including the low rate of complications, the short operative time, the absence of foreign material, the lack of gastrointestinal anastomosis and malabsorption, the patient's acceptance, and the feasibility of its conversion into various other bariatric procedures (*4,5*).

However, the enthusiasm for a growing sleeve practice has been met with concerns of de novo or worsening gastroesophageal reflux disease (GERD) after the procedure (*6-8*). GERD is a disorder of the upper gastrointestinal tract that is defined by heartburn and acid regurgitation, which develops when reflux of the stomach contents causes troublesome symptoms and/or complications, according to the evidence-based consensus of the Montreal definition and the classification of GERD, issued in 2006 (*9*). Although obesity and other patient-related and environmental factors have been found to be independently associated with a higher incidence of GERD, certain anatomic and physiologic alterations resulting from SG are being recognized as potential etiologies of worsening of reflux disease (*10*). The prevalence of GERD following SG can be fairly high. Several studies have noted an incidence between 6% and 47% (*11-15*). This has prompted discussion among the surgical community with regard to the underlying pathomechanisms of GERD after SG and the postoperative management of reflux disease (*16-19*). So far, a number of new techniques have been reported to yield more encouraging results with regard to reflux symptoms after SG, but most evidence originates from retrospective studies with a small number of cases or is based on experts' opinions. The available data are limited, and very heterogeneous. As is often the case in surgery, when there are many solutions to one problem,

it is typically because no single solution is adequate for all patients. The aim of the current work is to review the contemporary literature and summarize the latest knowledge on GERD after SG in order to offer more conclusive insights into this controversial condition.

2. Obesity and GERD: The pathophysiology of GERD in obese individuals

GERD is undoubtedly a disease directly related to obesity. Overweight doubles the chance of GERD, and the prevalence of GERD symptoms in morbidly obese patients is as high as 50% (*20*). Moreover, the prevalence of GERD is proportional to the severity of obesity (*21*). The pathophysiology of GERD in obese individuals needs to be understood in order to adequately treat both GERD and obesity.

2.1. A defective gastroesophageal barrier

The most important pathophysiologic abnormality in GERD has been thought to be a decrease in lower esophageal sphincter (LES) pressure, either in the resting state or in association with a transient lower esophageal sphincter relaxation (TLESR). The number of episodes of TLESR is higher in the obese (*22,23*), and there is a correlation between the number of TLESR with BMI and abdominal circumference (*24,25*). Moreover, other studies have noted an increased basal pressure in the obese that is probably linked to compensatory mechanisms due to the increased intra-abdominal pressure (*26,27*). The angle of His is an important antireflux mechanism. The more acute this angle, the more the gastric fundus will be projected toward the esophagus as gastric distension occurs during a meal. The deposition of fat in the gastroesophageal junction, common and excessive in obese individuals, can result in an obtuse angle. Hiatal hernia (HH) is more frequent in the obese (*28*). Obese women are two and a half times more likely to have HH than non-obese women (*29*).

2.2. Inadequate esophageal clearance

Esophageal clearance is affected by the production of saliva, gravity, and esophageal peristalsis. Obese patients have decreased salivation (*30*), and esophageal peristalsis may be impaired in as much as a quarter of obese individuals. In addition, studies in obese individuals have found that sleep is associated with decreased swallowing and longer esophageal acid clearance time (*28,31*).

2.3. The trans-diaphragmatic pressure gradient

Abdominal pressure is increased in obese individuals due to the deposition of abdominal fat and its effect on gastric pressure. For each point of increase in the BMI, there is a 10% increase in intragastric pressure (*32*). Obese patients may also have a more negative intrathoracic pressure due to diaphragm elevation secondary to abdominal fat and a consequent decrease in pulmonary expansion. Negative intrathoracic pressure may also be increased by the frequent incidence of obstructive apnea. Obstructive sleep apnea (OSA) is closely associated with obesity. One can easily forget that OSA itself may be a cause of GERD due to an increase in TLESR (*33*).

2.4. Diet

Consumption of a high-fat diet increases the incidence of GERD symptoms compared to a high-fiber diet, regardless of caloric intake, due to a decrease in gastric emptying, a decrease in LES pressure, and an increase in the number of TLESRs (*34*). "Junk" foods, such as candy, chocolate, cookies, ice cream and cakes, are consumed more frequently by obese individuals and can induce reflux.

3. Mechanisms of new-onset or worsening GERD after SG

3.1. Increased intragastric pressure (IGP)

The shape of the sleeve likely plays a major role in the pathophysiology of post-SG GERD. When a gastric sleeve is created, a large, compliant stomach is converted into a long and narrow tube, resulting in a lack of gastric compliance and an increased IGP that correlates inversely with the diameter of the gastric tube and that increases when the pylorus is closed. The final shape of the sleeve also plays a role as it may encourage GERD and regurgitation when it is funnel-shaped (*35-37*). In addition, the vagovagal reflex diminishes after resection of the fundus, and the physiological postprandial relaxation of the stomach is eliminated. This results in an even higher IGP, pushing the gastric content in a retrograde direction (*38*). Moreover, a sleeve stenosis or an overly narrow SG can easily aggravate postoperative GERD symptoms. Sleeve stenosis is mostly due to postoperative edema, kinking, angulation, and/or cicatrization of the sleeve. Most stenoses are located in the middle portion of the sleeve, although they can occur at other locations (*39,40*). Sleeve stenosis is responsible for a considerable number of conversions from SG to Roux-en-Y gastric bypass (RYGB).

3.2. Disruption of the anatomical antireflux mechanisms

Several anatomical structures of the gastroesophageal junction comprise the antireflux barrier. The most important of these are the lower esophageal sphincter (LES) and the sling fibers at the cardia, along with the diaphragmatic crura. Alterations in the anatomy of either

Table 1. Possible mechanisms, preventive measures, and related preoperative examinations for GERD after SG

Proposed mechanisms leading to GERD after SG	Preventive measures	Targeted preoperative examinations
Hypotension of the lower esophageal sphincter	Maintain the integrity of the sling fibers of Helvetius at the esophagogastric junction	Symptom reporting
Blunting of the angle of His	Stapling should not be too close to the angle of His	Upper gastrointestinal radiography/CT/ Endoscopy
Decreased gastric compliance and volume (leading to increased intragastric pressure)	Avoid twisting/ narrowing of the sleeve; Do not place excessive tension on the stomach when stapling	CT/Endoscopy
Gastric shape	Attention to sleeve size and volume; Avoid a small bougie; Do not oversuture with overly big bites	Upper gastrointestinal radiography/CT/ Endoscopy
Concomitant presence of a hiatal hernia	Repair the concomitant hiatal hernia	Symptom reporting/Esophagogram/CT/ Endoscopy/High-resolution manometry
Fundal dilatation with distal narrowing	Avoid leaving an excessive posterior gastric fundus; Avoid narrowing the gastric body or pylorus	Upper gastrointestinal radiography/CT/ Endoscopy

of these are thought to be associated with the incidence of reflux symptoms (*41,42*).

Another aspect of the antireflux barrier at the gastroesophageal junction seems to be an acute Angle of His. To preserve this natural barrier during surgery, a careful dissection at the angle of His must be maintained in order to spare the sling fibers and avoid blunting the angle of His (*43*). During creation of the sleeve, the gastric sling fibers are frequently transected near the angle of His, particularly if the transection line is very close to this anatomic landmark. These sling fibers contribute significantly to the function of the LES (*44*). Disruption of these fibers can sometimes result in the herniation of part of the gastric sleeve into the posterior mediastinum (*45*). Table 1 summarizes the possible mechanisms, preventive measures, and related preoperative examinations for GERD after SG

4. Management of GERD after SG

4.1. Conservative treatment

While up to 30% of patients may experience some GERD symptoms after SG, most do not require surgery and can be treated successfully with medication (*46*). First-line therapy is similar to that used in the general population, with recommended lifestyle changes including abstinence from alcohol, cessation of smoking, and dietary modifications (*47*). Second-line therapy is the taking of medications to reduce stomach acid. Proton pump inhibitors (PPIs) are the preferred drugs for treatment of reflux, though promotility agents can also be used (*48*). If GERD symptoms persist despite maximal medical therapy, more invasive therapy should be considered.

4.2. Endoscopic interventions

4.2.1. Balloon dilation/endoluminal stent

If the cause of GERD is more of a technical nature, for example, sleeve stenosis, twisting, kinking, or cicatrization, an endoscopic or surgical intervention should be considered. Sleeve stenosis is first treated endoscopically with balloon dilation or endoluminal stent (*49*). Balloon dilation is the main form of treatment, and it has a good success rate in evident stenosis, but many technical aspects of this technique are still vigorously debated. Endoscopic stenting is the second line of endoscopic treatment, and it yields promising results when performed by an experienced surgeon (50). However, most patients refuse stenting after counseling because of its cost and risk of intolerance. The use of endoscopic stenting to treat sleeve stenosis should differ from its use to deal with leakage in terms of the duration of stenting and the limitation of the procedure to an experienced endoscopist (51).

4.2.2. Antireflux mucosectomy (ARMS)

Although no endoscopic procedure has been widely accepted as standard treatment of GERD, the ARMS procedure has come to the forefront in recent years. This effective and novel technique involves performing a mucosectomy of three quarters of the circumference at the gastro-esophageal junction (GEJ) in order to reduce the diameter due to scarring retraction (*52*). This procedure has yielded promising results in about 70% of patients with GERD in the available case series (*53,54*).

4.2.3. Endoscopic radiofrequency therapy

Endoscopic radiofrequency (Stretta) is a type of

radiofrequency ablation therapy utilizing temperature-controlled radiofrequency energy that is endoscopically delivered to the lower esophageal sphincter. The therapy is thought to increase the thickness of the muscular layer, providing an increase in the barrier mechanism of the LES and thereby decreasing acid exposure and the number of transient inappropriate relaxations of the sphincter (*55*). The device does not leave behind a permanent implant. The therapy is thought to remodel the muscles of the LES and gastric cardia. Studies have reported that the procedure is a safe and effective treatment for GERD, with a morbidity rate of less than 0.6 %, and the procedure can be performed on an outpatient basis. It has now been studied with up to a 10-year follow-up in non-bariatric patients and it has resulted in a significant improvement in quality of life and decreased use of proton pump inhibitors (PPIs) 10 years after the procedure (*56*). Complications include mucosal injury, bleeding, and perforation of the esophagus (*49*).

4.3. Surgical management

Surgical management is based on the following causes of reflux after SG: a lack of gastric compliance, increased intraluminal pressure, and the LES pressure. Technical/anatomical problems such as any narrowing or twisting during the sleeve dilation of the fundus and the persistence of hiatal hernias need to be addressed (*57*). A number of techniques can be used to mitigate the severity of reflux, either by maintaining the normal anatomic structures that limit reflux or by supplementing these structures with a plication or gastroplasty. Individuals with existing severe reflux should not be eligible for SG. New techniques that incorporate plication during the index SG have resulted in some improvement, but these involve small cases series that need to be evaluated further. The only proven method of treating intractable reflux after SG is conversion to RYGB.

4.3.1. Conversion to Roux

RYGB is still the best approach to avoid the incidence of GERD symptoms and to alleviate preoperative reflux (*58*). Conversion to RYGB effectively reduces GERD and has been found to alleviate symptoms in most patients. A RYGB limits acid production to the small gastric pouch and it reduces esophageal reflux because of the Roux-en Y anatomy, which also retains the physical activity of the esophagus and gastric pouch within the abdomen (*59*).

Several studies have confirmed that an RYGB decreases the esophagus' exposure to gastric acid. Curell *et al.* (*60*) evaluated conversion from SG to RYGB due to GERD using a prospective bariatric surgery database (2010-2018), and they found that conversion to RYGB was effective in almost all patients. They proposed that the focus should be on an exhaustive examination and aggressive approach to a hiatus. Matar *et al.* (*61*) and Lim *et al.* (*62*) obtained similar results for a RYGB for GERD. Felsenreich *et al.* (*63*) evaluated RYGB as treatment for Barrett's esophagus and reflux after SG. They concluded that RYGB is an effective therapy for patients with Barrett's esophagus and reflux after SG. In a bid to define the best practice guidelines, an international expert panel consensus statement declared that the entire panel agreed that patients who develop intractable GERD following LSG are best treated with a conversion to RYGB (*64*).

4.3.2. Magnetic sphincter augmentation (MSA)

Since the rise of SG and the known rate of postoperative GERD, MSA has been recognized as an effective treatment option for patients with GERD after SG; MSA recreates a physiological LES by means of a titanium bead ring around the gastro-esophageal junction (*65*). The device can be implanted laparoscopically, and the procedure can be done on an outpatient basis. Patients are allowed to return to a normal diet on the first day postoperatively, for as much as they can tolerate. This procedure has been found to reduce the esophagus' exposure to gastric acid, to alleviate the symptoms of GERD, and to decrease the need for antireflux medications, improving the quality of life of patients (*66*). Broderick *et al.* (*67*) reported that patients with GERD after SG had an overall satisfaction after MSA as high as 100% (13/13). A study by Kuckelman *et al.* (*68*) compared therapeutic benefits in a standard eligible group and a post-bariatric surgery group. Kuckelman *et al.* contended that MSA can provide surgeons with a new and much needed tool in their armory to combat refractory or de novo GERD developing after bariatric procedures. Although studies have suggested promising results, they have only reported on a small group of patients followed for a short period. The potential for erosion of the LINX device as well as the difficulty in dealing with these erosions surgically should also be considered (*69*). We propose the following algorithm of management for GERD after SG (Figure 1).

5. Conclusion

Obesity is associated with both symptoms and complications of GERD, and the associated risks seem to increase with increasing weight. The true incidence of clinically significant GERD following SG is unclear, but there is evidence indicating an increase in its incidence. As SG continues to be the form of bariatric surgery most often performed worldwide, further research is needed to provide clear guidance regarding the optimal preoperative evaluation of eligible patients and to ascertain technical aspects that can help to potentially

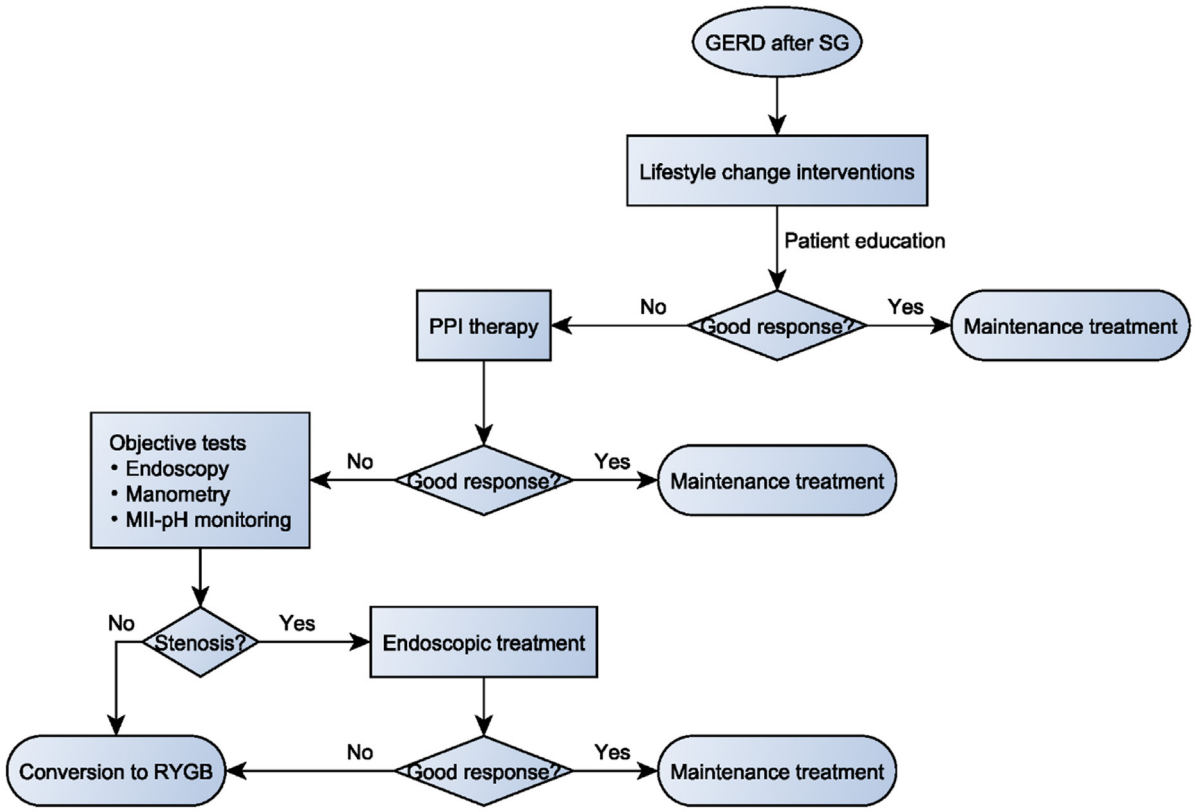

Figure 1. Current management algorithm for GERD after SG in the opinion of the authors. GERD, gastroesophageal reflux disease; SG, sleeve gastrectomy; PPI, proton pump inhibitor; RYGB, Roux-en-Y gastric bypass.

decrease the prevalence of this complication. GERD after SG is a complex problem if medical management fails. The first line of therapy is the use of antireflux medications. Currently available endoscopic antireflux procedures cannot be considered as an alternative to traditional surgical approaches in their current state, but they remain important weapons in the practitioner' s armory. The only evidence-based salvage operation for GERD after SG is RYGB. Numerous techniques have been proposed to mitigate the severity of reflux, either by maintaining the normal anatomic structures that limit reflux or by supplementing these structures with some type of plication or gastroplasty. Several of these new alternatives have yielded satisfactory results. Nevertheless, most evidence originates from retrospective studies with a small number of cases or is based on experts' opinions. The available data are limited, very heterogeneous, and need to be further evaluated.

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References

1. Blüher M. Obesity: Global epidemiology and pathogenesis. Nat Rev Endocrinol. 2019; 15:288-298.
2. Jaacks LM, Vandevijvere S, Pan A, McGowan CJ, Wallace C, Imamura F, Mozaffarian D, Swinburn B, Ezzati M. The obesity transition: stages of the global epidemic. Lancet Diabetes Endocrinol. 2019; 7:231-240.
3. Wee CC. Bariatric surgery for patients with obesity: The earlier the better. Ann Intern Med. 2020; 173:758-759.
4. Puzziferri N, Almandoz JP. Sleeve gastrectomy for weight loss. JAMA. 2018; 319:316.
5. Brajcich BC, Hungness ES. Sleeve gastrectomy. JAMA. 2020; 324:908.
6. van Rutte PW, Smulders JF, de Zoete JP, Nienhuijs SW. Outcome of sleeve gastrectomy as a primary bariatric procedure. Br J Surg. 2014; 101:661-668.
7. Dalboh A, Al-Shehri DM, Abd El Maksoud WM, Abbas KS, Alqahtani AJ, Al-Malki AQ, Al-Shahrani KA. Impact of Laparoscopic Sleeve Gastrectomy on Gastroesophageal Reflux Disease and Risk Factors

Associated with Its Occurrence Based Upon Quality of Life. Obes Surg. 2021; 31:3065-3074.
8. Yeung KTD, Penney N, Ashrafian L, Darzi A, Ashrafian H. Does Sleeve Gastrectomy Expose the Distal Esophagus to Severe Reflux?: A Systematic Review and Meta-analysis. Ann Surg. 2020; 271:257-265.
9. Vakil N, van Zanten SV, Kahrilas P, Dent J, Jones R; Global Consensus Group. The Montreal definition and classification of gastroesophageal reflux disease: A global evidence-based consensus. Am J Gastroenterol. 2006; 101:1900-1920; quiz 1943.
10. Althuwaini S, Bamehriz F, Aldohayan A, Alshammari W, Alhaidar S, Alotaibi M, Alanazi A, Alsahabi H, Almadi MA. P. Prevalence and predictors of gastroesophageal reflux disease after laparoscopic sleeve gastrectomy. Obes Surg. 2018; 28:916-922.
11. Alvarenga ES, Lo Menzo E, Szomstein S, Rosenthal RJ. Safety and efficacy of 1020 consecutive laparoscopic sleeve gastrectomies performed as a primary treatment modality for morbid obesity. A single-center experience from the metabolic and bariatric surgical accreditation quality and improvement program. Surg Endosc. 2016; 30:2673-2678.
12. Gorodner V, Buxhoeveden R, Clemente G, Solé L, Caro L, Grigaites A. Does laparoscopic sleeve gastrectomy have any influence on gastroesophageal reflux disease? Preliminary results. Surg Endosc. 2015; 29:1760-1768.
13. Moon RC, Teixeira AF, Jawad MA. Is preoperative manometry necessary for evaluating reflux symptoms in sleeve gastrectomy patients. Surg Obes Relat Dis. 2015; 11:546-551.
14. Gärtner D, Stroh C, Hukauf M, Benedix F, Manger T; Obesity Surgery Working Group, Competence Network Obesity. Sleeve gastrectomy in the German Bariatric Surgery Registry from 2005 to 2016: Perioperative and 5-year results. Surg Obes Relat Dis. 2019; 15:187-193.
15. Felsenreich DM, Prager G, Kefurt R, Eilenberg M, Jedamzik J, Beckerhinn P, Bichler C, Sperker C, Krebs M, Langer FB. Quality of life 10 years after sleeve gastrectomy: A multicenter study. Obes Facts. 2019; 12:157-166.
16. Johari Y, Wickremasinghe A, Kiswandono P, Yue H, Ooi G, Laurie C, Hebbard G, Beech P, Yap K, Brown W, Burton P. Mechanisms of esophageal and gastric transit following sleeve gastrectomy. Obes Surg. 2021; 31:725-737.
17. Patti MG, Schlottmann F. Gastroesophageal reflux after sleeve gastrectomy. JAMA Surg. 2018; 153:1147-1148.
18. Bevilacqua LA, Obeid NR, Yang J, Zhu C, Altieri MS, Spaniolas K, Pryor AD. Incidence of GERD, esophagitis, Barrett's esophagus, and esophageal adenocarcinoma after bariatric surgery. Surg Obes Relat Dis. 2020; 16:1828-1836.
19. Bou Daher H, Sharara AI. Gastroesophageal reflux disease, obesity and laparoscopic sleeve gastrectomy: The burning questions. World J Gastroenterol. 2019; 25:4805-4813.
20. Chang P, Friedenberg F. Obesity and GERD. Gastroenterol Clin North Am. 2014; 43:161-173.
21. Pandolfino JE. The relationship between obesity and GERD: "Big or overblown". Am J Gastroenterol. 2008; 103:1355-1357.
22. Wu JC, Mui LM, Cheung CM, Chan Y, Sung JJ. Obesity is associated with increased transient lower esophageal sphincter relaxation. Gastroenterology. 2007; 132:883-889.
23. Schneider JH, Küper M, Königsrainer A, Brücher B. Transient lower esophageal sphincter relaxation in morbid obesity. Obes Surg. 2009; 19:595-600.
24. Lee YY, McColl KE. Pathophysiology of gastroesophageal reflux disease. Best Pract Res Clin Gastroenterol. 2013; 27:339-351.
25. Richter JE, Rubenstein JH. Presentation and epidemiology of gastroesophageal reflux disease. Gastroenterology. 2018; 154:267-276.
26. Herbella FA, Sweet MP, Tedesco P, Nipomnick I, Patti MG. Gastroesophageal reflux disease and obesity. Pathophysiology and implications for treatment. J Gastrointest Surg. 2007; 11:286-290.
27. Valezi AC, Herbella FA, Junior JM, de Almeida Menezes M. Esophageal motility after laparoscopic Roux-en-Y gastric bypass: The manometry should be preoperative examination routine. Obes Surg. 2012; 22:1050-1054.
28. Suter M, Dorta G, Giusti V, Calmes JM. Gastro-esophageal reflux and esophageal motility disorders in morbidly obese patients. Obes Surg. 2004; 14:959-966.
29. Herbella FA, Patti MG. Gastroesophageal reflux disease: From pathophysiology to treatment. World J Gastroenterol. 2010; 16:3745-3749.
30. Côté-Daigneault J, Leclerc P, Joubert J, Bouin M. High prevalence of esophageal dysmotility in asymptomatic obese patients. Can J Gastroenterol Hepatol. 2014; 28:311-314.
31. Koppman JS, Poggi L, Szomstein S, Ukleja A, Botoman A, Rosenthal R. Esophageal motility disorders in the morbidly obese population. Surg Endosc. 2007; 21:761-764.
32. Nadaleto BF, Herbella FA, Patti MG. Gastroesophageal reflux disease in the obese: Pathophysiology and treatment. Surgery. 2016; 159:475-486.
33. Shepherd K, Hillman D, Holloway R, Eastwood P. Mechanisms of nocturnal gastroesophageal reflux events in obstructive sleep apnea. Sleep Breath. 2011; 15:561-570.
34. Mion F, Dargent J. Gastro-oesophageal reflux disease and obesity: Pathogenesis and response to treatment. Best Pract Res Clin Gastroenterol. 2014; 28:611-622.
35. F, Chand B, Grimminger P, Mikami D, Schoppmann SF, Müller-Stich B. Do we understand the pathophysiology of GERD after sleeve gastrectomy? Ann N Y Acad Sci. 2020; 1482:26-35.
36. Alhaj Saleh A, Janik MR, Mustafa RR, Alshehri M, Khan AH, Kalantar Motamedi SM, Rahim S, Patel I, Aryaie A, Abbas M, Rogula T, Khaitan L. Does sleeve shape make a difference in outcomes? Obes Surg. 2018; 28:1731-1737.
37. Wang Y, Yi XY, Gong LL, Li QF, Zhang J, Wang ZH. The effectiveness and safety of laparoscopic sleeve gastrectomy with different sizes of bougie calibration: A systematic review and meta-analysis. Int J Surg. 2018; 49:32-38.
38. Johari Y, Lim G, Wickremasinghe A, Yue H, Seah J, Ooi G, Playfair J, Laurie C, Beech P, Yap K, Hebbard G, Brown W, Burton P. Pathophysiological Mechanisms of GASTRO-ESOPHAGEAL Reflux Following Sleeve Gastrectomy. Ann Surg. 2020; doi: 10.1097/SLA.0000000000004637.
39. Ferraz ÁAB, da Silva JD, Santa-Cruz F, Aquino MR, Siqueira LT, Kreimer F. The impact of the gastric twist on esophagitis progression after sleeve gastrectomy:

Mid-term endoscopic findings. Obes Surg. 2020; 30:4452-4458.
40. Csendes A, Orellana O, Martínez G, Burgos AM, Figueroa M, Lanzarini E. Clinical, endoscopic, and histologic findings at the distal esophagus and stomach before and late (10.5 years) after laparoscopic sleeve gastrectomy: Results of a prospective study with 93% follow-up. Obes Surg. 2019; 29:3809-3817.
41. Coupaye M, Gorbatchef C, Calabrese D, Sami O, Msika S, Coffin B, Ledoux S. Gastroesophageal reflux after sleeve gastrectomy: A prospective mechanistic study. Obes Surg. 2018; 28:838-845.
42. Hesse UJ. What causes gastroesophageal reflux following sleeve gastrectomy. Obes Surg. 2020; 30:759.
43. Emile SH. Gastroesophageal reflux disease after sleeve gastrectomy: The need to predict its onset and prevent its consequences. Obes Surg. 2019; 29:2625-2626.
44. Tolone S, Savarino E, Yates RB. The impact of bariatric surgery on esophageal function. Ann N Y Acad Sci. 2016; 1381:98-103.
45. Borbély Y, Bouvy N, Schulz HG, Rodriguez LA, Ortiz C, Nieponice A. Electrical stimulation of the lower esophageal sphincter to address gastroesophageal reflux disease after sleeve gastrectomy. Surg Obes Relat Dis. 2018; 14:611-615.
46. Kindel TL, Oleynikov D. The improvement of gastroesophageal reflux disease and Barrett's after bariatric surgery. Obes Surg. 2016; 26:718-720.
47. Arman GA, Himpens J, Dhaenens J, Ballet T, Vilallonga R, Leman G. Long-term (11+years) outcomes in weight, patient satisfaction, comorbidities, and gastroesophageal reflux treatment after laparoscopic sleeve gastrectomy. Surg Obes Relat Dis. 2016; 12:1778-1786.
48. Barr AC, Frelich MJ, Bosler ME, Goldblatt MI, Gould JC. GERD and acid reduction medication use following gastric bypass and sleeve gastrectomy. Surg Endosc. 2017; 31:410-415.
49. Ganz RA. A review of new surgical and endoscopic therapies for gastroesophageal reflux disease. Gastroenterol Hepatol (N Y). 2016; 12:424-431.
50. Agnihotri A, Barola S, Hill C, Neto MG, Campos J, Singh VK, Schweitzer M, Khashab MA, Kumbhari V. An algorithmic approach to the management of gastric stenosis following laparoscopic sleeve gastrectomy. Obes Surg. 2017; 27:2628-2636.
51. Vilallonga R, Sanchez-Cordero S, Umpiérrez Mayor N, Molina A, Cirera de Tudela A, Ruiz-Úcar E, Carrasco MA. GERD after Bariatric Surgery. Can We Expect Endoscopic Findings? Medicina (Kaunas). 2021; 57:506.
52. Yoo IK, Ko WJ, Kim HS, Kim HK, Kim JH, Kim WH, Hong SP, Yeniova AÖ, Cho JY. Anti-reflux mucosectomy using a cap-assisted endoscopic mucosal resection method for refractory gastroesophageal disease: A prospective feasibility study. Surg Endosc. 2020; 34:1124-1131.
53. Inoue H, Tanabe M, de Santiago ER, Abad MRA, Shimamura Y, Fujiyoshi Y, Ueno A, Sumi K, Tomida H, Iwaya Y, Ikeda H, Onimaru M. Anti-reflux mucosal ablation (ARMA) as a new treatment for gastroesophageal reflux refractory to proton pump inhibitors: A pilot study. Endosc Int Open. 2020; 8:E133-133E138.
54. Monino L, Gonzalez JM, Vitton V, Barthet M. Antireflux mucosectomy band in treatment of refractory gastroesophageal reflux disease: A pilot study for safety, feasibility and symptom control. Endosc Int Open. 2020; 8:E147-147E154.
55. Khidir N, Angrisani L, Al-Qahtani J, Abayazeed S, Bashah M. Initial experience of endoscopic radiofrequency waves delivery to the lower esophageal sphincter (Stretta procedure) on symptomatic gastroesophageal reflux disease post-sleeve gastrectomy. Obes Surg. 2018; 28:3125-3130.
56. Fass R, Cahn F, Scotti DJ, Gregory DA. Systematic review and meta-analysis of controlled and prospective cohort efficacy studies of endoscopic radiofrequency for treatment of gastroesophageal reflux disease. Surg Endosc. 2017; 31:4865-4882.
57. Crawford C, Gibbens K, Lomelin D, Krause C, Simorov A, Oleynikov D. Sleeve gastrectomy and anti-reflux procedures. Surg Endosc. 2017; 31:1012-1021.
58. Assalia A, Gagner M, Nedelcu M, Ramos AC, Nocca D. Gastroesophageal reflux and laparoscopic sleeve gastrectomy: Results of the First International Consensus Conference. Obes Surg. 2020; 30:3695-3705.
59. Carandina S, Soprani A, Montana L, Murcia S, Valenti A, Danan M, d'Agostino J, Rivkine E, Nedelcu M. Conversion of sleeve gastrectomy to Roux-en-Y gastric bypass in patients with gastroesophageal reflux disease: Results of a multicenter study. Surg Obes Relat Dis. 2020; 16:732-737.
60. Curell A, Beisani M, García Ruiz de Gordejuela A, Vilallonga R, Verdaguer Tremolosa M, González López Ó, Caubet Busquet E, Fort López-Barajas JM. Outcomes of Conversion from Sleeve Gastrectomy to Roux-en-Y Gastric Bypass Due to GERD-a Retrospective Analysis of 35 Patients. Obes Surg. 2021; doi: 10.1007/s11695-021-05541-4.
61. Curell A, Beisani M, García Ruiz de Gordejuela A, Vilallonga R, Verdaguer Tremolosa M, González López Ó, Caubet Busquet E, Fort López-Barajas JM. Outcomes of Conversion from Sleeve Gastrectomy to Roux-en-Y Gastric Bypass Due to GERD-a Retrospective Analysis of 35 Patients. Obes Surg. 2021; doi: 10.1007/s11695-021-05541-4.
62. Lim CH, Lee PC, Lim E, Eng A, Chan WH, Tan HC, Ho E, Kovalik JP, Ganguly S, Tan J. Resolution of erosive esophagitis after conversion from vertical sleeve gastrectomy to Roux-en-Y gastric bypass. Obes Surg. 2020; 30:4751-4759.
63. Felsenreich DM, Langer FB, Bichler C, Eilenberg M, Jedamzik J, Kristo I, Vock N, Gensthaler L, Rabl C, Todoroff A, Prager G. Roux-en-Y gastric bypass as a treatment for Barrett's esophagus after sleeve gastrectomy. Obes Surg. 2020; 30:1273-1279.
64. Kichler K, Rosenthal RJ, DeMaria E, Higa K. Reoperative surgery for nonresponders and complicated sleeve gastrectomy operations in patients with severe obesity. An international expert panel consensus statement to define best practice guidelines. Surg Obes Relat Dis. 2019; 15:173-186.
65. Ndubizu GU, Petrick AT, Horsley R. Concurrent magnetic sphincter augmentation and hiatal hernia repair for refractory GERD after laparoscopic sleeve gastrectomy. Surg Obes Relat Dis. 2020; 16:168-170.
66. Clapp B, Dodoo C, Harper B, Kim J, Castro C, Hamdan M, Grasso S, Davis B. Magnetic sphincter augmentation at the time of bariatric surgery: An analysis of the MBSAQIP. Surg Obes Relat Dis. 2021; 17:555-561.
67. Broderick RC, Smith CD, Cheverie JN, Omelanczuk P,

Lee AM, Dominguez-Profeta R, Cubas R, Jacobsen GR, Sandler BJ, Fuchs KH, Horgan S. Magnetic sphincter augmentation: A viable rescue therapy for symptomatic reflux following bariatric surgery. Surg Endosc. 2020; 34:3211-3215.
68. Kuckelman JP, Phillips CJ, Derickson MJ, Faler BJ, Martin MJ. Esophageal magnetic sphincter augmentation as a novel approach to post-bariatric surgery gastroesophageal reflux disease. Obes Surg. 2018; 28:3080-3086.
69. DeMarchi J, Schwiers M, Soberman M, Tokarski A. Evolution of a novel technology for gastroesophageal reflux disease: A safety perspective of magnetic sphincter augmentation. Dis Esophagus. 2021.

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Mini-Review

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Prevention and treatment strategies for type 2 diabetes based on regulating intestinal flora

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SUMMARY Diabetes along with related comorbidities associated with high disability rates severely threatens human health. The etiology of diabetes is complex. Genetics, environmental factors, eating habits, drug usage, aging, and lack of movement play important roles in the development of diabetes. Intestinal flora is reportedly closely related to the occurrence and development of type 2 diabetes. Herein, we review changes in abundance and proportion of intestinal flora in patients with type 2 diabetes and regulation of intestinal flora through diet, drugs, and surgery to prevent and treat type 2 diabetes. A more appropriate clinical diagnosis and treatment plan could be made considering changes in intestinal flora in the future.

Keywords type 2 diabetes mellitus, intestinal microbiota, insulin resistance, research progress

1. Introduction

With the development of the economy and society, the global urbanization process has accelerated, and people's lifestyle and dietary habits have changed dramatically. Excessive salt, sugar, and fat in the diet increase the incidence of chronic metabolic diseases, such as obesity and diabetes. According to the latest data released by the International Diabetes Federation, there were approximately 463 million people aged 20-79 years with diabetes worldwide in 2019. China has the highest number of adults with diabetes (116.4 million), accounting for a quarter of the world's diabetes-affected population. According to forecasting models, diabetes will affect 700.2 million people by 2045 (*1*). Type 2 diabetes mellitus (T2DM) accounts for more than 90% of the total number of diabetic patients. Hence, it is of great significance to study the pathophysiological mechanisms and effective prevention and treatment of T2DM.

T2DM is a metabolic syndrome caused by the combined effects of genetic and environmental factors, and is characterized by an absolute or relative deficiency of insulin secretion and a decrease in insulin sensitivity in target organs. Glucose metabolism disorder is the primary manifestation of T2DM, followed by metabolic disorders of fat, protein, water, and electrolytes. Insulin resistance and dysfunction of islet β cells are considered to be the leading causes of the occurrence and development of T2DM (*2*). The intestinal flora is known as "the second human genome". As an internal environmental factor of the body, it plays a vital role in regulating metabolism, immunity, inflammation, and other physiological and pathological processes. There is increasing evidence that abnormal intestinal flora is closely associated with the occurrence and development of T2DM (*3*).

More than 1,000 species of bacteria inhabit the human gut, and the total number is approximately 10^{14}, which is 10-fold more than the number of human cells. These microbes weigh up to 1.2 kg in total and account for about 80% of microbes in the human body. Bacteroidetes and Firmicutes are the two main phyla, followed by Actinomycetes, Proteobacteria, and Verrucomicrobia (*4*). Depending on their relationship with the host, intestinal flora can be divided into commensal bacteria, opportunistic pathogens, and harmful bacteria. In the physiological state, these organisms are mutually dependent and restricted. They have a symbiotic relationship with the human body and maintain a dynamic balance. Furthermore, commensals are a component of the natural defense line to maintain human health. When pathological factors break this balance, many diseases occur. Herein, we review the characteristics of intestinal flora in patients with T2DM and strategies for preventing and treating T2DM by regulating intestinal flora to provide a relevant reference for clinical diagnosis and treatment of diabetes.

2. Differences in intestinal flora between T2DM and non-diabetic populations

Although it is still uncertain whether there is a causal

Table 1. Differences in the intestinal flora among patients with T2DM, prediabetic population, and normal population in some studies

Study	Intestinal flora	Patients with T2DM	Prediabetic population
Zhang *et al*. (*5*)	*Akkermansia muciniphila* and *Faecalibacterium prausnitzii*	–	↓
	Bacteroides	↓	–
	Verrucomicrobia	↓	↓
Egshatyan *et al*. (*6*)	*Blautia and Serratia*	↑↑	↑
Larsen *et al*. (*7*)	Firmicutes	↓	–
	Proteobacteria and Bacteroidetes	↑	–
Wu *et al*. (*8*)	*Bifidobacterium* and *Bacteroides vulgatus*	↓	–
Sedighi *et al*. (*10*)	*Lactobacillus*	↑	–
	Bifidobacterium	↓	–
Hartstra *et al*. (*13,14*)	*Roseburia*, *Eubacterium hallii*, and *Faecalibacterium prausnitzii*	↓	–
	Lactobacillus gasseri, *Streptococcus mutans*, and *Escherichia coli*	↑	–

↑:The abundance of the intestinal flora increased in patients with T2DM/prediabetic population. ↓:The abundance of the intestinal flora decreased in patients with T2DM/prediabetic population. – :Not mentioned.

relationship between intestinal flora alteration and T2DM, the changes in the intestinal flora in patients with T2DM have been confirmed (Table 1). Zhang *et al*. found that the abundance of butyric acid-producing bacteria, such as *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* in the normal population was higher than that in the prediabetic population, and the abundance of Bacteroides in patients with T2DM was only half of that in the normal population and prediabetic population. The abundance of Verrucomicrobia in the prediabetic population and patients with T2DM was significantly lower than that in the normal population, and it may be a potential marker of T2DM (*5*). Egshatyan *et al*. reported that the abundance of *Blautia* and *Serratia* in the gut of the prediabetic population was lower than that in patients with T2DM, and people with normal glucose tolerance have the lowest abundance (*6*). Larsen *et al*. used real-time quantitative PCR to analyze the fecal flora in 18 patients with T2DM and 18 non-diabetic individuals and found that the abundance of Firmicutes decreased in patients with T2DM, whereas the abundance of Proteobacteria and Bacteroidetes increased. Besides, the ratio of Bacteroidetes to Firmicutes significantly positively correlated with blood glucose concentration (*7*). Wu *et al*. found that the abundance of *Bifidobacterium* and *Bacteroides vulgatus* in patients with T2DM was significantly lower than that in non-diabetic individuals (*8*). Karlsson *et al*. isolated the fecal microbiota from 53 patients with T2DM, 49 people with impaired glucose tolerance, and 43 healthy European women for metagenomic sequencing. They found that compared with the intestinal flora in non-diabetic individuals, 4 *Lactobacillus* species and 5 *Clostridium* species were increased and decreased, respectively, in diabetic patients. *Lactobacillus* positively correlated with blood glucose and glycosylated hemoglobin (HbA1c), whereas *Clostridium* negatively correlated with blood glucose, HbA1c, insulin, C-peptide, and triacylglycerol, and positively correlated with adiponectin and high-density lipoprotein (HDL) (*9*). Sedighi *et al*. analyzed the microbiome in fecal samples of patients with T2DM and normal populations. They confirmed that intestinal flora of patients with T2DM had a high abundance of *Lactobacillus*, whereas abundance of Bifidobacterium in healthy individual's intestinal flora was relatively high (*10*). Pedersen *et al*. analyzed the difference in serum metabiome and metagenome between 75 patients with T2DM and 291 healthy individuals. They found that when the proportion of *Prevotella copri* and *Bacteroides vulgatus* increased, the content of branched-chain amino acids (BCAAs) in serum increased, which induced insulin resistance and aggravated impaired glucose tolerance (*11*). Lambeth *et al*. studied the intestinal microbiota characteristics in patients with prediabetes or T2DM and healthy individuals. Compared to patients with T2DM, the abundance of Chloracido members in the prediabetic group was high, and an unknown genus of *Pseudonocardiaceae* was identified in the prediabetic group. The abundance of *Collinsella* and an unknown genera of family Enterobacteriaceae in patients with T2DM significantly increased compared with that of the other groups (*12*). Hartstra *et al*. reported that the abundance of *Roseburia*, *Eubacterium hallii*, and *Faecalibacterium prausnitzii* decreased in patients with T2DM, whereas that of *Lactobacillus gasseri*, *Streptococcus mutans*, and *Escherichia coli* increased (*13,14*). Reitmeier *et al*. analyzed the correlation between rhythmic changes in intestinal microbes and incidence of T2DM. The study involved fecal flora data from more than 4000 people in three German cohorts. It was found that both diversity of intestinal flora and relative abundance of specific flora fluctuated

at a periodicity of 24 h, and 13 operational taxonomic units (OTUs) that affect microbial rhythm disorders in T2DM were identified, which can accurately identify and predict T2DM (*15*). Sroka-Oleksiak *et al*. analyzed the duodenal flora and multiple clinical indicators of 17 obese individuals, 22 obese patients with T2DM, and 27 healthy individuals, and the results suggested that *Bifidobacterium* may be a biomarker for the occurrence and development of T2DM and obesity in the future (*16*). Gurung *et al*. summarized 42 studies on the relationship between intestinal flora and T2DM and concluded that *Bacteroides*, *Faecalibacterium*, *Akkermansia*, and *Roseburia* negatively correlated with T2DM, whereas *Ruminococcus*, *Fusobacterium*, and *Blautia* positively correlated with T2DM (*3*). Thus, the number and diversity of intestinal flora in patients with T2DM undergo different degrees of changes, and a regular analysis of the changes in intestinal flora has realistic directive significance.

3. Prevention and treatment of T2DM based on regulating intestinal flora (Figure 1)

3.1. Probiotics and prebiotics

Probiotics are a class of active microorganisms that have beneficial effects on host health. They affect host energy and substance metabolism by improving the host microbiota (*17*). At present, there are three main types of probiotics: strictly anaerobic *Bifidobacterium*, aerotolerant *Lactobacillus*, and facultative anaerobic cocci. Probiotic functions are mainly ascribed to six main aspects: promoting digestion and absorption, enhancing immune cells, protecting intestinal mucosa, curtailing cancer risk, reducing cholesterol absorption, assisting oxidation resistance, and loosening the bowel to relieve constipation (*18,19*). Prebiotics are food components that cannot be digested or difficult to digest, and these components are beneficial to the health of the host as they selectively stimulate the proliferation and/or activity of bacteria in the colon. Probiotics play a major role in defending against pathogens, regulating immune function, increasing absorption of minerals, improving intestinal function, regulating metabolism, regulating appetite, *etc*. (*20*). Several studies have reported that prebiotics (such as fructooligosaccharide an insulin-like fructan) and probiotics (such as *Saccharomyces boulardii*) can change the composition of intestinal flora, increase the relative abundance of *Bifidobacterium* and *Lactobacillus*, and improve glucose tolerance and lipid metabolism (*21-23*). A study showed that a daily intake of a 200 mL milkshake containing 4×10^8 CFU·100 mL^{-1} *Lactobacillus acidophilus*, 4×10^8 CFU·100 mL^{-1} *Bifidobacterium*, and 10 $g \cdot L^{-1}$ fructooligosaccharides can lead to a significant decrease in blood glucose level in patients with T2DM. In another study by the same team, prebiotic supplements for pregnant women with diabetes reduced blood sugar levels during pregnancy and 12 months after delivery, lowered insulin concentrations, and improved insulin sensitivity (*24,25*). Perraudeau *et al*. conducted a 12-week intervention in 76 patients with T2DM and found that probiotic supplementation with WBF-011 (containing inulin,

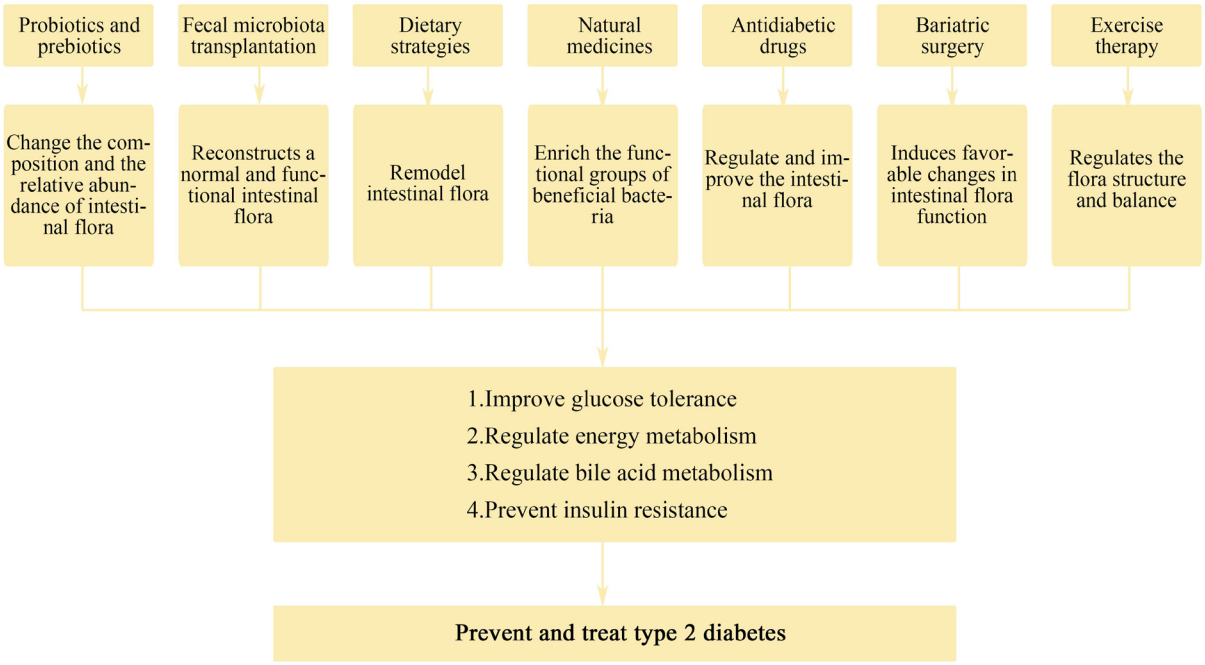

Figure 1. The possible mechanism of prevention and treatment of type 2 diabetes based on the effect of different strategies on the intestinal flora in various studies.

Akkermansia muciniphila, *Clostridium beijerinckii*, *Clostridium butyricum*, *Bifidobacterium infantis*, and *Anaerobutyricum hallii*) can significantly reduce postprandial blood glucose, HbA1c, and incremental glucose area under the curve with good safety and tolerability (*26*). Rittiphairoj *et al*. conducted a randomized controlled trial in approximately 2,000 patients with prediabetes or T2DM. The results showed that short-term or long-term probiotic use significantly reduced fasting blood glucose (FBG), HbA1c, and serum total cholesterol (TC). They also reported that probiotics more effectively reduced the HbA1c or FBG levels in patients not receiving insulin therapy (*27*). It is speculated that appropriate use of probiotics/probiotics can effectively prevent the occurrence and development of T2DM.

3.2. Fecal microbiota transplantation (FMT)

FMT has become a research hotspot in the medical field as a new treatment strategy. It is a technical system for treating intestinal diseases or extra-intestinal diseases by transplanting the functional flora from the feces of healthy people into the gastrointestinal tract of patients to reconstruct a normal and functional intestinal flora. FMT can be used to treat *Clostridium difficile* infection, irritable bowel syndrome, chronic constipation, inflammatory bowel disease, metabolic syndrome (hypertension, diabetes, fatty liver, obesity, *etc*.), autism, anxiety, depression, tumors, *etc*. (*28*). Vrieze *et al*. and Kootte *et al*. conducted controlled clinical trials to study whether the intestinal flora of thin people could improve blood glucose and lipid metabolism in men with metabolic syndrome. Six weeks after FMT, subjects who received FMT from thin people had significantly enhanced insulin sensitivity and increased abundance of intestinal flora compared with the control group. The results showed that the number of butyric acid-producing bacteria increased significantly. In addition, the subjects' plasma metabolites (such as γ-aminobutyric acid [GABA]) change, and butyric acid can regulate energy metabolism and prevent insulin resistance (*29,30*). Accordingly, FMT may be a good treatment option for patients with T2DM.

3.3. Dietary strategies

The major causes of T2DM are weight gain and abnormal visceral fat accumulation. They manifest as a large waistline and lead to metabolic syndrome and multiple complications. The control of obesity is more conducive to treat T2DM than drug intervention, and it can mitigate the progression of the disease at an early stage (*31*). Therefore, dietary therapy is key to the prevention and treatment of diabetes. Matacchione *et al*. reported that a diet rich in dietary polyphenols could effectively prevent T2DM by inhibiting the activity of α-amylase, α-glucosidase, and glucose transporters, stimulating insulin secretion, balancing hepatic glucose, preventing oxidative stress and inflammation-related hyperglycemia, and remodeling intestinal flora to improve blood glucose. The risk of developing T2DM is reduced by inhibiting or reducing intestinal transport of cholesterol and triglycerides, reducing serum cholesterol, triglyceride, and lipoprotein levels, and interacting with the synthesis and elimination of cholesterol and triglycerides to regulate lipid metabolism (*32*). Jiang *et al*. found that higher fruit intake is associated with a lower T2DM risk mediated by specific intestinal flora and metabolites. They found a correlation between fruit intake and the abundance of 31 OTUs belonging to *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, Ruminococcaceae members, *Clostridium*, *Acidaminococcus*, *Prevotella stercorea*, *Prevotella copri*, *Fusobacterium*, and *Enterobacteriaceae*. The fruit-flora index (FMI) was calculated based on the 31 OTUs associated with fruit intake. The FMI negatively correlated with the HbA1c level, and the risk of T2DM decreased by 17% for each additional unit of the FMI. Fecal metabolite sebacic acid positively associated with the FMI but negatively with T2DM risk. However, several other fecal metabolites negatively associated with the FMI were positively associated with T2DM risk. In a validation cohort of 6626 participants, T2DM risk was reduced by 10% for every additional unit of the FMI (*33*). Khursheed *et al*. reported that polysaccharides in mushrooms could play the role of prebiotics by regulating intestinal flora, metabolizing short-chain fatty acids (SCFAs) to increase the secretion of glucagon-like peptide (GLP)-1, and inhibiting gastric emptying to reduce appetite, thus playing an antidiabetic role (*34*). Therefore, cultivating good dietary habits (such as increasing the intake of fruits and vegetables) has an obvious effect on the prevention and treatment of T2DM.

3.4. Natural medicines

Xu *et al*. evaluated the clinical efficacy of different doses of *Gegen Qinlian* decoction in 187 patients with T2DM. The levels of FBG and HbA1c in patients treated with high and medium doses were significantly lower than those in the placebo and low-dose groups, and the therapeutic effect was dose-dependent. By analyzing the bacterial DNA in fecal samples of the subjects before and after treatment, they found a type of *Faecalibacterium prausnitzii*, which is closely related to the improvement of diabetes, was significantly enriched in the gut of patients after treatment. This increased bacterial load negatively correlated with levels of HbA1c and FBG, suggesting that alteration in intestinal bacteria is one of the crucial reasons for improvement in diabetes (*35*). Tong *et al*. randomly divided 450 patients with T2DM and hyperlipidemia into metformin and Chinese

herbal compound (AMC) treatment groups. After 12 weeks of treatment, 100 patients were randomly selected for evaluation of clinical efficacy. The results showed that AMC improved homeostasis model assessment of insulin resistance (HOMA-IR) and plasma triglycerides compared with metformin, and the increase in co-enriched bacteria represented by *Blautia* species was significantly associated with improvement in glycolipid homeostasis, which may improve hyperglycemia and hyperlipidemia by enriching functional groups of beneficial bacteria such as *Blautia* and *Faecalibacterium* (*36*). Tong *et al*. reported that Chinese herbal medicine and Chinese herbal prescriptions might improve glucose homeostasis and diabetes through the intestinal flora-mucosal immunity-inflammation-diabetes axis (*37*). Therefore, AMC may be beneficial to patients with T2DM, through its effect on the intestinal flora.

3.5. Antidiabetic drugs

In recent years, studies have found that some commonly used hypoglycemic drugs may regulate and improve intestinal flora in patients with T2DM to some extent, especially metformin (*38*). Metformin is recommended as a first-line oral drug to control blood glucose levels in patients with T2DM, and a meta-analysis showed that metformin increases abundance of bacteria that produce SCFAs in subjects with T2DM (*39*). Sun *et al*. treated patients newly diagnosed with T2DM with metformin and found that composition of intestinal flora changed significantly (Figure 2). *Bacteroides fragilis* decreased most significantly among other species; and levels of glycoursodeoxycholic acid (GUDCA) and tauroursodeoxycholic acid (TUDCA) in the gut increased. GUDCA and TUDCA are farnesoid X receptor (FXR) antagonists, and metformin increases the GUDCA level by reducing abundance of *Bacteroides fragilis* to inhibit activity of bile salt hydrolase. Metformin inhibits intestinal FXR signals independently of intestinal adenosine monophosphate-activated protein kinase, significantly increases the production of active GLP-1, and improves glucose homeostasis (*40*). According to the results of Forslund *et al*., metformin can improve the intestinal flora of patients with T2DM, promote the production of SCFAs such as butyric acid and propionic acid, stimulate intestinal gluconeogenesis, and increase insulin sensitivity in the body (*41*). In a clinical trial, 95 patients with T2DM were randomly divided into two groups: group A received acarbose 150 mg/d and group B received the same treatment as group A but without acarbose. The results showed that compared with group B, intestinal *Bifidobacterium* increased, and serum lipopolysaccharides and prothrombin activator inhibitor 1 significantly decreased in group A. Acarbose therapy can significantly increase intestinal *Bifidobacterium* in patients with T2DM and reduce the levels of some inflammatory factors besides

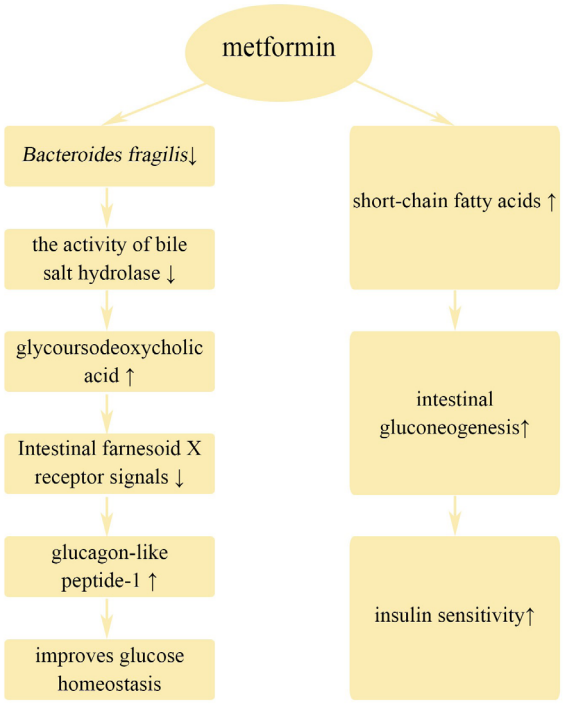

Figure 2. The mechanism of metformin in the treatment of diabetes by regulating intestinal flora. ↑:increase; ↓:decrease.

reducing blood glucose (*42*). Gu *et al*. randomly divided 106 patients with new-onset T2DM into two groups: those treated with acarbose 300 mg/d or glipizide 5-10 mg/d for 3 months. The results showed that acarbose was superior to glipizide in reducing glucose and lipid levels, body weight, and insulin resistance. Treatment with acarbose significantly increased the abundance of various probiotics (such as *Bifidobacterium* and *Lactobacillus*) and substantially reduced the abundance of *Clostridium* and *Bacteroides*, while there was no significant change in the glipizide group before and after treatment. Acarbose likely regulates glucose and lipid metabolism by changing the bile acid (BA) metabolism of intestinal microbes and affecting the host BA signal, thus achieving benefits other than the hypoglycemic effect (*43*). Accordingly, when choosing antidiabetic drugs, a more beneficial treatment plan for patients can be developed by taking into account the effects of drugs on intestinal flora.

3.6. Bariatric surgery

Linner and Kremen performed the world's first jejunoileal bypass surgery in 1954, pioneering the surgical treatment for obesity. A large number of randomized clinical trials comparing various surgical interventions with non-surgical interventions for diabetes have consistently demonstrated the former's advantage in improving all glucose variables and other metabolic aspects (*44*). At present, the widely accepted surgical procedures in metabolic and bariatric surgery include

laparoscopic sleeve gastrectomy (LSG), laparoscopic Roux-en-Y gastric bypass (LRYGB), and biliopancreatic diversion with duodenal switch. Among them, LSG and LRYGB are the most common types of bariatric surgery. For patients with T2DM, bariatric surgery plus medication is more effective than medical therapy alone. Magouliotis *et al*. found that after metabolic surgery, patients' blood glucose, insulin, triglyceride, TC, low-density lipoprotein, and HDL levels; HOMA-IR; food intake; and the rate of diabetes remission were significantly improved. Postoperatively, the levels of BCAA decreased, whereas those of trimethylamine-N-oxide (TMAO), GLP-1, GLP-2, and peptide YY (PYY) increased (*45*). LRYGB and LSG can reduce blood glucose level and body weight in obese patients with T2DM and increase *Roseburia* species. Compared with LSG, LRYGB can induce more favorable changes in intestinal flora function. LRYGB led to an increase in the abundance of Firmicutes and Actinobacteria phyla and a decrease in Bacteroidetes phyla, whereas LSG led to an increase in the abundance of Bacteroidetes phyla (*46*). Reports have suggested that bariatric surgery may change the microbiome; furthermore, it has been suggested that intestinal flora may play a role by increasing the absorption of energy in the diet and altering signaling pathways of metabolism and appetite. Clinical studies have shown that circulating BA concentration increases after bariatric surgery, and the total circulating BA concentration in LRYGB patients positively correlates with serum GLP-1 concentration and negatively correlates with postprandial blood glucose concentration. The increased concentration of circulating BA may be due to changes in the anatomical structure and intestinal microbiota caused by bariatric surgery (*47*). Bariatric surgery may be one of the most effective treatments for T2DM.

3.7. Exercise therapy

Exercise therapy in diabetes is mainly suitable for patients with mild and moderate T2DM, especially in obese individuals with T2DM. Exercise can increase the abundance of beneficial intestinal flora and regulate the flora structure and balance. It also significantly enhances the intestinal flora's ability to synthesize SCFAs, decompose BCAAs, and promote secretion of hormone PYY. Furthermore, it inhibits appetite and plays a key role in weight loss, improving insulin resistance, and regulating blood glucose. Low-grade inflammation is a characteristic of T2DM. Moderate running can prevent excessive activation of the immune system, and regular exercise such as endurance training in the form of stationary exercise bikes, aerobics, dumbbell, and other forms, and flexibility training, such as static stretching, can significantly improve the index of glycolipid metabolism and inflammation in patients with T2DM. In addition, the abundance of fungi, *Candida albicans*, and mycotoxins can be significantly reduced by exercise without affecting beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* (*48*). Liu *et al*. randomly divided 39 overweight men with prediabetes into two groups: one group performed 12 weeks of intense exercise and the other group maintained a sedentary lifestyle. After 12 weeks of one-to-one exercise training, all participants had significant and comparable reductions in body weight and body fat percentage without any drug and dietary interventions, while the individual differences in fasting glucose, insulin, and HOMA-IR were significant. Further studies showed that the ability to synthesize SCFAs and GABA and decompose BCAAs was significantly enhanced in the intestinal flora of exercise responders. Whereas, the intestinal flora of non-responders synthesized a large number of products that were not conducive to glucose metabolism, such as BCAAs and aromatic amino acids. These results suggest that intestinal flora and its metabolites mediate improvement in insulin sensitivity and glucose homeostasis by exercise. They are expected to be biomarkers for evaluating and predicting the efficacy of exercise intervention in future studies. Thus, intestinal flora intervention may help maximize the health benefits of exercise (*49*).

4. Summary and outlook

A growing number of studies have shown that intestinal flora is closely related to occurrence and development of T2DM. Intestinal flora may be involved in T2DM pathogenesis *via* multiple metabolic pathways, such as those mediated by SCFAs, BA metabolism, endotoxin, and TMAO. Regulating intestinal flora by bariatric surgery, probiotics, FMT, exercise, establishing healthy eating habits, antidiabetic drugs and other methods can improve insulin resistance and regulate blood glucose. Although new therapeutic methods are being developed, many uncertainties and potential risks must be accounted for, warranting further investigation.

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References

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga

S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K, Shaw JE, Bright D, Williams R; IDF Diabetes Atlas Committee. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019; 157:107843.
2. Taddeo EP, Alsabeeh N, Baghdasarian S, *et al*. Mitochondrial Proton Leak Regulated by Cyclophilin D Elevates Insulin Secretion in Islets at Nonstimulatory Glucose Levels. Diabetes. 2020; 69.
3. Gurung M, Li Z, You H, Rodrigues R, Jump DB, Morgun A, Shulzhenko N. Role of gut microbiota in type 2 diabetes pathophysiology. EBioMedicine. 2020; 51:102590.
4. Junjie Q, Ruiqiang L, Jeroen R, *et al*. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010; 464:59-65.
5. Zhang X, Shen D, Fang Z, Jie Z, Qiu X, Zhang C, Chen Y, Ji L. Human Gut Microbiota Changes Reveal the Progression of Glucose Intolerance. PloS One. 2013; 8:e71108.
6. Lilit E, Daria K, Anna P, Olga T, Alexander T, Dmitry A, Natalia K, Elena K, Vladislav B, Maria V, Sergey B. Gut microbiota and diet in patients with different glucose tolerance. Endocr Connect. 2016; 5:1-9.
7. Larsen N, Vogensen FK, Berg FWJvd, Nielsen DS, Andreasen AS, Pedersen BK, Al-Soud WA, Sørensen SJ, Hansen LH, Jakobsen M. Gut Microbiota in Human Adults with Type 2 Diabetes Differs from Non-Diabetic Adults. PloS One. 2010; 5:e9085.
8. Wu X, Ma C, Han L, Nawaz M, Gao F, Zhang X, Yu P, Zhao Ca, Li L, Zhou A, Wang J, Moore JE, Millar BC, Xu J. Molecular Characterisation of the Faecal Microbiota in Patients with Type II Diabetes. Curr Microbiol. 2010 ;61:69-78.
9. Karlsson FH, Tremaroli V, Nookaew I, Bergström G, Behre CJ, Fagerberg B, Nielsen J, Bäckhed F. Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature. 2013; 498:99-103.
10. Sedighi M, Razavi S, Navab-Moghadam F, Khamseh ME, Alaei-Shahmiri F, Mehrtash A, Amirmozafari N. Comparison of gut microbiota in adult patients with type 2 diabetes and healthy individuals. Microb Pathog. 2017; 111:362-369.
11. Pedersen HK, Gudmundsdottir V, Nielsen HB, *et al*. Human gut microbes impact host serum metabolome and insulin sensitivity. Nature. 2016; 535:376-381.
12. Lambeth SM, Carson T, Lowe J, Ramaraj T, Leff JW, Luo L, Bell CJ, Shah VO. Composition, Diversity and Abundance of Gut Microbiome in Prediabetes and Type 2 Diabetes. J Diabetes Obes. 2015; 2:1-7.
13. McLean MH, Dieguez D Jr, Miller LM, Young HA. Does the microbiota play a role in the pathogenesis of autoimmune diseases? Gut. 2015; 64:332-341.
14. Hartstra AV, Bouter KE, Bäckhed F, Nieuwdorp M. Insights into the role of the microbiome in obesity and type 2 diabetes. Diabetes Care. 2015; 38:159-165.
15. Reitmeier S, Kiessling S, Clavel T, *et al*. Arrhythmic Gut Microbiome Signatures Predict Risk of Type 2 Diabetes. Cell Host Microbe. 2020; 28:258-272.e6.
16. Sroka-Oleksiak A, Młodzińska A, Bulanda M, Salamon D, Major P, Stanek M, Gosiewski T. Metagenomic Analysis of Duodenal Microbiota Reveals a Potential Biomarker of Dysbiosis in the Course of Obesity and Type 2 Diabetes: A Pilot Study. J Clin Med. 2020; 9:369.
17. McFarland LV. From yaks to yogurt: the history, development, and current use of probiotics. Clin Infect Dis. 2015; 60 Suppl 2:S85-90.
18. Palacios T, Vitetta L, Coulson S, Madigan CD, Denyer GS, Caterson ID. The effect of a novel probiotic on metabolic biomarkers in adults with prediabetes and recently diagnosed type 2 diabetes mellitus: study protocol for a randomized controlled trial. Trials. 2017; 18:7.
19. Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP, Shulman GI, Gordon JI, Hoffman HM, Flavell RA. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature. 2012; 482:179-185.
20. Sanders ME, Merenstein DJ, Reid G, Gibson GR, Rastall RA. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. Nat Rev Gastroenterol Hepatol. 2019; 16:605-616.
21. Everard A, Lazarevic V, Derrien M, Girard M, Muccioli GG, Neyrinck AM, Possemiers S, Van Holle A, François P, de Vos WM, Delzenne NM, Schrenzel J, Cani PD. Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. Diabetes. 2011; 60:2775-2786.
22. Everard A, Matamoros S, Geurts L, Delzenne NM, Cani PD. Saccharomyces boulardii administration changes gut microbiota and reduces hepatic steatosis, low-grade inflammation, and fat mass in obese and type 2 diabetic db/db mice. mBio. 2014; 5:e01011-14.
23. Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, Gibson GR, Delzenne NM. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia. 2007; 50:2374-2383.
24. Moroti C, Souza Magri LF, de Rezende Costa M, Cavallini DC, Sivieri K. Effect of the consumption of a new symbiotic shake on glycemia and cholesterol levels in elderly people with type 2 diabetes mellitus. Lipids Health Dis. 2012; 11:29.
25. Laitinen K, Poussa T, Isolauri E; Nutrition, Allergy, Mucosal Immunology and Intestinal Microbiota Group. Probiotics and dietary counselling contribute to glucose regulation during and after pregnancy: a randomised controlled trial. Br J Nutr. 2009; 101:1679-1687.
26. Perraudeau F, McMurdie P, Bullard J, *et al*. Improvements to postprandial glucose control in subjects with type 2 diabetes: a multicenter, double blind, randomized placebo-controlled trial of a novel probiotic formulation. BMJ Open Diabetes Res Care. 2020; 8:e001319.
27. Rittiphairoj T, Pongpirul K, Janchot K, Mueller NT, Li T. Probiotics Contribute to Glycemic Control in Patients with Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis. Adv Nutr. 2021;1 2:722-734.
28. Borody TJ, Khoruts A. Fecal microbiota transplantation and emerging applications. Nat Rev Gastroenterol Hepatol. 2011; 9:88-96.
29. Kootte RS, Levin E, Salojärvi J, *et al*. Improvement of Insulin Sensitivity after Lean Donor Feces in Metabolic Syndrome Is Driven by Baseline Intestinal Microbiota Composition. 2017; 26:611-619.e6.
30. Vrieze A, Nood EV, Holleman F, *et al*. Transfer of

Intestinal Microbiota From Lean Donors Increases Insulin Sensitivity in Individuals With Metabolic Syndrome. Gastroenterology. 2012; 143:913-916.e7.
31. Lean MEJ. Low-calorie diets in the management of type 2 diabetes mellitus. Nat Rev Endocrinol. 2019; 15:251-252.
32. Matacchione G, Gurău F, Baldoni S, Prattichizzo F, Silvestrini A, Giuliani A, Pugnaloni A, Espinosa E, Amenta F, Bonafè M, Procopio AD, Rippo MR, Olivieri F, Sabbatinelli J. Pleiotropic effects of polyphenols on glucose and lipid metabolism: Focus on clinical trials. Ageing Res Rev. 2020; 61:101074.
33. Jiang Z, Sun TY, He Y, *et al*. Dietary fruit and vegetable intake, gut microbiota, and type 2 diabetes: results from two large human cohort studies. BMC Med. 2020; 18:371.
34. Khursheed R, Singh SK, Wadhwa S, Gulati M, Awasthi A. Therapeutic potential of mushrooms in diabetes mellitus: Role of polysaccharides. Int J Biol Macromol. 2020; 164:1194-1205.
35. Xu J, Lian F, Zhao L, Zhao Y, Chen X, Zhang X, Guo Y, Zhang C, Zhou Q, Xue Z, Pang X, Zhao L, Tong X. Structural modulation of gut microbiota during alleviation of type 2 diabetes with a Chinese herbal formula. ISME J. 2015; 9:552-562.
36. Tong X, Xu J, Lian F, *et al*. Structural Alteration of Gut Microbiota during the Amelioration of Human Type 2 Diabetes with Hyperlipidemia by Metformin and a Traditional Chinese Herbal Formula: a Multicenter, Randomized, Open Label Clinical Trial. mBio. 2018; 9:e02392-17.
37. Gao Z, Li Q, Wu X, Zhao X, Zhao L, Tong X. New Insights into the Mechanisms of Chinese Herbal Products on Diabetes: A Focus on the "Bacteria-Mucosal Immunity-Inflammation-Diabetes" Axis. J Immunol Res. 2017; 2017:1813086.
38. Morita Y, Nogami M, Sakaguchi K, Okada Y, Hirota Y, Sugawara K, Tamori Y, Zeng F, Murakami T, Ogawa W. Enhanced Release of Glucose Into the Intraluminal Space of the Intestine Associated With Metformin Treatment as Revealed by [18F]Fluorodeoxyglucose PET-MRI. Diabetes Care. 2020; 43:1796-1802.
39. McCreight LJ, Bailey CJ, Pearson ER. Metformin and the gastrointestinal tract. Diabetologia. 2016; 59:426-435.
40. Sun L, Xie C, Wang G, *et al*. Gut microbiota and intestinal FXR mediate the clinical benefits of metformin. Nat Med. 2018; 24:1919-1929.
41. Forslund K, Hildebrand F, Nielsen T, *et al*. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature. 2015; 528:262-266.
42. Su B, Liu H, Li J, Sunli Y, Liu B, Liu D, Zhang P, Meng X. Acarbose treatment affects the serum levels of inflammatory cytokines and the gut content of bifidobacteria in Chinese patients with type 2 diabetes mellitus. J Diabetes. 2015; 7:729-739.
43. Gu Y, Wang X, Li J, *et al*. Analyses of gut microbiota and plasma bile acids enable stratification of patients for antidiabetic treatment. Nat Commun. 2017; 8:1785.
44. Cummings DE, Rubino F. Metabolic surgery for the treatment of type 2 diabetes in obese individuals. Diabetologia. 2018; 61:257-264.
45. Magouliotis DE, Tasiopoulou VS, Sioka E, Chatedaki C, Zacharoulis D. Impact of Bariatric Surgery on Metabolic and Gut Microbiota Profile: a Systematic Review and Meta-analysis. Obes Surg. 2017; 27:1345-1357.
46. Murphy R, Tsai P, Jüllig M, Liu A, Plank L, Booth M. Differential Changes in Gut Microbiota After Gastric Bypass and Sleeve Gastrectomy Bariatric Surgery Vary According to Diabetes Remission. Obes Surg. 2017; 27:917-925.
47. Kaska L, Sledzinski T, Chomiczewska A, Dettlaff-Pokora A, Swierczynski J. Improved glucose metabolism following bariatric surgery is associated with increased circulating bile acid concentrations and remodeling of the gut microbiome. World J Gastroenterol. 2016; 22:8698-8719.
48. Pasini E, Corsetti G, Assanelli D, Testa C, Romano C, Dioguardi FS, Aquilani R. Effects of chronic exercise on gut microbiota and intestinal barrier in humans with type 2 diabetes. Minerva Med. 2019; 110:3-11.
49. Liu Y, Wang Y, Ni Y, Cheung CKY, Lam KSL, Wang Y, Xia Z, Ye D, Guo J, Tse MA, Panagiotou G, Xu A. Gut Microbiome Fermentation Determines the Efficacy of Exercise for Diabetes Prevention. Cell Metab. 2020; 31:77-91.e5.

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Review

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Metformin acts on the gut-brain axis to ameliorate antipsychotic-induced metabolic dysfunction

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SUMMARY Antipsychotic-induced metabolic dysfunction (AIMD) is an intractable clinical challenge worldwide. The situation is becoming more critical as second-generation antipsychotics (SGAs), to a great extent, have replaced the role of first-generation antipsychotics in managing major psychiatric disorders. Although the exact mechanisms for developing AIMD is intricate, emerging evidence has indicated the involvement of the microbiota-gut-brain axis in AIMD. SGAs treatment may change the diversity and compositions of intestinal flora (*e.g.*, decreased abundance of *Bacteroidetes* and *Akkermansia muciniphila*, and increased *Firmicutes*). Short-chain fatty acids and other metabolites derived from gut microbiota, on the one hand, can regulate the activity of intestinal endocrine cells and their secretion of satiety hormones (*e.g.*, glucagon-like peptide 1, peptide YY, cholecystokinin and ghrelin); on the other hand, can activate the vagus nerve or transport into the brain to exert a central modulation of foraging behaviors via binding to neuropeptide receptors. Interestingly, metformin, a classical antidiabetic agent, is capable of alleviating AIMD possibly by regulating the microbiota-gut-brain axis. That is, metformin can not only partially reverse the alterations of gut microbial communities due to SGAs treatment, but also play a positive role in rectifying the disturbances of peripheral and central satiety-related neuropeptides. Current evidence has indicated a promising role for metformin on ameliorating AMID, but further verifications in well-designed clinical trials are still warranted.

Keywords antipsychotic-induced metabolic dysfunction, gut-brain axis, gut microbiota, metformin, hypothalamus, neuropeptide

1. Introduction

In recent decades, second-generation antipsychotics (SGAs) have gradually replaced classic antipsychotics as the first-line choice for treatment of psychotics and related disorders due to their favorable therapeutic outcomes, fewer extrapyramidal events, and lower recurrence rate (*1*). However, many animal and human studies have found that taking SGAs (especially olanzapine [OLZ] and risperidone [RIS]) can cause significant weight gain and metabolic dysfunction (*2,3*). The longer the drug is taken, the more severe the metabolic side effect seems to be (*3,4*), and first-time users of SGAs are more likely to gain weight (*5*). Antipsychotic-induced metabolic dysfunction (AIMD), such as obesity and insulin resistance, also leads to decreased treatment compliance, which makes the treatment more difficult and increases treatment cost. Clinical studies have shown that metformin combined with SGAs can effectively ameliorate AIMD (*6*), but its mechanism is not fully elucidated. Interestingly, consumption of non-antibiotics, such as antidiabetics (metformin) and SGAs, has been associated with alterations in gut microbiome composition, which may be attributed to their antibiotic-like effects (*7*). Joint actions of metformin and antipsychotics not only can affect the composition and metabolism of intestinal microbes, but also modulate the expression of neuropeptides and neurotransmitter receptors in the brain, which may help to alleviate AIMD. In this

review, we discuss the AIMD induced by SGAs, its relationship with the microbiota-gut-brain axis, and the potential mechanisms of metformin in alleviating AIMD *via* the gut-brain axis.

2. Second-generation antipsychotic-induced metabolic disorder

Antipsychotic drugs are mainly used in managing mental disorders with psychotic symptoms, such as schizophrenia, schizoaffective psychosis and mania. According to the time sequence of their emergence and pharmacological characteristics, antipsychotics are mainly classified into two categories, SGAs and first-generation antipsychotics (FGAs). The former gradually replaces FGAs because of their less extrapyramidal adverse effects within the treatment dosage. SGAs exert antipsychotic effects predominately by blocking dopamine 2 (D_2) receptor and 5-hydroxytryptamine 2A ($5\text{-}HT_{2A}$) receptor. However, accumulating studies have indicated that patients receiving antipsychotic treatment encountered an increased risk of developing metabolic abnormalities (such as weight gain and type 2 diabetes) (*2*). Albeit following a short treatment period (≤ 6 weeks), the weight of patients receiving antipsychotics for the first time increased significantly (*5*). In this study, the average weight gain of OLZ, quetiapine and RIS treatment was 3.42 kg, 1.91 kg and 2.68 kg, respectively (*5*). Based on an integrated clinical trial database, a study compared short-term (4-12 weeks, N = 1,742) versus long-term (1 year, N = 1,649) effects of different antipsychotics on patient weight within a standard treatment dose, and found significant differences among different SGAs (*3*). Compared to placebo, patients receiving OLZ or RIS had a greater incidence of weight gain, but not in those with haloperidol or ziprasidone treatment (3). Median weight increase per month was similar for the short-term and long-term exposure cohorts (*3*). Another meta-analysis including 11 studies also investigated the difference of short-term and long-term antipsychotic treatment on weight gain (*4*). In this study, drug-free patients were chosen for comparison, and antipsychotic drug exposure duration was selected for three periods (4-8 weeks, 10-12 weeks, and 24-48 weeks). Compared to short-term intervention, long-term consumption of antipsychotic drugs could result in more apparent weight gain (*4*). A more recent meta-analysis of 150 double-blind studies found that OLZ, chlorine azepine, amisulpride, and RIS were superior to FGAs in overall efficacy, and patients taking OLZ and RIS had a lower probability of recurrence than those taking FGAs. The incidence of extrapyramidal effects caused by SGAs was also lower than haloperidol, a classic FGA. However, compared with haloperidol, the weight gain effects of OLZ, clozapine, amisulpride, RIS, and quetiapine were apparently more significant (*8*). Similar findings were shown in other studies that clozapine and OLZ had the highest risk of weight gain, and other drugs (*e.g.*, RIS, quetiapine, and amisulpride) had a relatively low or moderate risk, while ziprasidone and aripiprazole (ARI) showed lowest risk (*9*). Interestingly, compared to those who were in a chronic course, patients diagnosed with first-episode psychotic disorder (FEP) were more likely to have weight gain (*10*). Taken together, these findings reveal that AIMD is a highly prevalent and inevitable clinical challenge. Although the mechanisms underlying AIMD were not fully understood, recently more and more pieces of evidence have suggested that AIMD may be closely related to the gut-brain axis.

3. Metabolism and Gut-brain axis

Accumulating evidence has shown that the densest flora niches in the human gut participate in regulating brain function and even host behavior. The two-way communication between the brain and intestinal flora, that is, the flora-gut-brain axis, operates predominantly through microbe-derived metabolites, vagus nerve, neuroimmune and neuroendocrine pathways. The afferent fibers of sympathetic and parasympathetic nerves link directly to the brain. Intestinal epithelial cells, including intestinal endocrine cells, keep close contact with the intestinal flora (*11,12*). For instance, metabolites of intestinal flora affect the secretion of satiety hormones by intestinal endocrine cells, such as glucagon-like peptide 1 (GLP-1), peptide YY (PYY), and cholecystokinin (CCK) (*12*). These hormones on the one hand activate the vagus nerve, transmit signals to the hypothalamus to control eating behavior, and on the other hand, enter the circulatory system to regulate eating behavior and other physiological processes. Studies in mice also showed that PYY and CCK could enhance feeding behavior (*13*).

To date, adequate shreds of evidence have shown that gut microbiota constitutes an important part of the gut-brain axis regulation. The main gut flora can be divided into five categories: *Bacteroidetes*, *Firmicutes*, *Verrucomicrobia*, *Proteobacteria*, and *Actinobacteria*. For healthy individuals, *Bacteroidetes* and *Firmicutes* are the main microbiota producing short-chain fatty acids (SCFAs) and occupy a major position in the gastrointestinal tract (GIT). SCFAs are one of the main bioactive metabolites produced in the gut (*14*). Acetate and propionate are mainly produced by *Bacteroidetes* phyla, whereas butyrate is mainly produced by *Firmicutes*. SCFAs influence lipid, glucose, and cholesterol metabolism in various tissues (*15*). Several clinical and preclinical studies indicate that SCFAs can regulate the release of satiety hormones in the GIT and manipulate the expression of anorectic neuropeptide (*e.g.*, proopiomelanocortin [POMC], neuropeptide Y [NPY], and agouti-related peptide [AgRP]) in the brain (*16-20*). Hunger and satiety signals initiate in the GIT and are delivered ascendingly into the hypothalamus.

Ghrelin, one of the "hunger hormones", is secreted in the GIT and bound to its receptor in the hypothalamus to regulate appetite. Leptin, another satiety hormone functionally opposite to ghrelin, also acts on its receptor in the hypothalamus to regulate energy consumption. Therefore, gut microbiota may be critical for maintaining metabolic homeostasis *via* the gut-brain axis regulation (*21)*.

4. Mechanisms of AIMD

4.1. AIMD and gut microbiota

Pre-clinic studies have found that traditional mice treated with OLZ and high-fat diet had more significant weight gain than those treated with high-fat diet only, but no significant difference was found in the same treatment of sterile mice (*22*). Transplanting feces from RIS-treated mice to traditional mice can cause significant weight gain and decrease in total resting metabolic rate compared to those without fecal transplantation (*23*). These preliminary findings suggest that gut microbiota is involved in the weight gain caused by antipsychotic drugs.

Antipsychotic treatment may cause alterations in the compositions of gut microbiota. It was found that the fecal abundance of *Actinobacteria* and *Proteobacteria* in patients receiving OLZ was decreased, accompanied by an increased *Firmicutes*/*Bacteroidetes* (*F/B*) ratio (*24*). An animal study in mice also found *Erysipelotrichi* (*Firmicutes* phylum) and *Gammaproteobacteria* (*Proteobacteria* phylum) was increased, and *Bacteroidia* (in *Bacteroidetes* phylum) was decreased in the OLZ-treated group. In addition, approximately 0.71% of body weight gain was accompanied by a 1% increase of abundance in *Erysipelotrichi*, indicating that *Erysipelotrichi* may mediate OLZ-induced weight gain (*22*). Bahr *et al*. compared flora composition, energy intake and consumption and weight between RIS-treated and placebo-treated female mice (*23*). They found that RIS treatment caused an increase in weight, together with alterations in the microbiome composition. The abundance of *Firmicutes* in RIS-treated mice increased by 32.6%, while the abundance of *Bateroidetes* decreased by 22.4%. Under *Firmicutes* phylum, compared with control mice, the abundance of *Lactobacillus* in mice treated with RIS was reduced, while *Allobaculum* was increased. Under *Bacteroidetes* phylum, RIS-treated mice had decreased *Alistipes* species and increased *Bacteroides* species (*23*). Flowers *et al*. also found that gut *Alistipes* was preferentially increased in patients with bipolar disorder or schizophrenia free from atypical antipsychotics (*25*). Interestingly, some studies found that AIMD could be alleviated by antibiotic treatment. The number of *Firmicutes* in the rats treated with antibiotics and OLZ was decreased and the number of Bacteroides increased, while the number of *Firmicutes* in rats treated with OLZ alone was increased and Bacteroides was decreased (*26*). These findings indicate that the increased abundance of *Firmicutes* and decreased abundance of *Bateroidetes* may help explain why SGAs can induce weight gain. Other clinical studies found that in patients treated with RIS, the weight, gut microbial diversity, *F/B* ratio, the abundance of *Escherichia coli* and *Bifidobacterium* were significantly increased, but the abundance of *Lactobacillus* and *Clostridium coccoides* group was decreased compared to that of the untreated group (*27,28*). Another study has found that the *Lachnospiraceae* abundance was increased, whereas the *Akkermansia* abundance was decreased in patients receiving SGAs (*29*). Studies of different age groups treated with RIS all reported an increase in the *F/B* ratio (*28-30*). Notably, a newly published animal study revealed that *Akkermansia muciniphila* could be a promising probiotic agent for alleviating systemic inflammation and OLZ-induced insulin resistance and hyperglycemia *via* regulation of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase - key enzymes in hepatic gluconeogenesis (*31*). Therefore, preclinical and clinical studies congruously observed the role of gut microbiota on AIMD, but its biological mechanism needs to be further elucidated.

4.2. AIMD and neurotransmitter receptors

SGAs act not only on dopamine receptors (DRs) in the brain but also on adrenergic α, cholinergic M1, histamine (H), and serotonin receptors. Through the comparison of blood glucose, DRs expression levels, and the weight between Roman high-avoidance or Roman low-avoidance mice treated with OLZ and traditional mice, Evers *et al*. found that only Roman high-avoidance mice showed a substantial increase in blood glucose, DRs expression, and weight (*30*), suggesting that AIMD may be associated with the expression of DRs. OLZ-induced weight gain and hyperphagia was blunted in serotonin 2C receptor (HTR_{2C}) knockout mice or treatment with the HTR2C-specific agonist (*32*). Another study found that in mice treated with OLZ, but not aripiprazole or haloperidol, showed a significant increase in weight and foraging behavior, while the binding density of the histamine receptor (HR) in the arcuate hypothalamic nucleus was decreased, suggesting that AIMD was also associated with H1 receptors *(33,34*).

White adipose tissue is now perceived as an endocrine organ that can release various peptides, such as adiponectin, leptin, resistin and visfatin, which are related to weight gain caused by SGAs. Elevated serum insulin levels were presented even in antipsychotic-free FEP patients, along with alterations in appetite regulating hormones, indicating predisposed metabolic dysfunction in FEP patients before antipsychotic treatment (*35*). Besides, SGAs also affect the expression of neuropeptides. OLZ treatment not only influenced body

weight, blood glucose level, and fatty acid community activity, but also up-regulated neuropeptide Y (NPY) mRNA expression and down-regulated POMC mRNA expression (*34*). Clinical studies have also found that AIMD is associated with the polymorphism of satiety-related hormone receptor genes. For example, one study reported that the blood leptin level was increased while ghrelin was decreased in AIMD patients (*36*). These findings indicate that several important neuropeptides are linked to AIMD.

Based on the above evidence, we speculate that, on the one hand, SGAs may affect the production of SCFAs in the intestine by changing the composition of intestinal flora. SCFAs mediate the secretion of PYY, GLP-1, CCK, and other peptides in intestinal endocrine cells, which activate the vagus nerve or transport through the blood-brain barrier (BBB), and regulate the expression of NPY receptors and POMC receptors in the hypothalamus, thus controlling foraging behavior. On the other hand, SGAs control foraging behavior by altering production of intestine-derived leptin and ghrelin and the expression of corresponding receptors in the brain. Summarized findings from animal and human studies related to mechanisms of AIMD in the gut-brain axis are listed in Table 1.

5. Metformin alleviates AIMD

Metformin, a biguanide component, is the first-line drug for type 2 diabetes. Metformin directly regulates the metabolism of glucose, promotes anaerobic fermentation of sugars, and increases absorption and utilization of glucose in peripheral tissues, such as muscle and fat. Besides, it can increase insulin sensitivity. Previous studies have shown that metformin can cause weight loss (*37*). An animal study found that metabolic dysfunction, including hyperglycemia, hyperlipidemia, insulin resistance, and white fat accumulation, was potently attenuated in rodents treated with both metformin and SGA compared with those with SGA only (*6*). Clinical studies also revealed that metformin combined with SGA could alleviate the problems of weight gain, body mass index (BMI) increase, hyperglycemia, and hyperlipidemia induced by SGA alone in pediatrics, adolescents, and adults as well, regardless of first-time treatment or retreatment (*38-40*). A systematic review and meta-analysis including 7 studies with 151 cases of antipsychotics plus metformin and 154 cases of antipsychotics plus placebo, found that weight and BMI were significantly reduced with the metformin supplement (*41*). Another meta-analysis on metformin for clozapine-associated obesity again found that in terms of weight loss (-3.12 kg, 95% CI: -4.88 kg to -1.37 kg) or BMI (-1.18 kg/m^2, 95%:CI: -1.76 kg/m^2 to -0.61 kg/m^2), metformin was overall superior to placebo (40). Similar results have been documented on metformin supplement in overweight patients with RIS treatment (*42*). Other meta-analytical evidence showed that in

Table 1. Animal and human studies related to mechanisms of AIMD in the gut-brain axis

Author, year	Drugs	Study subjects	Results	*Ref.*
Morgan, 2014	OLZ	Mouse	Traditional mice treated with OLZ and high-fat diet had more significant weight gain.	*22*
Bahr, 2015	RIS	Mouse	Weight gain and total resting metabolic rate decreased in mice that received fecal transplants from RIS-treated mice.	*23*
Davey, 2012	OLZ	Patient	*Actinobacteria* and *Proteobacteria* ↓, *F/B* ↑	*24*
Flowers, 2019	Antipsychotics	Patient	*Alistipes* ↓	*25*
Davey, 2013	OLZ	Rat	*Firmicutes* ↑ and *Bacteroidetes* ↓	*26*
Bahr, 2015	RIS	Female mouse	*Firmicutes* abundance (*Lactobacillus* species and *Allobaculum*) ↑, *Bateroidetes* abundance (*Bacteroides* species and *Alistipes* species) ↓	*23*
Bahr, 2015; Flowers, 2017	RIS	Patient	Weight ↑, Gut microbiota diversity ↑, *F/B* ↑, *Bifidobacterium* ↑, *Escherichia coli* ↑, *Clostridium coccoides* group and *Lactobacillus* ↓	*27* *28*
Yuan, 2018	RIS	Patient	*Lachnospiraceae* ↑, *Akkermansia* ↓	*29*
Evers, 2017	OLZ	Mouse	Roman high-avoidance mice showed increased body weight, blood glucose, and dopaminergic expression levels	*30*
Huang, 2021	OLZ	Mouse	*Akkermansia muciniphila* improves OLZ-related hyperglycemia and insulin resistance	*31*
Lord, 2017	OLZ	Mouse	AIMD was related to HTR_{2C}	*32*
Han, 2008	OLZ, RIS, ARI	Mouse	Weight and feeding behavior increased significantly in mice treated with OLZ or RIS, not ARI, while the binding density of the histamine receptor significantly decreased	*33*
Lian, 2014	OLZ, RIS, ARI	Rat	NPY mRNA expression ↑, POMC mRNA expression ↓	*34*
Potvin, 2015	Antipsychotics	Patient	Leptin ↑, ghrelin ↓	*36*

Abbreviations: OLZ, olanzapine; RIS, risperidone; ARI, aripiprazole; *F/B*, *Firmicutes*/*Bacteroidetesin*; AIMD, antipsychotic-induced metabolic dysfunction; HTR2C, serotonin 2C receptor; POMC, proopiomelanocortin; AMPK, AMP-activated protein kinase; NPY, Neuropeptide Y.

children and adolescents, whose weight was increased due to SGAs treatment, after 4, 12, or 16 weeks of continuous treatment with metformin, the weight of the metformin group was significantly reduced compared to placebo (*43*). Based on a rat model of schizophrenia established by consecutive injections of MK801 during the neurodevelopmental period, Luo *et al*. found that metformin attenuated OLZ- and RIS-induced metabolic dysfunctions in these rats without weakening the therapeutic effects of the antipsychotics (*44*). These clinical and pre-clinical findings together indicate the favorable outcome of metformin in managing AMID.

6. Mechanisms of metformin ameliorating AIMD

6.1. Metformin and gut microbiota

Previous studies have indicated that metformin plays its role in treating metabolic dysfunction possibly by changing the composition of gut microbiota. A study by Na-Ri Shin *et al*. showed that metformin could cause an increase in *Akkermansia* spp. population, indicating metfomin may regulate blood glucose *via* altering intestinal microbial composition (*45*). Meanwhile, Lee H *et al*. found that metformin treatment enriched *A. muciniphila in vitro*, which was in agreement with the results reported in *in vivo* mouse models. Therefore, metformin perhaps affects metabolic processes by increasing the *Akkermansia* spp. population (*46*). In addition, another study identified 22 enriched microbes and 24 depleted microbes in healthy mice treated with metformin. Within the class level, the relative abundances of *Prevotellaceae*, *Verrucomicrobiaceae*, *Rikenellaceae*, and *Porphyromonadaceae* were increased, while *Rhodobacteraceae* and *Lachnospiraceae* were decreased (47). Other clinical studies have also shown that metformin treatment could elevate the relative abundance of *Escherichia coli* (under phylum *Proteobacteria*) and reduce *Intestinibacter* (*48,49*), which was in line with findings from cross-sectional cohorts that compared untreated patients to metformin-treated patients with T2D (*50*). Pastor-Villaescusa *et al*. conducted a multicenter, double-blind, and randomized control trial including 160 obese children. It was found that metformin treatment resulted in a decrease in *Actinobacteria* abundance, while the *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* abundances were different between the metformin and placebo treatment groups (*51*). Therefore, the effects of metformin on AMID may be associated with alterations in gut microbial composition.

6.2. Metformin acts on the brain

Existing evidence indicates that metformin may cross the BBB to act on the brain and directly influence the central nervous system (*52*). Hypothalamic AMP-activated protein kinase (AMPK) is a dominant adjustment factor of food intake, and preclinical studies found that metformin could act on cultured hypothalamic neurons *via* blocking the increase of AMPK phosphorylation and NPY expression, and thus decrease glucose levels (*53-55*). Meanwhile, intracerebroventricular injection of metformin promoted POMC expression and induced anorexia in a rat model (*56*). The hypothalamic phosphorylated signal transducer and activator of transcription 3 (pSTAT3) signaling to the central mechanisms are critical for regulating energy intake and consumption. A previous study showed that a decrease in hypothalamic STAT3 signaling initiated weight gain by hyperphagia and adiposity with changes in glucose homeostasis (*57*). A Lee *et al*. study suggested that metformin could directly act on the hypothalamus and cause an increase in pSTAT3 expression, and changes in POMC mRNA had a similar tendency with pSTAT3, eventually inducing an anorexic status (*56*). Moreover, metformin could down-regulate expression of AgRP mRNA, an orexigenic neuropeptide mainly expressed in the arcuate neurons that robustly stimulates food intake (*58,59*).

In addition, metformin also affects the expression of hypothalamic neurotransmitters. Some researchers have observed that metformin could play a selective 5-HT receptor activator-like role in mouse neuroblastoma N1E-115 cells, and activate the 5-HT3 receptor to regulate gastrointestinal motility. Additionally, metformin can increase the expression of receptors in the hypothalamus to improve individual sensitivity to leptin and insulin, thus playing an anorexic role (*60*). Detailed findings in studies investigating the mechanisms of metformin on AMID *via* the gut-brain axis are listed in Table 2.

7. Conclusion

In summary, accumulating studies have proved that both AIMD and metformin are closely related to the gut-brain axis (Figure 1). SGAs act on the GIT to alter the composition and metabolism of gut microbiota, mainly causing an increase in *F/B* ratio, and a decrease in *Proteobacteria* and *Akkermansia*. Interestingly, metformin plays an opposite role by increasing the abundance of *Akkermansia*. Changes in microbiota composition consequently caused changes in SCFA production and other microbe-derived metabolites. SGAs also act on various receptors and regulate neuropeptides in the hypothalamus, including up-regulation of NPY, AgRP mRNA expression, and down-regulation of POMC expression, while metformin can down-regulate the expression of NPY, AgRP mRNA and up-regulate POMC expression, and block the activation of hypothalamic AMPK signaling, thus coordinating the gut-brain axis to regulate the balance between leptin and ghrelin to maintain metabolic homeostasis and foraging behavior. In conclusion, metformin may be a promising agent for controlling AIMD. More well-

Table 2. Multiple mechanisms of metformin ameliorating AIMD via the gut-brain axis

Author, year	Study subjects	Results	*Ref.*
Na-Ri, 2019	Mouse	*Akkermansia* spp ↑	*45*
Lee, 2014	Mouse	*Akkermansia* spp. (*A.muciniphila*) ↑	*46*
Ma, 2018	Mouse	Healthy mice treated with metformin showed 46 significantly changed microbes, including 22 enriched and 24 depleted microbes identified within the class level Prevotellaceae, *Porphyromonadaceae*, *Verrucomicrobiaceae*, *Rikenellaceae* ↑ and *Lachnospiraceae*, *Rhodobacteraceae* ↓	*47*
Elbere, 2018; Forslund, 2015	Patient	*Escherichia coli* ↑, *Intestinibacter* ↓	*48, 49*
Mirshamsi, 2004	Rat, mouse	NPY expression ↓, pSTAT3 ↓	*57*
Lee, 2012	Rat	pSTAT3 ↑, POMC ↓	*56*
Cubeddu, 2000; Bing, 2006	Patient	AgRP mRNA ↓	*58 59*
Auber, 2011	Rat	5-HT receptor, leptin receptor ↑	*60*

Abbreviations: SCFAs, short-chain fatty acids; AMPK, AMP-activated protein kinase; NPY, neuropeptide Y; POMC, proopiomelanocortin; AgRP, agouti-related peptide; pSTAT3, phosphorylated signal transducer and activator of transcription 3; 5-HT, 5-hydroxytryptamine.

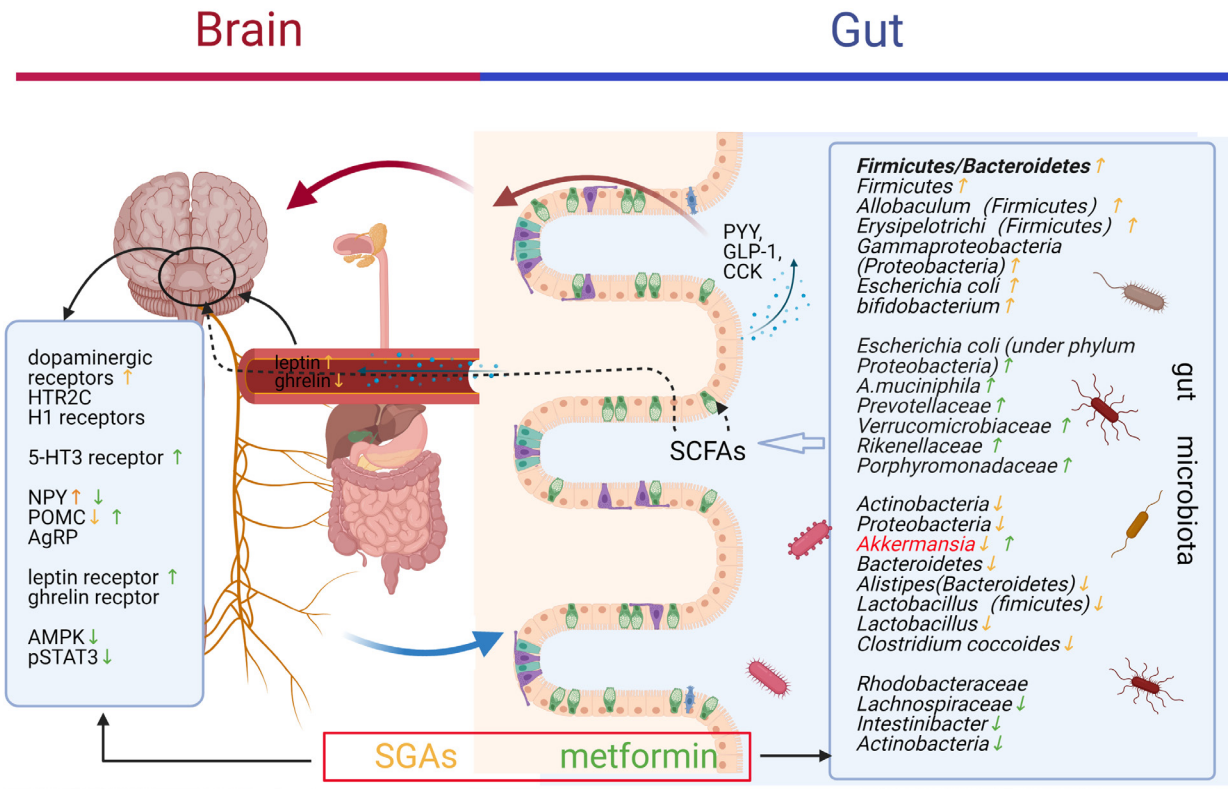

Figure 1. The mechanisms of AIMD and metformin ameliorating AIMD. SGAs act on GIT to alter the composition and metabolism of gut microbiota (marked by an orange arrow). Metformin plays an opposite role on *Akkermansia* (marked with red; both SGAs and metformin alter its abundance), and also influences the abundances of other microbes (marked by a green arrow). Changes in microbiota composition consequently cause changes in SCFAs production. SCFAs act on the intestinal endocrine cell to regulate the secretion of PYY, GLP-1, and CCK, which subsequently activate the vagus nerve to send signals to the brain. SGAs also acts on various receptors and regulate neuropeptides in the hypothalamus (marked by an orange arrow), while metformin can down-regulate the expression of NPY, AgRP mRNA and up-regulate POMC expression, and inhibit hypothalamic AMPK signals (marked by a green arrow). Other receptors are also related to the development of AIMD and metformin treatment, but the detailed correlation is still unclear (not marked by any arrow). Therefore, metformin coordinates the gut-brain axis to regulate the balance between leptin and ghrelin to maintain metabolic homeostasis and modulate foraging behavior.

designed clinical trials on this topic are warranted.

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References

1. Zhang JP, Gallego JA, Robinson DG, Malhotra AK, Kane JM, Correll CU. Efficacy and safety of individual second-generation vs. first-generation antipsychotics in first-episode psychosis: a systematic review and meta-analysis. Int J Neuropsychopharmacol. 2013; 16:1205-1218.
2. Lochmann van Bennekom MW, Gijsman HJ, Zitman FG. Antipsychotic polypharmacy in psychotic disorders: a critical review of neurobiology, efficacy, tolerability and cost effectiveness. J Psychopharmacol. 2013; 27:327-336.
3. Parsons B, Allison DB, Loebel A, Williams K, Giller E, Romano S, Siu C. Weight effects associated with antipsychotics: a comprehensive database analysis. Schizophr Res. 2009; 110:103-110.
4. Tarricone I, Ferrari Gozzi B, Serretti A, Grieco D, Berardi D. Weight gain in antipsychotic-naive patients: a review and meta-analysis. Psychol Med. 2010; 40:187-200.
5. Barton BB, Segger F, Fischer K, Obermeier M, Musil R. Update on weight-gain caused by antipsychotics: a systematic review and meta-analysis. Expert Opin Drug Saf. 2020; 19:295-314.
6. Adeneye AA, Agbaje EO, Olagunju JA. Metformin: an effective attenuator of risperidone-induced insulin resistance hyperglycemia and dyslipidemia in rats. Indian J Exp Biol. 2011; 49:332-338.
7. Maier L, Pruteanu M, Kuhn M, Zeller G, Telzerow A, Anderson EE, Typas A. Extensive impact of non-antibiotic drugs on human gut bacteria. Nature. 2018; 555:623-628.
8. Leucht S, Corves C, Arbter D, Engel RR, Li C, Davis JM. Second-generation versus first-generation antipsychotic drugs for schizophrenia: a meta-analysis. Lancet. 2009; 373:31-41.
9. Newcomer JW. Second-generation (atypical) antipsychotics and metabolic effects: a comprehensive literature review. CNS Drugs. 2005; 19:1-93.
10. Alvarez-Jiménez M, González-Blanch C, Crespo-Facorro B, Hetrick S, Rodríguez-Sánchez J M, Pérez-Iglesias R, Vázquez-Barquero JL. Antipsychotic-induced weight gain in chronic and first-episode psychotic disorders: a systematic critical reappraisal. CNS Drugs. 2008; 22:547-562.
11. Furness JB, Rivera LR, Cho HJ, Bravo DM, Callaghan B. The gut as a sensory organ. Nat Rev Gastroenterol Hepatol. 2013; 10:729-740.
12. Latorre R, Sternini C, De Giorgio R, Greenwood-Van Meerveld B. Enteroendocrine cells: a review of their role in brain-gut communication. Neurogastroenterol Motil. 2016; 28:620-630.
13. Sandhu KV, Sherwin E, Schellekens H, Stanton C, Dinan TG, Cryan JF. Feeding the microbiota-gut-brain axis: diet, microbiome, and neuropsychiatry. Transl Res. 2017; 179:223-244.
14. Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. Nature. 2011; 474:327-336.
15. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res. 2013; 54:2325-2340.
16. Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion *via* the G-protein-coupled receptor FFAR2. Diabetes. 2012; 61:364-371.
17. Sakakibara S, Yamauchi T, Oshima Y, Tsukamoto Y, Kadowaki T. Acetic acid activates hepatic AMPK and reduces hyperglycemia in diabetic KK-A(y) mice. Biochem Biophys Res Commun. 2006; 344:597-604.
18. Frost G, Sleeth ML, Sahuri-Arisoylu M, Lizarbe B, Cerdan S, Brody L. The short-chain fatty acid acetate reduces appetite *via* a central homeostatic mechanism. Nat Commun.2014; 5:3611.
19. Chambers ES, Viardot A, Psichas A, Morrison DJ, Murphy KG, Zac-Varghese SE. Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. Gut. 2015; 64:1744-1754.
20. Byrne CS, Chambers ES, Alhabeeb H, Chhina N, Morrison DJ, Preston T. Increased colonic propionate reduces anticipatory reward responses in the human striatum to high-energy foods. Am J Clin Nutr. 2016; 104:5-14.
21. Yang Y, Li W, Zhao J, Zhang H, Song X, Xiao B. Association between ghrelin gene (GHRL) polymorphisms and clinical response to atypical antipsychotic drugs in Han Chinese schizophrenia patients. Behav Brain Funct. 2012; 8:11.
22. Morgan AP, Crowley JJ, Nonneman RJ, Quackenbush CR, Miller CN, Ryan AK. The antipsychotic olanzapine interacts with the gut microbiome to cause weight gain in mouse. PLoS One. 2014; 9:e115225.
23. Bahr SM, Weidemann BJ, Castro AN, Walsh JW, deLeon O, Burnett CM. Risperidone-induced weight gain is mediated through shifts in the gut microbiome and suppression of energy expenditure. EBioMedicine.2015; 2:1725-1734.
24. Davey KJ, O'Mahony SM, Schellekens H, O'Sullivan O, Bienenstock J, Cotter PD. Gender-dependent consequences of chronic olanzapine in the rat: effects on body weight, inflammatory, metabolic and microbiota parameters. Psychopharmacology (Berl). 2012; 221:155-169.
25. Flowers SA, Baxter NT, Ward KM, Kraal AZ, McInnis MG, Schmidt TM, Ellingrod VL. Effects of Atypical Antipsychotic Treatment and Resistant Starch Supplementation on Gut Microbiome Composition in a Cohort of Patients with Bipolar Disorder or Schizophrenia. Pharmacotherapy. 2019; 39:161-170.
26. Davey KJ, Cotter PD, O'Sullivan O, Crispie F, Dinan TG, Cryan JF, O'Mahony SM. Antipsychotics and the gut microbiome: olanzapine-induced metabolic dysfunction is attenuated by antibiotic administration in the rat. Transl Psychiatry. 2013; 3:e309.
27. Bahr SM, Tyler BC, Wooldridge N, Butcher BD, Burns TL, Teesch LM. Use of the second-generation antipsychotic, risperidone, and secondary weight gain are associated with an altered gut microbiota in children. Transl Psychiatry. 2015; 5:e652.
28. Flowers SA, Evans SJ, Ward KM, McInnis MG,

Ellingrod VL. Interaction Between Atypical Antipsychotics and the Gut Microbiome in a Bipolar Disease Cohort. Pharmacotherapy. 2017; 37:261-267.
29. Yuan X, Zhang P, Wang Y, Liu Y, Li X, Kumar BU. Changes in metabolism and microbiota after 24-week risperidone treatment in drug naïve, normal weight patients with first episode schizophrenia. Schizophr Res. 2018; 201:299-306.
30. Evers SS, Boersma GJ, Tamashiro KL, Scheurink AJ, van Dijk G. Roman high and low avoidance rats differ in their response to chronic olanzapine treatment at the level of body weight regulation, glucose homeostasis, and cortico-mesolimbic gene expression. J Psychopharmacol. 2017; 31:1437-1452.
31. Huang D, Gao J, Li C, Nong C, Huang W, Zheng X. A potential probiotic bacterium for antipsychotic-induced metabolic syndrome: mechanisms underpinning how *Akkermansia muciniphila* subtype improves olanzapine-induced glucose homeostasis in mice. Psychopharmacology (Berl). 2021.7.27
32. Lord CC, Wyler SC, Wan R, Castorena CM, Ahmed N, Mathew D. The atypical antipsychotic olanzapine causes weight gain by targeting serotonin receptor 2C. J Clin Invest. 2017; 127:3402-3406.
33. Han M, Deng C, Burne TH, Newell KA, Huang XF. Short- and long-term effects of antipsychotic drug treatment on weight gain and H1 receptor expression. Psychoneuroendocrinology. 2008; 33:569-580.
34. Lian J, Huang XF, Pai N, Deng C. Betahistine ameliorates olanzapine-induced weight gain through modulation of histaminergic, NPY and AMPK pathways. Psychoneuroendocrinology. 2014; 48:77-86.
35. Lis M, Stańczykiewicz B, Liśkiewicz P, Misiak B. Impaired hormonal regulation of appetite in schizophrenia: A narrative review dissecting intrinsic mechanisms and the effects of antipsychotics. Psychoneuroendocrinology. 2020; 119:104744.
36. Potvin S, Zhornitsky S, Stip E. Antipsychotic-induced changes in blood levels of leptin in schizophrenia: a meta-analysis. Can J Psychiatry. 2015; 60:S26-34.
37. Diabetes Prevention Program Research Group. Long-term safety, tolerability, and weight loss associated with metformin in the Diabetes Prevention Program Outcomes Study. Diabetes Care. 2012; 35:731-737.
38. Morrison JA, Cottingham EM, Barton BA. Metformin for weight loss in pediatric patients taking psychotropic drugs. Am J Psychiatry. 2002; 159:655-657.
39. Baptista T, Martínez J, Lacruz A, Rangel N, Beaulieu S, Serrano A. Metformin for prevention of weight gain and insulin resistance with olanzapine: a double-blind placebo-controlled trial. Can J Psychiatry. 2006; 51:192-196.
40. Wu RR, Zhao JP, Guo XF, He YQ, Fang MS, Guo WB. Metformin addition attenuates olanzapine-induced weight gain in drug-naive first-episode schizophrenia patients: a double-blind, placebo-controlled study. Am J Psychiatry. 2008; 165:352-358.
41. Siskind DJ, Leung J, Russell AW, Wysoczanski D, Kisely S. Metformin for Clozapine Associated Obesity: A Systematic Review and Meta-Analysis. PLoS One. 2016; 11:e0156208.
42. Xia JX, Wang YB, Gan JG, Cao SL, Duan D, Qian PH, Shen F. Efficacy of metformin combined behavior intervention in the treatment of metabolic disorders caused by risperidone. The Chinese Journal of Clinical Pharmacology. 2011; 27:417-419. (in Chinese)
43. Ellul P, Delorme R, Cortese S. Metformin for Weight Gain Associated with Second-Generation Antipsychotics in Children and Adolescents: A Systematic Review and Meta-Analysis. CNS Drugs. 2018; 32:1103-1112.
44. Luo C, Wang X, Mao X, Huang H, Liu Y, Zhao J. Metformin attenuates antipsychotic-induced metabolic dysfunctions in MK801-induced schizophrenia-like rats. Psychopharmacology (Berl). 2020; 237:2257-2277.
45. Shin NR, Lee JC, Lee HY, Kim MS, Whon TW, Lee MS, Bae JW. An increase in the *Akkermansia* spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. Gut. 2014; 63:727-735.
46. Lee H, Ko G. Effect of metformin on metabolic improvement and gut microbiota. Appl Environ Microbiol. 2014; 80:5935-5943.
47. Ma W, Chen J, Meng Y, Yang J, Cui Q, Zhou Y. Metformin Alters Gut Microbiota of Healthy Mice: Implication for Its Potential Role in Gut Microbiota Homeostasis. Front Microbiol. 2018; 9:1336.
48. Elbere I, Kalnina I, Silamikelis I, Konrade I, Zaharenko L, Sekace K. Association of metformin administration with gut microbiome dysbiosis in healthy volunteers. PLoS One. 2018; 13:e0204317.
49. Forslund K, Hildebrand F, Nielsen T, Falony G, Le Chatelier E, Sunagawa S. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature. 2015; 528:262-266.
50. Weersma RK, Zhernakova A, Fu J. Interaction between drugs and the gut microbiome. Gut. 2020; 69:1510-1519.
51. Pastor-Villaescusa B, Plaza-Díaz J, Egea-Zorrilla A, Leis R, Bueno G, Hoyos R. Evaluation of the gut microbiota after metformin intervention in children with obesity: A metagenomic study of a randomized controlled trial. Biomed Pharmacother. 2021; 134:111117.
52. Lv WS, Wen J P, Li L, Sun RX, Wang J, Xian YX. The effect of metformin on food intake and its potential role in hypothalamic regulation in obese diabetic rats. Brain Res. 2012; 1444:11-19.
53. Andersson U, Filipsson K, Abbott CR, Woods A, Smith K, Bloom SR. AMP-activated protein kinase plays a role in the control of food intake. J Biol Chem. 2004; 279:12005-12008.
54. Coyral-Castel S, Tosca L, Ferreira G, Jeanpierre E, Rame C, Lomet D. The effect of AMP-activated kinase activation on gonadotrophin-releasing hormone secretion in GT1-7 cells and its potential role in hypothalamic regulation of the oestrous cyclicity in rats. J Neuroendocrinol. 2008; 20:335-346.
55. Minokoshi Y, Alquier T, Furukawa N, Kim YB, Lee A, Xue B. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature. 2004; 428:569-574.
56. Lee CK, Choi YJ, Park SY, Kim JY, Won KC, Kim YW. Intracerebroventricular injection of metformin induces anorexia in rats. Diabetes Metab J. 2012; 36:293-299.
57. Mirshamsi S, Laidlaw HA, Ning K, Anderson E, Burgess LA, Gray A. Leptin and insulin stimulation of signalling pathways in arcuate nucleus neurons: PI3K dependent actin reorganization and KATP channel activation. BMC Neurosci. 2004; 5:54.
58. Cubeddu LX, Bönisch H, Göthert M, Molderings G, Racké K, Ramadori G. Effects of metformin on intestinal 5-hydroxytryptamine (5-HT) release and on 5-HT3

receptors. Naunyn Schmiedebergs Arch Pharmacol. 2000; 361:85-91.
59. Li B, Lee K, Martin RJ. Overexpression of glucose transporter 2 in GT1-7 cells inhibits AMP-activated protein kinase and agouti-related peptide expression. Brain Res., 2006; 1118:1-5.
60. Aubert G, Mansuy V, Voirol MJ, Pellerin L, Pralong FP. The anorexigenic effects of metformin involve increases in hypothalamic leptin receptor expression. Metabolism. 2011; 60:327-334.

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Original Article

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Soluble β-amyloid impaired the GABA inhibition by mediating KCC2 in early APP/PS1 mice

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SUMMARY Alzheimer's disease (AD) is a neurodegenerative disorder, which has become the leading cause of dementia cases globally. Synaptic failure is an early pathological feature of AD. However, the cause of synaptic failure in AD, especially the GABAergic synaptic activity remains unclear. Extensive evidence indicates that the presence of soluble amyloid-β is an early pathological feature in AD, which triggers synaptic dysfunction and cognitive decline. Our recent study explored the relation of GABAergic transmission and soluble Aβ in early APP/PS1 mice. Firstly, we found soluble Aβ42 levels were significantly increased in serum, hippocampus and prefrontal cortex in 3-4 months APP/PS1 mice, which was much earlier than Aβ plagues formation. In addition, we found TNF-α and BDNF expression levels were increased, while KCC2 and $GABA_AR$ expression were decreased in 3-4 months APP/PS1 hippocampus. When we treated 3-4 months APP/PS1 mice with a potent γ-secretase inhibitor, LY411575, which can reduce the soluble Aβ42 levels, the TNF-α and BDNF protein levels were decreased, while KCC2 and $GABA_AR$ levels were increased. In conclusion, our study suggested soluble Aβ may impaired the GABA inhibition by mediating KCC2 levels in early APP/PS1 mice. KCC2 may be served as a potential biomarker for AD.

Keywords Alzheimer's disease, soluble β-amyloid, TNF-α, BDNF, KCC2, $GABA_AR$

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder, which characterized by progressive memory and cognitive impairment, and as the disease advances, symptoms include the loss of memory and motor skills, disorientation, failure to perform self-care, and finally the loss of bodily functions, which leads to the death (*1,2*). Although the cause of AD isn't completely understood, two major players that are often cited in its progression are plaques and tangles (*3*). Amyloid pathogenesis starts with altered cleavage of amyloid precursor protein (APP), an integral protein on the plasma membrane, by β-secretases (BACE1) and γ-secretases to produce insoluble Aβ fibrils, and Aβ then oligomerizes, diffuses into synaptic clefts, and interferes with synaptic signaling (*4,5*).

Microglial cells can clear soluble and fibrillar Aβ, however, continued interactions of these cells with Aβ can lead to an inflammatory response resulting in neurotoxicity (*6*). Aβ oligomers and fibrils are capable of priming microglial cells through interactions with various receptors, which enhance the production of inflammatory cytokines and chemokines (interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor α (TNF-α), C1q, *etc.*) and make microglia more susceptible to secondary stimuli, promoting microglial activation (*7,8*). Numerous studies indicated that TNF-α levels are higher in the cerebrospinal fluid (CSF) of AD patients compared to cognitively normal controls (*9-11*). Aβ can induce the release of high levels of TNF-α from primary cultures of rodent and human microglial cells (*9*). High levels of TNF-α increase the production of other pro-inflammatory cytokines, such as IL-1, IL-6, and IL-8, that can participate in the development of chronic neuroinflammation (*9,10*). TNF-α overproduction has also been associated with lower hippocampal volume and with a greater likelihood of mild cognitive impairment (MCI) patient conversion to AD (*12-14*). TNF-α, a pivotal role in neuroinflammation, is a very attractive pharmacological target, which used to reduce TNF-α signaling in rodent models of AD showed a significant reduction in AD-like brain pathology accompanied by an

amelioration of cognitive function (*9,12*).

Brain-derived neurotrophic factor (BDNF), an neurotrophins, can promote nervous system development, maintain neuronal plasticity and repair damaged neurons, but its excess can cause the opposite effect (*15,16*). TNF-α can up-regulate the expression of exon-IV-BDNF mRNA and BDNF protein in nerve injury tissues and astrocytes (*17-19*). BDNF-induced TrkB inhibited $GABA_A$ synaptic responses by down-regulates the expression of K^+-Cl^- cotransporter KCC2 and impairs neuronal Cl^- extrusion (*15,20-22*). The BDNF that secreted from activated microglias and peri-lesion damaged neurons act on damaged neuron and inhibiting KCC2 mRNA transcription by the BDNF-TrkB pathway (*15*).

In the vertebrate brain, $GABA_A$ receptors mediate the majority of fast inhibition in the brain, which depends on the intracellular Cl^- ($[Cl^-]_i$) concentration (*22,23*). KCC2, a K^+-Cl^- cotransporter, is a neuronal isoform that is broadly expressed in the adult. KCC2 functions in setting the proper $[Cl^-]_i$ by transporting Cl^- against the concentration gradient (*24*). Thus, KCC2 is a crucial regulator of GABA-mediated hyperpolarization. Loss of KCC2 activity orchestrates a depolarizing shift in E_{GABA} and is implicated in cortical development systems such as neuro- and synaptogenesis (*25,26*). The treatment of memantine, a drug that is currently prescribed for the treatment of AD, reduces the expression of KCC2 in the hippocampus and cerebral cortex and attenuates behavioral responses mediated by $GABA_AR$ activation in mice (*27*). There is a close linkage between the GABAergic signaling system and various aspect of AD pathology including Aβ toxicity (*28*), tau hyperphosphorylation and ApoE4 effect (*29*), which suggests that GABAergic system might undergo dynamic remodeling and play important roles in AD pathology.

Here, we found an increased soluble Aβ levels in 3-4 months APP/PS1 mice, which will lead to impaired GABA inhibition by mediating BDNF-TrkB-KCC2 pathway. When treated with γ-secretase inhibitors, these phenotypes can be rescued. The results indicate that KCC2 or $GABA_AR$ levels for the early diagnosis of AD.

2. Materials and Methods

2.1. Animals

All experimental procedures involving animals were performed in accordance with the guidelines for the humane treatment of animals by the Association of Laboratory Animal Sciences and were approved by the Center for Laboratory Animal Sciences at the Anhui Medical University. Every attempt was made to limit animal numbers and suffering. APP/PS1 double-transgenic mice were generated by crossing APP/PS1 and WT mice obtained from the Model Animal Research Center of Nanjing University (Nanjing, China). All mice were group kept in a clean laminar flow cabinet under SPF conditions at the Experimental Animal Center of Anhui Medical University (Anhui, China). The mice were housed in conventional cages at 20-25°C under a 12 h light/dark cycle, supplied with standard laboratory chow and water ad libitum. The mice were maintained under these conditions for at least 1 week for acclimatization before the commencement of experiments. All animals involved in experiments were 3-4 month-old unless otherwise indicated. At a certain age, mice were separately anesthetized. Brains were carefully dissected. Blood was collected. Brain tissues were preserved in 4% buffered paraformaldehyde solution at 4°C before the tissues were sliced at 4μm thickness (CRYOSTAR NX50, Thermo). Blood samples were centrifuged at 3500 rpm for 10 min (Hettich, Mikro 200R). Plasma was stored at -80°C until the ELISA was run.

2.2. Antibodies and reagents

KCC2 (Millipore, 07-432), $GABA_AR$ (Abcam, ab94585), $GABA_BR$ (Affinity, AF0162), BDNF (Affinity, DF6387), β-Amyloid 1-42 (Abcam, ab201060), TNF-α(Affinity, AF7014), IL-6 (Servicebio, GB11117), IL-1β (Affinity, AF5103), NLRP1 (Abcam, ab3683), NLRP3 (Abcam, ab214185), AMPAR (Abcam, ab31232), PSD95 (Thermo, MA1-045), Iba-1 (Servicebio, GB11105), GFAP (Servicebio, GB12096), β-Tubulin (Affinity, T0023), β-Actin (Affinity, AF7018), Goat Anti-Rabbit IgG (H+L) HRP (Affinity, S0001), Goat Goat Anti-Mouse IgG (H+L) HRP (Affinity, S0002).

2.3. Behavioral test

Open fields test (OFT) The open field test, also known as the open box test, is a commonly used animal behavior experiment. It is used to observe the spontaneous exploration of motor activity and anxiety behavior in mice. It is also a way to evaluate experimental animals' autonomous exploration of behavior and emotional tension in a new environment. The OFT instrument consists of two parts: an open field box (96 × 96 × 50 cm) and an automatic data acquisition system, the bottom of the open field box is equally divided into 9 squares. Install a camera about 2 meters directly above to record the experimental process in real time. First, place the mouse in the central area and let it crawl freely for 2 minutes to adapt to the environment. Then, record his total movement distance in 3 minutes, the number of times of threading, the time spent in the middle area and the distance moved. Before each test, clean the bottom of the open field box with ethanol to eliminate odor cues, and keep the room quiet.

Elevated-plus maze (EPM) High plus maze can be used to assess the anxiety level of animals. The height of the instrument is about 60 cm from the ground. It

consists of a set of open arms (30 × 5 cm) and a set of closed arms (30 × 5 × 15 cm) to cross vertically, and a central area (5 × 5 cm). Install a camera about 2 meters directly above to record the experimental process in real time. First, place the mouse in the central area facing the open arm, and let him explore freely for 5 minutes. The instrument will automatically record data, calculate the time and number of times the mouse enters the open arm. Before each test, clean the bottom of the open and closed arms with ethanol to eliminate odor cues and keep the room quiet.

Forced swim test (FST) Forced swim test (FST) is a behavioral test used to assess depression in mice. The mice are individually placed in a transparent plastic cylinder (height 50cm, 20cm in diameter, water depth 35cm, maintained at 25 ± 1℃) . Install a camera about 0.5 meters above to record in real time. Place the mouse in the water for 2 minutes, test the movement status of mice within 4 minutes, during the experiment, the mice will show three behaviors: struggling, swimming and standing still. Count the time the mice stayed still in the water during the experiment.

Tail suspension test (TST) The tail suspension test is a method used to evaluate depression in mice. Fix the tail of the mouse to the suspension rod with tape, make it head down. The suspension rod is fixed on a horizontal rod about 40 cm above the ground, each mouse is separated by opaque cardboard, the mouse is not less than 15 cm away from the surrounding space. First acclimate the mouse for 2 minutes, then test the mouse's twisting and struggling within 3 minutes, record its immobile time.

2.4. Hippocampal neuron cultures and treatment

HT22 neuron was maintained with Dulbecco's modified Eagle's medium (DMEM; Hyclone; Logan, USA) supplemented with 10% fetal bovine serum (FBS; Sijiqing, Zhejiang Tianhang Biological Technology Co., LTD; Huzhou, China) in a humidified incubator with an atmosphere of 5% CO_2 at 37°C. These cells at logarithmic growth were collected, counted and seeded at a density of 1×10^6 cells/25 cm^2 culture flask cultured in DMEM supplemented with 10% FBS. When these cells reached 80-90% confluency, the medium was replaced using the serum-free medium for 24 h and DMSO, TNF-α (PEPROTECH, AF-315-01A) and CLP257 (Sigma, SML1368-5MG) was added for 2 h, total proteins were extracted for followed western blot analysis.

2.5. Western blot

Hippocampus and prefrontal cortex tissue were obtained and homogenized using a lysis buffer and protease inhibitor. After 1 min of homogenization, the cellular debris was removed by centrifugation at 14000 rpm for 10 min at 4°C and supernatant were collected for denaturation for 20 min at 75°C. The lysate was then centrifuged on SDS-PAGE (Bio-Rad) under denaturing conditions and transferred to polyvinylidene difluoride (PVDF) microporous membrane. Membranes were blocked for 30 min using 5% non-fat dry milk in TBS containing 0.5% Tween-20 (TBST) and then incubated with specific primary antibodies against Aβ42, TNF-α, IL-6, IL-1β, BDNF, KCC2, $GABA_AR$, $GABA_BR$, NLRP1, NLRP3, AMPAR, PSD95. Anti β-Tubulin or β-Actin antibodies were used as the loading control. Antibodies were diluted at 1:1,000 to 1:2,000 at use. The next day, after three washes with TBST, the membranes were incubated with the corresponding secondary antibody (Affinity, S002) at room temperature (RT) for 1 h using 3% milk in TBST followed by three additional washes with TBST. Bands were visualized using a Chemi Doc TMMP Imaging System (Bio-Rad, USA) and analyzed with Gel Pro Analysis software. For the presentation in figures, digitalized blot images were processed and cropped using Adobe Illustrator to show the interested protein signals.

2.6. Immunofluorescent staining

The brain sections were sliced, antigen retrieved and blocked. Incubation of primary antibodies (Aβ42, Iba-1, GFAP) were done in carrier (PBS with 1% serum and 0.2% BSA) at 4°C overnight. After three washes with PBS, the sections were incubated in Rhodamine (TRITC)-Conjugated Goat anti-Rabbit IgG (ZSGB-BIO, ZF-0316) or FITC-Conjugated Goat anti-Mouse IgG (ZSGB-BIO, ZF-0312) at 37°C for 50 min. Washed with PBS, the sections were incubated in DAPI Staining Solution (Byotime, C1005) for 10 min. After three washes with PBS, the sections were mounted with Antifade Mounting Medium (Byotime, P0126-5ml), observed and photographed under a fluorescence microscope (Leica, Germany). The images were evaluated with ImageJ software (NIH, Bethesda, MD, USA).

2.7. Quantitative Real-time PCR (qRT-PCR)

Mice brains were dissected and immediately frozen in liquid nitrogen. Total RNA was extracted using Trizol reagent. RNA pellets were resuspended in diethylpyrocarbonate-treated water and RNA concentration was measured using a Nanodrop2000c spectrometer (Thermo Scientific). RNA was treated by the PrimeScript™ RT Master Mix (TaKaRa, Code No. RR036A) and then reverse-transcribed using the Bio-Rad T100TM thermal cycler. Relative quantification of gene expression was performed using the Bio-Rad CFX96™ Real-time System. Platinum Quantitative PCR P TB Green® Premix Ex Taq™ II (TaKaRa, Code No. RR820A) was used with the following primers and

probes:

TNF-α F-primer, 5'-CATGATCCGCGACGTGGAACTG-3';
TNF-α R-primer, 5'-AGAGGGAGGCCATTTGGGAACT-3';
BDNF F-primer, 5'-GGTATCCAAAGGCCAACTGA-3';
BDNF R-primer, 5'-CTTATGAATCGCCAGCCAAT-3';
Slc12a5 F-primer, 5'-GGGCAGAGAGTACGATGGC-3';
Slc12a5 R-primer, 5'-TGGGGTAGGTTGGTGTAGTTG-3';
β-Actin F-primer, 5'-GTGACGTTGACATCCGTAAAGA-3';
β-Actin R-primer, 5'- GTAACAGTCCGCCTAGAAGCAC-3';

Assay efficiencies were experimentally determined using a five-point dilution series of cDNA spanning a 100-fold range in concentration. 0.025 mg cDNA template was used per reaction. Statistical analysis was performed on $2^{-\Delta\Delta Cq}$ values.

2.8. Enzyme-linked immunosorbent assay (ELISA)

The supernatants of serum, hippocampus, and prefrontal cortex of WT and APP/PS1 mice were collected. The ELISA kit was used for the quantitative determination of Aβ42 (CUSABIO, CSB-E10787m), TNF-α (CUSABIO, CSB-E04741m), IL-6 (CUSABIO, CSB-E04639m), and IL-1β (CUSABIO, CSB-E08054m). Aβ42, TNF-α, IL-6, and IL-1β standards and samples were added to the wells of assay plates and incubated for 1 h at 37°C. Blank wells were added with standard diluent. The horseradish peroxidase (HRP) conjugated reagent (100 μL) was added to each well for 1 h at 37°C. Plates were washed four times with PBS, and chromogen solution (100 μL) was added to each well. The plates were gently mixed and incubated for 15 min at 37°C in the dark. Then, stop solution (50 μL) was added to each well and examined the absorbance at 450 nm with a microplate reader (SPECTRAMAX 190, USA) within 15 min.

2.9. Statistical analysis

Data were presented as mean ± standard error and analyzed using GraphPad Prism version 7.0 (GraphPad Software, La Jolla, CA, USA) by student's *t*-test or one-way ANOVA, as appropriate with $p < 0.05$ as statistically significant.

3. Results

3.1. Increased soluble Aβ42 in 3-4 months APP/PS1 mice

As shown in Figure 1, the ELISA result showed that the soluble Aβ42 were significantly increased in serum,

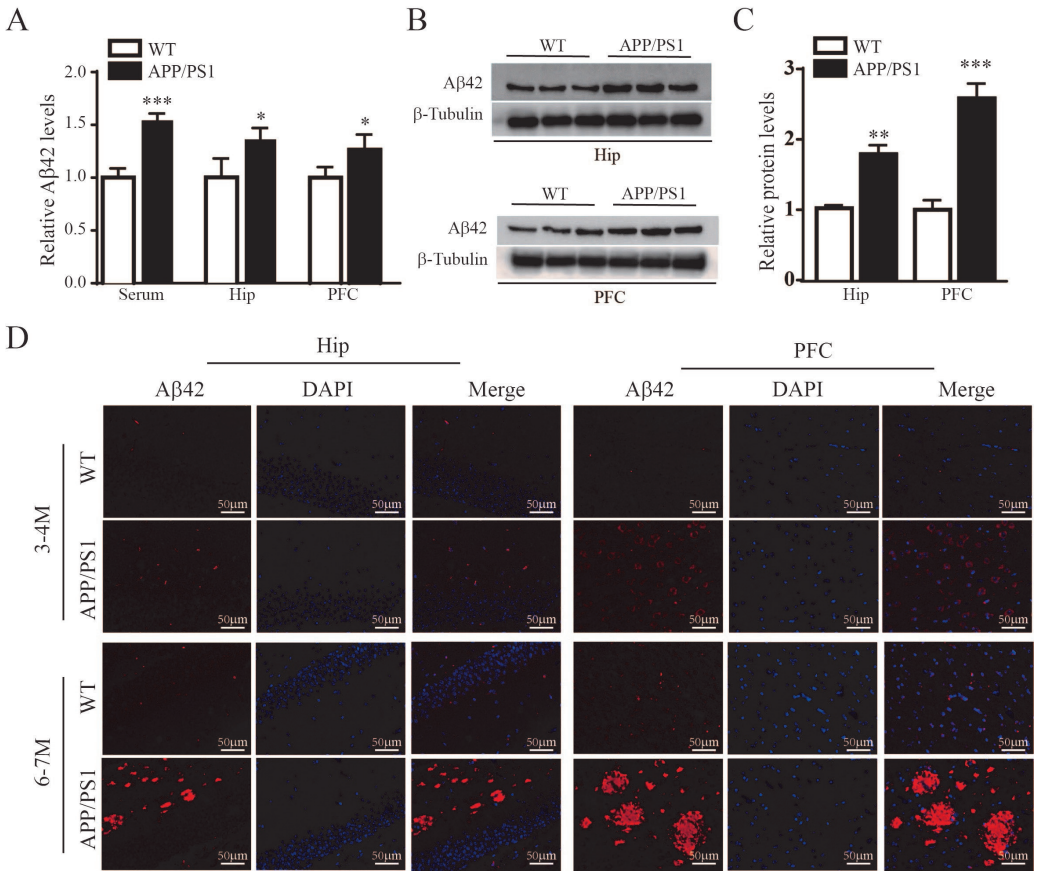

Figure 1. Increased soluble Aβ42 level in 3-4 months APP/PS1 mice. (A) The soluble Aβ42 levels in serum, hippocampus, and prefrontal cortex by ELISA. **(B)** Representative immunoblots of Aβ42 in the hippocampus and prefrontal cortex of WT and APP/PS1 mice. **(C)** Quantification of Aβ42 protein levels in (B). **(D)** Representative images of Aβ42 in the hippocampus and prefrontal cortex of 3-4 and 6-7 months WT and APP/PS1 mice. ($n = 6$ in each group, Student's *t*-test). All data are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

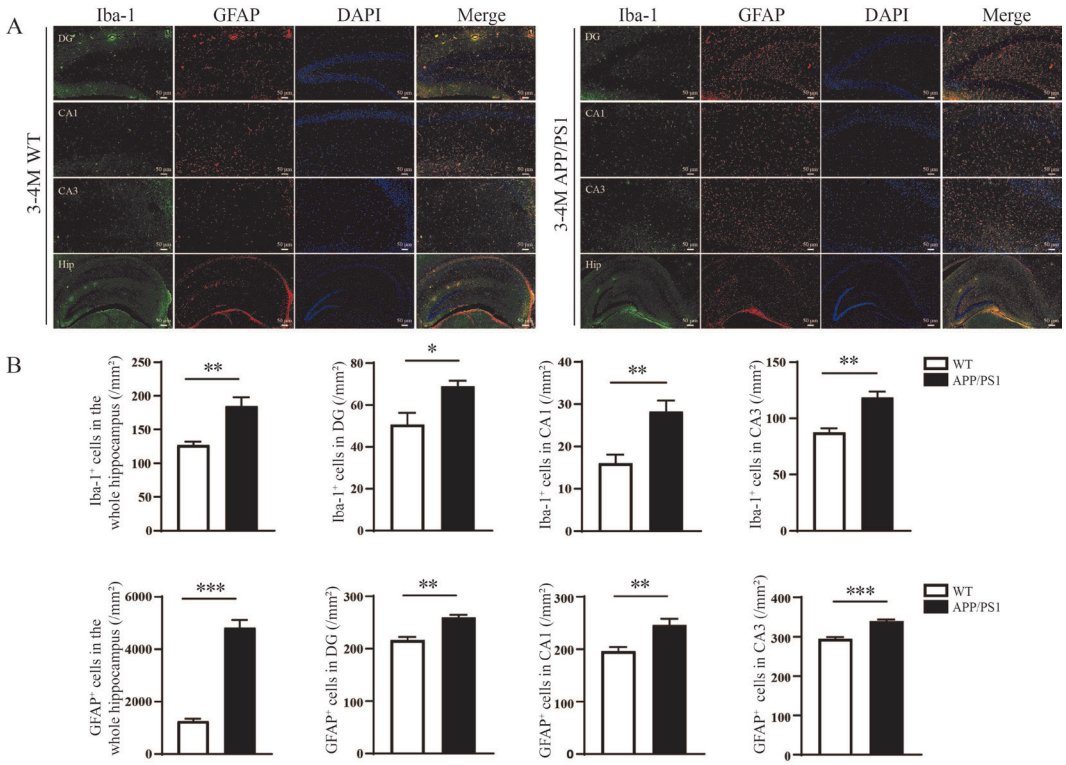

Figure 2. APP/PS1 mice exhibit activated astrocyte and microglia in hippocampus. (A) Representative images of Iba1+ and GFAP+ cells in 3-4 months APP/PS1 hippocampus. **(B)** Quantification of Iba1+ and GFAP+ cells in CA1, CA3, and DG of hippocampus in 3-4 months WT and APP/PS1 mice. ($n = 5$ in each group, Student's t-test). All data are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

hippocampus and prefrontal cortex in 3-4 months APP/PS1 mice (Figure 1A). In addition, we also performed western blotting to detect Aβ42 protein levels in hippocampus and prefrontal cortex, and found that Aβ42 protein levels were significantly increased in 3-4 months APP/PS1 mice (Figure 1B-C). Immunofluorescent staining results showed more Aβ plaques in hippocampus and prefrontal cortex in 6-7 months APP/PS1 mice, but not in 3-4 months APP/PS1 mice (Figure 1D).

3.2. Activated glia cells and increased neuroinflammation in 3-4 months APP/PS1 mice

Aβ could cause neuroinflammation in the brain (*6*). In order to detect whether increased Aβ42 levels could induce neuroinflammation, we performed immunofluorescent staining to label glia cells. Compared with WT mice, the number of microglia and astrocytes were both increased significantly in 3-4 months APP/PS1 hippocampus (Figure 2A-B). To further test if increased soluble Aβ42 can lead to neuroinflammation, we detected TNF-α, IL-6 and IL-1β levels by ELISA kits and found these inflammatory factors were all increased in 3-4 months APP/PS1 mice (Figure 3A). Western blotting and real-time PCR results also showed an increased IL-1β and TNF-α protein and mRNA levels in hippocampus and prefrontal cortex of 3-4 months APP/PS1 (Figure 3B-E; Figure 6). NLRP1 and NLRP3 belong to NLR family and are both expressed in CNS (*30*). Activation of

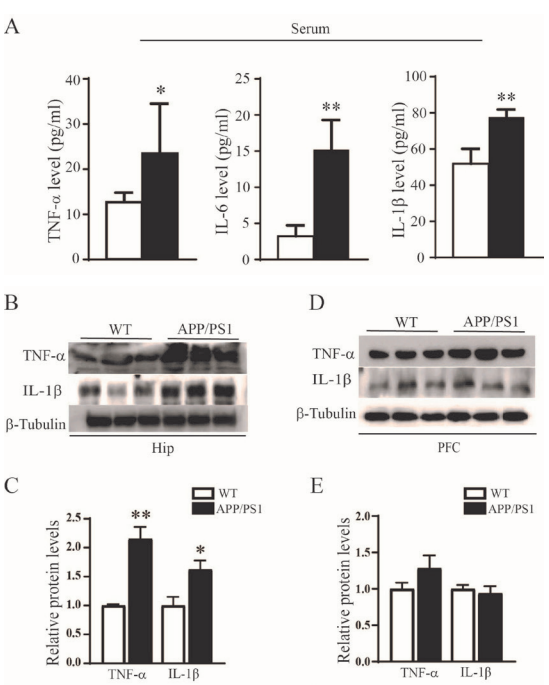

Figure 3. Increased neuroinflammation in 3-4 months APP/PS1 hippocampus. (A) TNF-α, IL-6, IL-1β levels in the serum of 3-4 months WT and APP/PS1 mice by ELISA. **(B)** Representative immunoblots of TNF-α and IL-1β in 3-4 months WT and APP/PS1 hippocampus. **(C)** Quantification of TNF-α and IL-1β protein levels in (B). **(D)** Representative immunoblots of TNF-α, and IL-1β in 3-4 months WT and APP/PS1 prefrontal cortex . **(E)** Quantification of TNF-α, and IL-1β protein levels in (D). ($n = 6$ in each group, Student's t-test). All data are presented as mean±SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

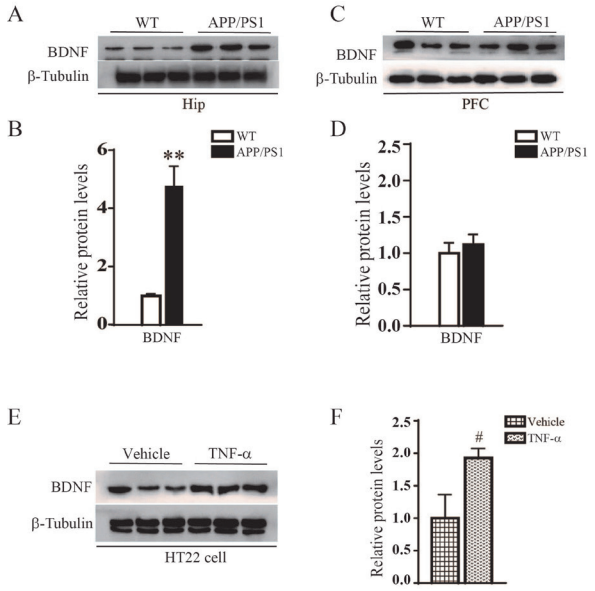

Figure 4. Increased BDNF level in 3-4 months APP/PS1 hippocampus. (A) Representative immunoblots of BDNF in 3-4 months WT and APP/PS1 hippocampus. **(B)** Quantification of BDNF protein levels in (A). **(C)** Representative immunoblots of BDNF in 3-4 months WT and APP/PS1 prefrontal cortex. **(D)** Quantification of BDNF protein levels in (C). **(E)** Representative immunoblots of BDNF in HT22 cells treated by TNF-α or vehicle control. **(F)** Quantification of BDNF protein levels in (E). (n = 3 in each group, Student's t-test). All data are presented as mean ± SEM. $^{**}p < 0.01$ *vs.* WT; $^{\#}p < 0.05$ *vs.* vehicle control.

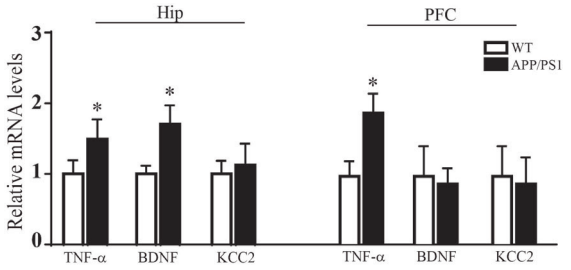

Figure 6. Increased mRNA levels of TNF-α and BDNF in 3-4 months APP/PS1 hippocampus. The QPCR results of TNF-α, BDNF and KCC2 mRNA levels in the hippocampus and prefrontal cortex of 3-4 months WT and APP/PS1 mice. (n = 6 in each group, Student's t-test). All data are presented as mean ± SEM. $^{*}p < 0.05$.

inflammasomes can promote the production and release of pro-inflammatory cytokines (*31*). We next examined whether increased inflammatory factors level was regulated by activation of inflammasomes. We performed western blotting to detect NLRP-1 and NLRP-3 protein levels in the hippocampus and prefrontal cortex, and found no significant difference between WT and APP/PS1 mice (Figure S1A-D, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=83*).These results demonstrated that increased soluble Aβ42 level may lead to increased neuroinflammation in 3-4 months APP/PS1 mice, which is much earlier than Aβ plagues formation.

3.3. Increased TNF-α levels induced an excess of BDNF level in 3-4 months APP/PS1 hippocampus

TNF-α can up-regulate BDNF protein levels in nerve injury tissues and astrocytes (*17-19*). While excess BDNF can damage neurons. As shown in Figure 4 and Figure 6, we found the protein and mRNA levels of BDNF were both increased in 3-4 months APP/PS1 hippocampus (Figure 4A-D; Figure 6). In addition, we used TNF-α (10 ng/mL) to treat HT22 neuron for 3 h, and found BDNF protein level was significantly increased when treated with TNF-α (Figure 4E-F). These results indicated that BDNF mRNA and protein levels were both increased in 3-4 months APP/PS1 hippocampus, which may be caused by increased neuroinflammation.

3.4. KCC2 and $GABA_AR$ levels were decreased in APP/PS1 hippocampus

BDNF-induced TrkB can inhibit $GABA_A$ synaptic responses by down-regulating the KCC2 expression and impairs neuronal Cl^- extrusion (*15,20-22*). By western blotting, we found KCC2 and $GABA_AR$ protein levels were significantly decreased in 3-4 months APP/PS1 hippocampus (Figure 5A-B), while no change in prefrontal cortex (Figure 5C-D). Real-time PCR results showed unchanged KCC2 mRNA level in hippocampus and prefrontal cortex (Figure 6). In contrast, levels of $GABA_BR$, AMPAR, and PSD95 were similar between WT and APP/PS1 mice. (Figure 5A-D; Figure S2A-D, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=83*). Next we used a selective KCC2 activator, CLP257 (100 μM) to treat HT22 neuron for 2 h, and found a significantly increased KCC2 and $GABA_AR$ protein levels when treated with CLP257 (Figure 5E-F). Furthermore, we also found that KCC2 and $GABA_AR$ protein levels were decreased in 1-2 months APP/PS1 hippocampus (Figure 5G-H). These results demonstrated that inhibitory function were impaired in early APP/PS1 hippocampus. Restore KCC2 can rescue $GABA_AR$ protein level by enhancing chloride extrusion. In order to make sure whether impaired GABA inhibition can lead to abnormal behavior in 3-4 months APP/PS1 mice, we performed open field test, elevated-plus maze, forced swim test, and tail suspension test. The results showed that APP/PS1 mice at 3-4 months not showed anxiety-like and depressive behavior compared to WT controls (Figure S3A-J, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=83*).

3.5. Increased inhibitory protein levels by inhibition of Aβ production

In order to explore whether these changes were caused by increased soluble Aβ42, 3-4 months APP/PS1 mice were treated with LY411575, a potent γ-secretase

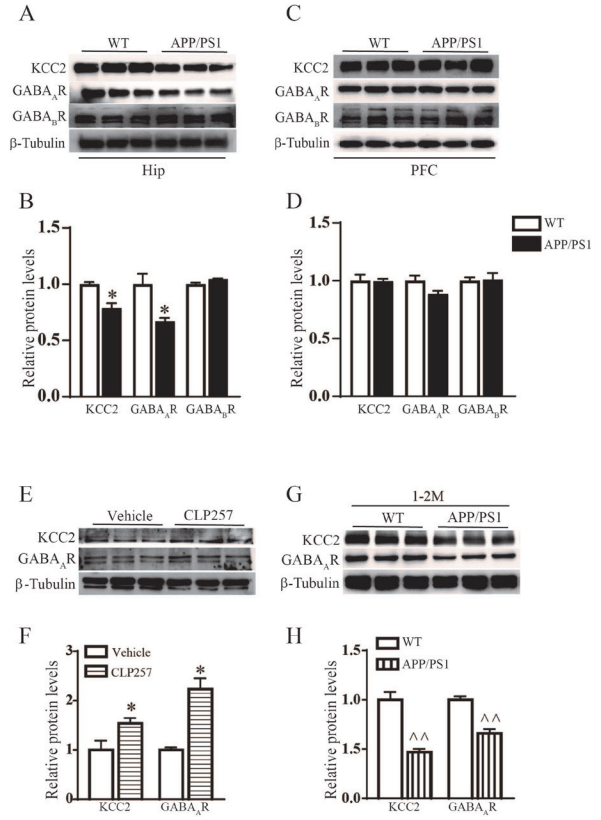

Figure 5. Decreased inhibitory protein levels in 3-4 months APP/PS1 hippocampus. (A) Representative immunoblots of KCC2, $GABA_AR$, and $GABA_BR$ in 3-4 months WT and APP/PS1 hippocampus. **(B)** Quantification of KCC2, $GABA_AR$, and $GABA_BR$ protein levels in (A). **(C)** Representative immunoblots of KCC2, $GABA_AR$, and $GABA_BR$ in 3-4 months WT and APP/PS1 prefrontal cortex. **(D)** Quantification of KCC2, $GABA_AR$, and $GABA_BR$ protein levels in (C). **(E)** Representative immunoblots of KCC2 and $GABA_AR$ protein levels in HT22 cell treated by CLP257 or vehicle control. **(F)** Quantification of KCC2 and $GABA_AR$ protein levels in (E). **(G)** Representative immunoblots of KCC2 and $GABA_AR$ in 1-2 months WT and APP/PS1 hippocampus. **(H)** Quantification of KCC2 and $GABA_AR$ protein levels in (G). (n = 6 in each group, Student's t-test). All data are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* 3-4 months WT; $^{\wedge\wedge}p < 0.01$ *vs.* 1-2 months WT.

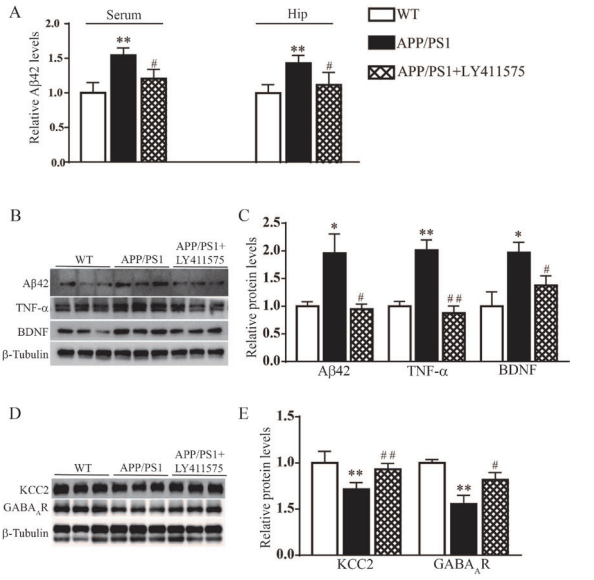

Figure 7. Reducing interstitial fluid levels of Aβ can reduce BDNF , increase inhibitory protein levels in APP/PS1 hippocampus. (A) The soluble Aβ42 levels in serum and hippocampus of 3-4 months APP/PS1 mice treated with LY411575 by ELISA. (n = 6 in each group). **(B)** Representative immunoblots of Aβ42, TNF-α, and BDNF. **(C)** Quantification of Aβ42, TNF-α, and BDNF protein levels in (B). **(D)** Representative immunoblots of KCC2 and $GABA_AR$. **(E)** Quantification of KCC2 and $GABA_AR$ protein levels in (D). (n = 3 in each group, one-way ANOVA). All data are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* WT; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* APP/PS1.

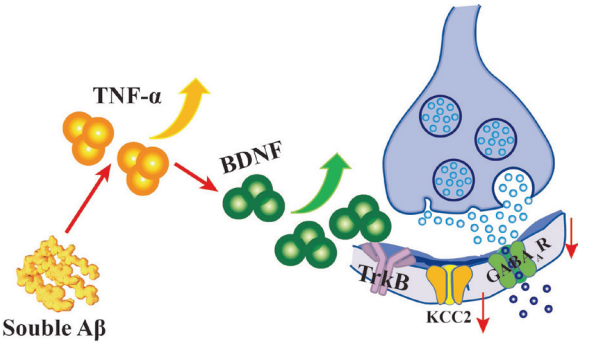

Figure 8. A working model shows the mechanism underlying soluble Aβ regulation of $GABA_AR$ mediated inhibition in the hippocampus. The soluble Aβ42 level were elevated in the hippocampus of the early stage of AD mice, which can lead to TNF-α release from astrocyte and microglia. Increased TNF-α up regulated BDNF mRNA and protein levels in hippocampus. Excess BDNF can lead to downregulated KCC2 expression which causes impaired GABA inhibition.

inhibitor by intraperitoneal injection, 2mg/kg/day, for 2 days. ELISA result showed that the soluble Aβ42 was remarkably decreased in serum and hippocampus of 3-4 months APP/PS1 mice when treated with LY411575 (Figure 7A). As shown in Figure 7B-E, western blot result showed that Aβ42, TNF-α, and BDNF protein levels were significantly decreased, while KCC2 and $GABA_AR$ protein levels were increased when treated with LY411575 (Figure 7B-E). These results indicated that soluble Aβ impaired the GABA inhibition most likely by mediating KCC2 level in early APP/PS1 mice.

4. Discussion

In this study, we found an impaired GABA inhibition in the early AD mice, which may be caused by increased soluble Aβ42 as summarized in (Figure 8). This conclusion is supported by several lines of evidence: 1) In 3-4 months APP/PS1 mice, soluble Aβ42 was significantly increased without plaque formation. 2) In 3-4 months APP/PS1 hippocampus, TNF-α and BDNF levels were significantly increased, while KCC2 and $GABA_AR$ were decreased. 3) After treated with LY411575, a potent γ-secretase inhibitor, the levels of soluble Aβ42, TNF-α, BDNF, KCC2 and $GABA_AR$ levels can be rescued.

In the last decade, extensive studies indicated that the presence of soluble amyloid-β, an early pathological feature, triggers synaptic dysfunction and cognitive decline in AD (*32-34*). It was reported that the soluble Aβ content of human brain is better correlated with the severity of the disease than are plaques (*35,36*) and biochemically-measured levels of soluble Aβ, including soluble oligomers, correlate much better with the presence and degree of cognitive deficits than do simple plaque counts (*36-38*). Haass, *et al.* (*39*) hypothesized that diffusible oligomers have the principal role, particularly during the earliest, even pre-symptomatic, stages of the AD process. Ample evidence points towards an Aβ-dependent impairment at both inhibitory (*40,41*) and excitatory synapses (*42,43*). The neurotoxic soluble Aβ oligomers, including the most toxic oligomeric Aβ42, have been shown to alter synaptic plasticity and synaptic transmission in various AD animal models *via* a variety of synaptic targets of Aβ (*44*). In our study, we found that soluble Aβ42 level was increased in serum, hippocampus and prefrontal cortex in 3-4 months APP/PS1 mice, which was much earlier than Aβ plaque formation.

Converging lines of evidence support that soluble Aβ are the principal cytotoxic agents that induce a complex array of downstream effects on neurons, microglia, astrocytes and cerebral microvessels in AD (*32*). Neuroinflammation in AD is thought to be primarily driven by microglial cells. Microglial cells continued interactions with soluble and fibrillar Aβ can lead to an inflammatory response resulting in neurotoxicity (*6*). Aβ oligomers and fibrils enhanced the production of inflammatory cytokines and chemokines (interleukin-1, interleukin-6, tumor necrosis factor α (TNF-α), C1q, *etc.*) by priming microglial cells through activated NF-κB and NLRP3 (*6-8*). Primary microglia of rodent and human cultures stimulated with Aβ showed the release of high levels of TNF-α (*9,45*). Numerous studies indicated that TNF-α levels are higher in the cerebrospinal fluid (CSF) of AD patients (*9-11*). TNF-α is a pivotal role in neuroinflammation and a very attractive pharmacological target (*9*). According to a study showed a reduction of TNF-α signaling in rodent models of AD significantly decreased AD-like brain pathology accompanied by an amelioration of cognitive function (*12*). Furthermore, overproduction of TNF-α associated with lower hippocampal volume and with a greater likelihood of mild cognitive impairment (MCI) patient conversion to AD (*12-14*). In our study, we found TNF-α protein and mRNA levels were both increased in hippocampus and prefrontal cortex of APP/PS1 mice. TNF-α capable increased the protein and mRNA expression of BDNF in nerve injury tissues and astrocytes (*17-19*). Astrocytes are one of the sources of BDNF expression and secretion (*17,46*). Our results showed that the BDNF level was increased in 3-4 months APP/PS1 hippocampus. BDNF can protect the nervous system and neuronal plasticity but its excess can cause the opposite effect (*15,16*). BDNF can regulate KCC2 expression by TrkB receptors, which regulated neuronal Cl^- extrusion (*15*). BDNF bound TrkB phosphotyrosine 816 (Y816) by the PLCγ1 pathway inhibits KCC2 mRNA transcription and bound TrkB phosphotyrosine 515 by the Shc pathway promotes KCC2 mRNA transcription (*47*). However, excess BDNF can activate both the PLCγ1 and Shc signaling which leads to KCC2 expression downregulated. KCC2 expression is downregulated following activation of both the PLCγ1 and Shc signaling cascades of the BDNF-TrkB pathway in damaged neurons (*48,49*). In mature neurons, BDNF activates neuronal m-Calpain, which irreversible inactivation of KCC2 *via* MAPK-mediated phosphorylation (*50,51*). We found KCC2 protein level was decreased in APP/PS1 hippocampus. These results demonstrated that increased TNF-α in the hippocampus of early AD mice can stimulate a large amount of BDNF expression and thus suppresses KCC2 expression.

Synaptic failure is an early pathological feature of AD (*52*), including GABAergic activity (*53*). γ-aminobutyric acid (GABA), which a principle inhibitory neurotransmitter in the mammalian central nervous system (CNS)mediates synaptic inhibition by acting on $GABA_A$ and $GABA_B$ receptors ($GABA_AR$, $GABA_BR$) (*22,54*). In the vertebrate brain, $GABA_AR$ mediate the majority of fast inhibition in the brain, and the $GABA_BR$ mediated inhibition is less efficient (*22,23*). $GABA_A$ receptors mediate inhibition depended on the intracellular Cl^- ($[Cl^-]_i$) concentration(*22*). KCC2, which set the proper $[Cl^-]i$ by transporting Cl^- against the concentration gradient is a crucial regulator of GABA-mediated hyperpolarization (*24*). Several previous studies indicated the loss of KCC2 activity orchestrates a depolarizing shift in E_{GABA} which implicated in cortical development systems such as neuro- and synaptogenesis (*25,26*). Previously, we found that restore KCC2 can increase $GABA_AR$ protein level by enhancing Cl^- extrusion (*55*). Our current data shows a decreased $GABA_AR$ protein level in APP/PS1 hippocampus, which may be result of reduced KCC2 level. Several studies showed that exogenous BDNF could decrease presynaptic GABA release and postsynaptic $GABA_AR$ response (*56-58*). When exposure to BDNF, the number of $GABA_A$ receptor rapid reduced in postsynaptic, which is responsible for the decline in GABAergic mIPSC amplitudes (*57,58*). It has been demonstrated that the subunit compositions of $GABA_ARs$ are altered in some regions of the cortex and hippocampus in AD independent of age-related changes (*59,60*). There is accumulating evidence, which indicates that GABAergic neurotransmission also undergoes profound pathological changes in AD (*61,62*) and may be a promising therapeutic target for this neurodegenerative disorder (*63,64*). These results demonstrated that excessive BDNF prevents neuronal Cl^- extrusion by inhibited the expression of KCC2, thereby impaired GABA inhibition.

Taken together, stimulated overproduction of TNF-α

by elevated soluble Aβ42 activated the BNDF-TrkB-KCC2 pathway and thus inhibited Cl^- extrusion, which causes impaired GABA inhibition in early AD mice. These phenotypes were much earlier than Aβ plaque formation. To our knowledge, our present study is the first to report that soluble Aβ42 impaired GABA inhibition *via* mediating KCC2. And our study provides a clue that the role of GABAergic synaptic transmission in the early AD and KCC2 may be a biomarker for the study of early diagnosis of AD.

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References

1. 2019 Alzheimer's disease facts and figures. Alzheimers Dement. 2019; 15:321-387.
2. Daulatzai MA. Early stages of pathogenesis in memory impairment during normal senescence and Alzheimer's disease. J Alzheimers Dis. 2010; 20:355-67.
3. Alber J, Goldfarb D, Thompson LI, Arthur E, Hernandez K, Cheng D, DeBuc DC, Cordeiro F, Provetti-Cunha L, den Haan J, Van Stavern GP, Salloway SP, Sinoff S, Snyder PJ. Developing retinal biomarkers for the earliest stages of Alzheimer's disease: What we know, what we don't, and how to move forward. Alzheimers Dement. 2020; 16:229-243.
4. Chen JX, Yan SS. Role of mitochondrial amyloid-beta in Alzheimer's disease. J Alzheimers Dis. 2010;20 Suppl 2:S569-578.
5. Crews L, Masliah E. Molecular mechanisms of neurodegeneration in Alzheimer's disease. Hum Mol Genet. 2010; 19R1:12-20.
6. Gold M, El Khoury J. beta-amyloid, microglia, and the inflammasome in Alzheimer's disease. Semin Immunopathol. 2015; 37:607-611.
7. Heppner FL, Ransohoff RM, Becher B. Immune attack: the role of inflammation in Alzheimer disease. Nat Rev Neurosci. 2015; 16:358-372.
8. Marttinen M, Takalo M, Natunen T, Wittrahm R, Gabbouj S, Kemppainen S, Leinonen V, Tanila H, Haapasalo A, Hiltunen M. Molecular Mechanisms of Synaptotoxicity and Neuroinflammation in Alzheimer's Disease. Front Neurosci. 2018; 12:963.
9. Decourt B, Lahiri DK, Sabbagh MN. Targeting Tumor Necrosis Factor Alpha for Alzheimer's Disease. Curr Alzheimer Res. 2017; 14:412-25.
10. Zhao M, Cribbs DH, Anderson AJ, Cummings BJ, Su JH, Wasserman AJ, Cotman CW. The induction of the TNFalpha death domain signaling pathway in Alzheimer's disease brain. Neurochem Res. 2003; 28:307-318.
11. Hennessy E, Gormley S, Lopez-Rodriguez AB, Murray C, Murray C, Cunningham C. Systemic TNF-alpha produces acute cognitive dysfunction and exaggerated sickness behavior when superimposed upon progressive neurodegeneration. Brain Behav Immun. 2017; 59:233-244.
12. Sudheimer KD, O'Hara R, Spiegel D, Powers B, Kraemer HC, Neri E, Weiner M, Hardan A, Hallmayer J, Dhabhar FS. Cortisol, cytokines, and hippocampal volume interactions in the elderly. Front Aging Neurosci. 2014; 6:153.
13. Tarkowski E, Andreasen N, Tarkowski A, Blennow K. Intrathecal inflammation precedes development of Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2003; 74:1200-5.
14. Liu Y, Zhou LJ, Wang J, *et al.* TNF-alpha Differentially Regulates Synaptic Plasticity in the Hippocampus and Spinal Cord by Microglia-Dependent Mechanisms after Peripheral Nerve Injury. J Neurosci. 2017; 37:871-881.
15. Lee-Hotta S, Uchiyama Y, Kametaka S. Role of the BDNF-TrkB pathway in KCC2 regulation and rehabilitation following neuronal injury: A mini review. Neurochem Int. 2019; 128:32-38.
16. Ziemlinska E, Kugler S, Schachner M, Wewior I, Czarkowska-Bauch J, Skup M. Overexpression of BDNF increases excitability of the lumbar spinal network and leads to robust early locomotor recovery in completely spinalized rats. PLoS One. 2014; 9:e88833.
17. Saha RN, Liu X, Pahan K. Up-regulation of BDNF in astrocytes by TNF-alpha: a case for the neuroprotective role of cytokine. J Neuroimmune Pharmacol. 2006; 1:212-222.
18. Su WF, Wu F, Jin ZH, Gu Y, Chen YT, Fei Y, Chen H, Wang YX, Xing LY, Zhao YY, Yuan Y, Tang X, Chen G. Overexpression of P2X4 receptor in Schwann cells promotes motor and sensory functional recovery and remyelination *via* BDNF secretion after nerve injury. Glia. 2019; 67:78-90.
19. Bucker J, Fries GR, Kapczinski F, Post RM, Yatham LN, Vianna P, Bogo Chies JA, Gama CS, Magalhaes PV, Aguiar BW, Pfaffenseller B, Kauer-Sant'Anna M. Brain-derived neurotrophic factor and inflammatory markers in school-aged children with early trauma. Acta Psychiatr Scand. 2015; 131:360-368.
20. Rivera C, Li H, Thomas-Crusells J, Lahtinen H, Viitanen T, Nanobashvili A, Kokaia Z, Airaksinen MS, Voipio J, Kaila K, Saarma M. BDNF-induced TrkB activation down-regulates the K^+-Cl^-cotransporter KCC2 and impairs neuronal Cl^- extrusion. J Cell Biol. 2002; 159:747-752.
21. Tanaka T, Saito H, Matsuki N. Inhibition of $GABA_A$ synaptic responses by brain-derived neurotrophic factor

(BDNF) in rat hippocampus. J Neurosci. 1997; 17:2959-2966.
22. Porcher C, Medina I, Gaiarsa JL. Mechanism of BDNF Modulation in GABAergic Synaptic Transmission in Healthy and Disease Brains. Front Cell Neurosci. 2018; 12:273.
23. Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R. GABA: A pioneer transmitter that excites immature neurons and generates primitive oscillations. Physiol Rev. 2007; 87:1215-1284.
24. Ben-Ari Y. Excitatory actions of gaba during development: the nature of the nurture. Nature Reviews Neuroscience. 2002; 3:728-739.
25. Vanhatalo S, Palva JM, Andersson S, Rivera C, Voipio J, Kaila K. Slow endogenous activity transients and developmental expression of K^+-Cl^- cotransporter 2 in the immature human cortex. The European journal of neuroscience. 2005; 22:2799-2804.
26. Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K. The K^+/Cl^- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. Nature. 1999; 397: 251-255.
27. Molinaro G, Battaglia G, Riozzi B, Di Menna L, Rampello L, Bruno V, Nicoletti F. Memantine treatment reduces the expression of the K^+/Cl^- cotransporter KCC2 in the hippocampus and cerebral cortex, and attenuates behavioural responses mediated by GABA(A) receptor activation in mice. Brain Res. 2009; 1265:75-79.
28. Tang BL. Amyloid Precursor Protein (APP) and GABAergic Neurotransmission. Cells. 2019; 8:550.
29. Najm R, Jones EA, Huang Y. Apolipoprotein E4, inhibitory network dysfunction, and Alzheimer's disease. Mol Neurodegener. 2019; 14:24.
30. Kummer JA, Broekhuizen R, Everett H, Agostini L, Kuijk L, Martinon F, van Bruggen R, Tschopp J. Inflammasome components NALP 1 and 3 show distinct but separate expression profiles in human tissues suggesting a site-specific role in the inflammatory response. J Histochem Cytochem. 2007; 55:443-452.
31. Strowig T, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in health and disease. Nature. 2012; 481:278-286.
32. Walsh D, Selkoe D. Aβ Oligomers - A decade of discovery. J Neurochem. 2007; 101:1172-1184.
33. Mhillaj E, Morgese MG, Tucci P, Furiano A, Luongo L, Bove M, Maione S, Cuomo V, Schiavone S, Trabace L. Celecoxib Prevents Cognitive Impairment and Neuroinflammation in Soluble Amyloid beta-treated Rats. Neuroscience. 2018; 372:58-73.
34. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med. 2016; 8: 595-608.
35. Roher A, Chaney M, Kuo Y-M, Webster S, Stine W, J. Haverkamp L, Woods A, J. Cotter R, M. Tuohy J, Krafft G, S. Bonnell B, R. Emmerling M. Morphology and Toxicity of A -(1-42) Dimer Derived from Neuritic and Vascular Amyloid Deposits of Alzheimer's Disease. J Biol Chem. 1996; 271:20631-35.
36. Lue LF, Kuo YM, Roher AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH, Rydel RE, Rogers J. Soluble Amyloid β Peptide Concentration as a Predictor of Synaptic Change in Alzheimer's Disease. Am J Pathol. 1999; 155:853-862.
37. McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, Bush AI, Masters CL. Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. Ann Neurol. 1999; 46:860-866.
38. Näslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P, Buxbaum JD. Correlation between elevated levels of amyloid β-peptide in the brain and cognitive decline. JAMA. 2000; 283:1571-7.
39. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat Rev Mol Cell Biol. 2007; 8:101-112.
40. Verret L, O Mann E, B Hang G, M I Barth A, Cobos I, Ho K, Devidze N, Masliah E, Kreitzer A, Mody I, Mucke L, Palop J. Inhibitory Interneuron Deficit Links Altered Network Activity and Cognitive Dysfunction in Alzheimer Model. Cell. 2012; 149:708-721.
41. Palop JJ, Mucke L. Network abnormalities and interneuron dysfunction in Alzheimer disease. Nat Rev Neurosci. 2016; 17:777-792.
42. Lerdkrai C, Asavapanumas N, Brawek B, Kovalchuk Y, Mojtahedi N, Olmedillas Del Moral M, Garaschuk O. Intracellular Ca^{2+} stores control *in vivo* neuronal hyperactivity in a mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A. 2018; 115:E1279-E1288.
43. Zott B, Simon MM, Hong W, Unger F, Chen-Engerer H-J, Frosch MP, Sakmann B, Walsh DM, Konnerth A. A vicious cycle of β amyloid-dependent neuronal hyperactivation. Science. 2019; 365:559-565..
44. He Y, Wei M, Wu Y, Qin H, Li W, Ma X, Cheng J, Ren J, Shen Y, Chen Z, Sun B, Huang FD, Shen Y, Zhou YD. Amyloid β oligomers suppress excitatory transmitter release *via* presynaptic depletion of phosphatidylinositol-4,5-bisphosphate. Nat Commun. 2019; 10:1193.
45. Dhawan G, Floden AM, Combs CK. Amyloid-beta oligomers stimulate microglia through a tyrosine kinase dependent mechanism. Neurobiol Aging. 2012; 33:2247-2261.
46. Ohno Y, Kinboshi M, Shimizu S. Inwardly Rectifying Potassium Channel Kir4.1 as a Novel Modulator of BDNF Expression in Astrocytes. Int J Mol Sci. 2018; 19:3313.
47. Rivera C, Voipio J, Thomas-Crusells J, Li H, Emri Z, Sipila S, Payne JA, Minichiello L, Saarma M, Kaila K. Mechanism of activity-dependent downregulation of the neuron-specific K-Cl cotransporter KCC2. J Neurosci. 2004; 24:4683-4691.
48. Kaila K, Price TJ, Payne JA, Puskarjov M, Voipio J. Cation-chloride cotransporters in neuronal development, plasticity and disease. Nat Rev Neurosci. 2014; 15:637-654.
49. Beggs S, Trang T, Salter MW. P2X4R+ microglia drive neuropathic pain. Nat Neurosci. 2012; 15:1068-1073.
50. Puskarjov M, Ahmad F, Kaila K, Blaesse P. Activity-dependent cleavage of the K-Cl cotransporter KCC2 mediated by calcium-activated protease calpain. J Neurosci. 2012; 32:11356-11364.
51. Zadran S, Jourdi H, Rostamiani K, Qin Q, Bi X, Baudry M. Brain-derived neurotrophic factor and epidermal growth factor activate neuronal m-calpain *via* mitogen-activated protein kinase-dependent phosphorylation. J Neurosci. 2010; 30:1086-1095.
52. Selkoe DJ. Alzheimer's disease is a synaptic failure. Science. 2002; 298:789-791.
53. Verret L, Mann EO, Hang GB, Barth AM, Cobos I, Ho K, Devidze N, Masliah E, Kreitzer AC, Mody I, Mucke L, Palop JJ. Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. Cell. 2012; 149:708-721.
54. Succol F, Fiumelli H, Benfenati F, Cancedda L, Barberis A.

Intracellular chloride concentration influences the $GABA_A$ receptor subunit composition. Nat Commun. 2012; 3:738.
55. Chen M, Wang J, Jiang J, Zheng X, Justice NJ, Wang K, Ran X, Li Y, Huo Q, Zhang J, Li H, Lu N, Wang Y, Zheng H, Long C, Yang L. APP modulates KCC2 expression and function in hippocampal GABAergic inhibition. Elife. 2017; 6:e20142.
56. Brady ML, Pilli J, Lorenz-Guertin JM, Das S, Moon CE, Graff N, Jacob TC. Depolarizing, inhibitory GABA type A receptor activity regulates GABAergic synapse plasticity *via* ERK and BDNF signaling. Neuropharmacology. 2018; 128:324-339.
57. Lemtiri-Chlieh F, Levine ES. BDNF evokes release of endogenous cannabinoids at layer 2/3 inhibitory synapses in the neocortex. J Neurophysiol. 2010; 104:1923-1932.
58. Brunig I, Penschuck S, Berninger B, Benson J, Fritschy JM. BDNF reduces miniature inhibitory postsynaptic currents by rapid downregulation of $GABA_A$ receptor surface expression. Eur J Neurosci. 2001; 13:1320-1328.
59. Govindpani K, Calvo-Flores Guzmán B, Vinnakota C, Waldvogel HJ, Faull RL, Kwakowsky A. Towards a Better Understanding of GABAergic Remodeling in Alzheimer's Disease. Int J Mol Sci. 2017; 18:1813.
60. Kwakowsky A, Calvo-Flores Guzmán B, Pandya M, Turner C, Waldvogel HJ, Faull RL. $GABA_A$ receptor subunit expression changes in the human Alzheimer's disease hippocampus, subiculum, entorhinal cortex and superior temporal gyrus. J Neurochem. 2018; 145:374-392.
61. Gueli MC, Taibi G. Alzheimer's disease: amino acid levels and brain metabolic status. Neurological Sciences. 2013; 34: 1575-79.
62. Ramos-Miguel A, Hercher C, Beasley CL, Barr AM, Bayer TA, Falkai P, Leurgans SE, Schneider JA, Bennett DA, Honer WG. Loss of Munc18-1 long splice variant in GABAergic terminals is associated with cognitive decline and increased risk of dementia in a community sample. Molecular Neurodegeneration. 2015; 10: 65.
63. Vellas B, Sol O, Snyder P, Ousset PJ, Haddad R, Maurin M, Lemarie J-C, Desire L, P Pando M. EHT0202 in Alzheimers Disease: A 3-Month, Randomized, Placebo-Controlled, Double-Blind Study. Curr Alzheimer Res. 2011; 8:203-212.
64. Guerrini G, Ciciani G, Costanzo A, Daniele S, Martini C, Ghelardini C, Di Cesare Mannelli L, Ciattini S. Synthesis of novel cognition enhancers with pyrazolo[5,1-c][1,2,4] benzotriazine core acting at γ-aminobutyric acid type A ($GABA_A$) receptor. Bioorg Med Chem. 2013; 21:2186-2198.

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Commentary

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Considerations and guidance to control the rebound in COVID-19 cases

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SUMMARY Induced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its variants, the COVID-19 pandemic has caused a serious crisis for healthcare systems worldwide. COVID-19 vaccine coverage has increased in many countries, but the COVID-19 epidemic has rapidly expanded, with a daily increase of 30,390 COVID-19 cases and 9,761 deaths since August 12, 2021. This article provides a brief overview of growing concerns about a rebound of the COVID-19 pandemic caused by the Delta variant and public health epidemic control measures that have recently been relaxed. As of August 13, 2021, 465,679 cases of COVID-19 due to the Delta variant of SARS-CoV-2 have been detected in over 120 countries. Epidemic control measures were relaxed in some areas, such as allowing large gatherings and improper criteria for ending self-isolation. Even in China, where the epidemic was tightly controlled with strict non-pharmaceutical interventions (NPIs), new COVID-19 cases, and asymptomatic cases in particular, spiked in the first 13 days of August. More importantly, most of those cases were local, while most of the cases accounting for the previous increase were imported. Therefore, relaxed epidemic control measures and asymptomatic infections possibly caused by the Delta variant of SARS-CoV-2 may increase the risk of virus transmission. Accordingly, suggestions for COVID-19 containment, such as encouraging vaccination of the general population, using Internet of Things technology (loT) to reduce the possibility of contact with the asymptomatic infected, and enhancing disease surveillance, have been offered here.

Keywords COVID-19, SARS-CoV-2, non-pharmaceutical interventions, vaccination, Internet of Things technology (loT), vaccines

1. Introduction

COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This global threat has led to 205,338,159 confirmed cases, including 4,333,094 deaths, since August 13, 2021 (*1*). Although more than 4 billion vaccine doses have been administered, the daily confirmed cases continued to increase from 302,979 to 686,063 from June 23, 2021 to August 12, 2021 (*1*). To make matters worse, the B.1.617.2 (Delta) variant of SARS-CoV-2 spread from its origin in India, and this variant is more transmissible than other variants, especially in indoor sports settings and within households (*2*). Despite such troubling circumstances, epidemic control measures have been relaxed in some areas because vaccination coverage increased (*1*). Thus, the reason for the daily increase in new cases over the last two month and further suggestions for COVID-19 control have been discussed here.

2. The possibility of a new rebound in cases

2.1. The spread of the Delta variant

After the Delta variant (also known as B.1.617.2) emerged in India after December 2020, it spread to over 120 countries, and it has led to 465,679 confirmed cases as of August 13, 2021 (*2,3*). Compared to previous variants, the Delta variant is highly contagious (more than twice as contagious than previous variants), and the Delta variant leads to more infections and it spreads faster (*4*). The high transmissibility of the Delta variant was responsible for not only the deadly second wave of COVID-19 infections in April 2021 in India (*3,4*) but also the highest number of cases due to SARS-CoV-2, with 246,284 confirmed cases in UK as of August 13,

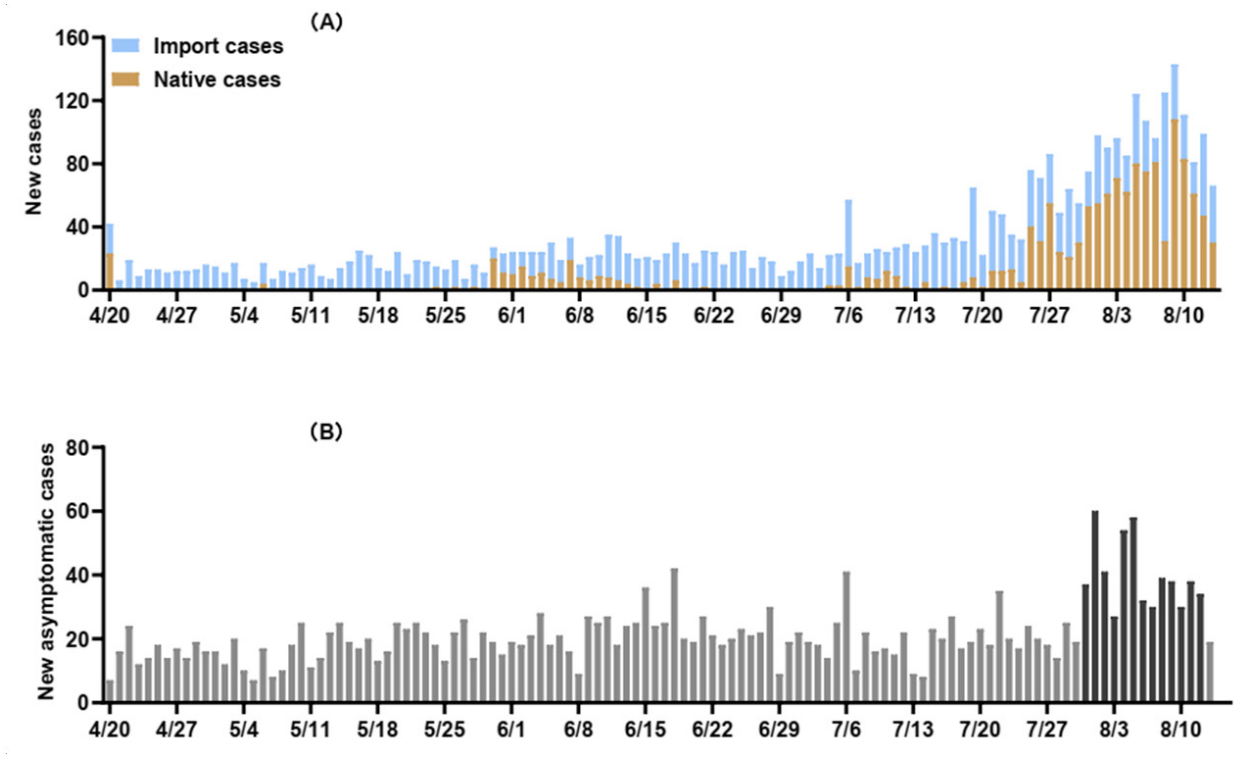

Figure 1. (A), The daily increase in new COVID-19 cases between April 20, 2021 and August 13, 2021. Blue bars indicate imported cases, and yellow bars indicate the number of local cases. **(B), The daily increase in new asymptomatic cases of COVID-19 between April 20, 2021 and August 13, 2021.** A darker color indicates a significant increase in new asymptomatic cases over the past few months.

2021 (*3,5*).

Although there have been great breakthroughs and promising vaccine coverage in some countries, cases have continued to rebound since late June 2020 (*1*). Various studies have examined the Delta variant's transmissibility and contagiousness in unvaccinated people and fully vaccinated people. Those studies revealed that even fully vaccinated people who are asymptomatic could be infectious and that unvaccinated people are much more likely to transmit the virus through contract. In addition, the Delta variant seems to produce the same high viral titer in unvaccinated and fully vaccinated people (*6-8*).

2.2. Public health epidemic control measures that have recently been relaxed

In addition to vaccination, tightening public health and social measures have also contributed substantially to control of the virus (*9*). However, epidemic control measures are a balance of infections and deaths and economic loss. A modelling study by Yang *et al* found that if lockdowns were lifted too early and too extensively across the UK, then there could be a risk of a second wave, possibly resulting in 23.4 million infected and 897,000 deaths (*10*). In countries and regions that have relaxed public health measures and self-isolation, lifting lockdowns too early (such as improper criteria for ending self-isolation) could be a major cause of a rebound in cases. Agreeing with those findings, a study by Zhang *et al*. stated that the combination of vaccination and interventions can effectively suppress the transmission of new COVID-19 variants (*11*). Thus, public health epidemic control measures that have recently been relaxed may also be responsible for the rebound in cases.

2.3. COVID-19 cases can even rebound in countries with stringent epidemic controls

As mentioned before, China has tightened its stringent epidemic controls and it has a promising vaccination rate, thus successfully controlling the epidemic during the first wave (*9*). However, cases have increased over the last month (Figure 1A). Unlike most of the previous sporadic cases that were imported, most of the cases from August 1, 2021 to August 12, 2021 were local. Based on this phenomenon, asymptomatic infection will presumably increase the risk of virus transmission. The daily increase in the asymptomatic infected from August 1, 2021 to August 12, 2021 was higher than previous increases (Figure 1B). After the first nadir in COVID-19 cases in China, the asymptomatic infected accounted for more than 65.8% (2521/3831) of the daily increase in cases from April 20, 2021 to August 13, 2021. Corroborating that fact, Snider *et al*. reported that asymptomatic cases were a hidden challenge in predicting confirmed cases of COVID-19 (*12*). Therefore, the public should pay

more attention to asymptomatic infections even after the pandemic has subsided.

3. Guidance to control the rebound in COVID-19 cases

COVID-19 has led to tremendous hardships and challenges worldwide. As the epidemic continues, various effective vaccines have been developed and administered despite the rise and spread of various variants. However, the asymptomatic infected, and especially those who self-quarantined or who were released prematurely after close contact, are a hidden challenge (*12*).

Suggestions to deal with those issues are as follows: First, to better ascertain the characteristics of asymptomatic COVID-19 infection and to reduce the harm to the public, more clinical research on the contagiousness, transmissibility, and mortality rate of the virus and its variants should be conducted in large populations and at multiple centers. Second, non-pharmaceutical interventions can protect not only the symptomatic infected but also the asymptomatic infected. Personal protective measures need to be maintained even after the COVID-19 epidemic. Measures such as wearing a mask, disinfecting one's hands, and social distancing should be continued for a prolonged period. Third, to avoid the infections caused by symptomatic individuals, vaccination of the general public is required to reduce the risk of infection.

Last, since asymptomatic cases are usually hard to detect and the contacts of those affected are difficult to track, the criteria for release have been lax. However, the Internet of things (IoT) can play a role in combating the COVID-19 pandemic (*13-15*). For example, remote monitoring of health data, long-term follow-up and data collection can be used to tackle COVID-19. The IoT provides an excellent integrated network and it supplies useful information that can aid healthcare decision-making and public health management.

In order to better control COVID-19, new IoT technologies should be improved and then implemented. Simpler follow-ups, nucleic acid testing for the self-quarantined, and integrating information on close contacts are several issues the IoT could tackle (*14*). Another issue is accurately and rapidly conducting nucleic acid testing. Predicting outbreaks and the rebound of the epidemic are additional issues loT could tackle. The security and privacy of patient data need to be monitored, and this issue is still a subject of debate (*16*).

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References

1. World Health Organization. Global influenza strategy 2019-2030. *https://apps.who.int/iris/bitstream/handle/10665/311184/9789241515320eng.pdf?sequence=18&isAllowed=y* (accessed August 15, 2021).
2. Dougherty K, Mannell M, Naqvi O, Matson D, Stone J. SARS-CoV-2 B.1.617.2 (Delta) variant COVID-19 outbreak associated with a gymnastics facility - Oklahoma, April-May 2021. MMWR Morb Mortal Wkly Rep. 2021; 70:1004-1007.
3. Statista. Number of SARS-CoV-2 Delta variant cases worldwide as of August 13, 2021, by country or territory. *http://www.statista.com/statistics/1245971/number-delta-variant-worldwide-by-country/* (accessed August 15, 2021).
4. Centers for Disease Control and Prevention. Delta Variant: What We Know About the Science. *https://www.cdc.gov/coronavirus/2019-ncov/variants/delta-variant.html* (accessed August 15, 2021).
5. Aleem A, Akbar Samad AB, Slenker AK. Emerging variants of SARS-CoV-2 and novel therapeutics against coronavirus (COVID-19). In StatPearls. Treasure Island (FL): StatPearls Publishing; 2021.
6. Musser JM, Christensen PA, Olsen RJ, Long SW, Subedi S, Davis JJ, Hodjat, D, Walley DR, Kinskey JC, Gollihar J. Delta variants of SARS-CoV-2 cause significantly increased vaccine breakthrough COVID-19 cases in Houston, Texas. MedRxiv. 2021; doi: https://doi.org/10.1101/2021.07.19.21260808
7. Nasreen S, Chung H, He S, *et al*. Effectiveness of COVID-19 vaccines against variants of concern in Ontario, Canada. MedRxiv. 2021; doi: https://doi.org/10.1101/2021.06.28.21259420.
8. Riemersma KK, Grogan BE, Kita-Yarbro A, Halfmann P, Kocharian A, Florek KR, Westergaard R, Bateman A, Jeppson GE, Kawaoka Y, O'Connor DH, Friedrich TC, Grande KM. Vaccinated and unvaccinated individuals have similar viral loads in communities with a high prevalence of the SARS-CoV-2 delta variant. MedRxiv. 2021; doi: https://doi.org/10.1101/2021.07.31.21261387.
9. Burki T. China's successful control of COVID-19. Lancet Infect Dis. 2020; 20:1240-1241.
10. Yang P, Yang G, Qi J, Sheng B, Yang Y, Zhang S, Bi G, Mao X. The effect of multiple interventions to balance healthcare demand for controlling COVID-19 outbreaks: A modelling study. Sci Rep. 2021; 11:3110.
11. Zhang S, Bi G, Yang Y, Qi J, Li S, Mao X, Peng R, Yang P. An extended COVID-19 epidemiological model with vaccination and multiple interventions for controlling COVID-19 outbreaks in the UK. MedRxiv. 2021; doi: https://doi.org/10.1101/2021.03.10.21252748.
12. Snider B, Patel B, McBean E. Asymptomatic cases, the hidden challenge in predicting COVID-19 caseload increases. Infect Dis Rep. 2021; 13:340-347.
13. Singh RP, Javaid M, Haleem A, Suman R. Internet of things (IoT) applications to fight against COVID-19

pandemic. Diabetes Metab Syndr. 2020; 14:521-524.
14. Tsikala Vafea M, Atalla E, Georgakas J, Shehadeh F, Mylona EK, Kalligeros M, Mylonakis E. Emerging technologies for use in the study, diagnosis, and treatment of patients with COVID-19. Cell Mol Bioeng. 2020; 13:249-257.
15. Swayamsiddha S, Mohanty C. Application of cognitive Internet of Medical Things for COVID-19 pandemic. Diabetes Metab Syndr. 2020; 14:911-915.
16. Yang Y, Wang X, Du R-H, *et al*. Serological investigation of asymptomatic cases of SARS-CoV-2 infection reveals weak and declining antibody responses. Emerg Microbes Infect. 2021;10:905-912.

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Commentary

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Update: Drug treatment options for coronavirus disease 2019 (COVID-19)

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SUMMARY Coronavirus disease 19 (COVID-19) continues to rage as a global pandemic. A number of potential therapeutic agents have been explored over the past year or two. However, numerous drugs that were expected to prove highly effective, such as lopinavir/ritonavir and remdesivir, have been found to have little benefit in large clinical trials. Interleukin-6 receptor antagonists, glucocorticoids, Janus kinase inhibitors, and some antivirals have been found to provide significant benefits in terms of reducing viral load, reducing the time of nucleic acid conversion, or improving survival. For example, bamlanivimab and etesevimab, which are newly designed monoclonal antibodies against the surface spike protein S1 subunit receptor-binding domain (RBD) of SARS-CoV-2, have a significant effect on reducing the viral load and the hospitalization rate of patients with mild COVID-19. Several vaccines against SARS-CoV-2 have been widely administered worldwide and have provided good protection. Nevertheless, the increasingly hardy variants of the virus have raised the requirements for vaccine design. Perhaps RBD-based vaccines are a viable way to defend against variants, but this still needs to be verified in a large sample. Therefore, this paper provides an update on the treatment options for COVID-19 based on three previously proposed dimensions of drug screening: standard assays of existing broad-spectrum antivirals, screening of chemical libraries, and redevelopment of new, specific drugs.

Keywords COVID-19, SARS-CoV-2, coronaviruses, drug therapy

As of August 14, 2021, there were 205 million cumulative diagnoses of coronavirus disease 19 (COVID-19) and 4.3 million deaths worldwide (*1*). The COVID-19 pandemic has triggered a global health crisis, and effective pharmacological interventions may be the solution to the existing dilemma. The current authors previously proposed three approaches to drug screening for the treatment of COVID-19 (*2*), and the current article updates the potential treatment options that are currently available (Table 1).

The first approach is to standardize existing broad-spectrum antivirals that are used to treat other viral infections. Representative drugs are interferon (INF) and ribavirin. *In vitro* experiments have confirmed that IFN-β effectively blocks the replication of SARS-CoV-2 (*3*). In a prospective, open-label, randomized, phase II trial in Hong Kong, 86 patients with COVID-19 were given subcutaneous INF β-1b along with lopinavir/ritonavir and ribavirin within 7 days of symptom onset for 14 days. The time to nucleic acid conversion (7 days verse 12 days) was significantly shorter in patients receiving the triple combination compared to the antiviral control group not receiving interferon (*4*). In the United Kingdom, a randomized, double-blind, placebo-controlled phase II trial that evaluated the effect of IFN β-1a, known as SNG001, after 14 days of continuous inhalation found a greater likelihood of improvement in patients receiving IFN β-1a than in the placebo group (*5*). However, another clinical trial of IFN beta-1a given subcutaneously or intravenously found that it did not reduce overall or subgroup mortality in patients with COVID-19 (*6*). Therefore, further clinical trials of IFN-β are urgently warranted.

The second method is to screen established chemical libraries and to then conduct antiviral trials.

Repurposing of older drugs has accelerated the deployment of new therapies for COVID-19. Based on previous experience with the treatment of SARS, Middle East respiratory syndrome, and other novel influenza viruses, numerous large-scale clinical trials have explored various drugs. Two studies have found that mortality and the need for invasive mechanical ventilation are reduced

Table 1. Potential COVID-19 drugs mentioned in this article

Drug therapy	Classification	Outcomes	Country/Research team	Ref.
INF β-1b +lopinavir/ritonavir +ribavirin	Broad-spectrum antiviral	Less time to nucleic acid conversion compared to that in the control group not receiving IFN.	China	(*4*)
IFN β-1a (SNG001)	Broad-spectrum antiviral	Patients who received SNG001 had greater odds of improvement and recovered more rapidly from SARS-CoV-2 infection.	The United Kingdom	(*5*)
Tocilizumab	Interleukin-6 receptor antagonist	Reduction in mortality and the need for invasive mechanical ventilation and improvement of outcomes, including survival.	RECOVERY Collaborative Group, REMAP-CAP Investigators, Netherlands	(*7-9*)
Sarilumab	Interleukin-6 receptor antagonist	Improvement of outcomes, including survival.	REMAP-CAP Investigators	(*8*)
Dexamethasone	Glucocorticoid	The 28-day mortality among those who were receiving respiratory support was lower.	RECOVERY Collaborative Group, COALITION COVID-19 Brazil III Investigators, Egypt	(*10-12*)
Nitazoxanide	Anti-parasite drug	Can induce a greater reduction in viral load.	SARITA-2 investigators	(*15*)
Molnupiravir	Nucleoside analogue	Highly effective at reducing nasopharyngeal SARS-CoV-2 and viral RNA.	United States	(*20*)
Imatinib	Tyrosine kinase inhibitor	May have a clinical benefit in terms of survival and the duration of mechanical ventilation.	Netherlands	(*21*)
Baricitinib	Janus kinase inhibitor	Baricitinib plus remdesivir was superior to remdesivir alone in reducing recovery time and accelerating improvement in clinical status.	ACTT-2 Study Group Members	(*22*)
Tofacitinib	Janus kinase inhibitor	Led to a lower risk of death or respiratory failure through day 28.	STOP-COVID Trial Investigators	(*23*)
Bamlanivimab +etesevimab	RBD monoclonal antibodies	Led to a lower incidence of hospitalization and death and accelerated the decline in the SARS-CoV-2 viral load.	BLAZE-1 Investigators, BLAZE-2 Investigators	(*25-26*)
REGN-COV2 (casirivimab +imdevimab)	RBD monoclonal antibodies	Reduced viral load.	Trial Investigators	(*28*)
Meplazumab	CD147 monoclonal antibody	Reduced the time to discharge, case severity, and the time to a negative test result.	China	(*30*)
CoronaVac	Inactivated vaccine	Effective in preventing COVID-19, including severe illness and death	Sinovac Research and Development Co., Ltd.	(*36*)
I-R-F vaccine (V-01)	Protein subunit	All vaccinated adults were positive for antibodies to the RBD after two doses	China	(*38*)

in patients receiving interleukin-6 receptor antagonists (*e.g.*, tocilizumab and sarilumab); the duration of mechanical ventilation and hospitalization may also be reduced (*7-9*). Similarly, a benefit from dexamethasone was also noted (*10-12*). The previous Solidarity trial, a global study led by the World Health Organization, found that remdesivir, hydroxychloroquine, and lopinavir had little or no effect on hospitalized patients with COVID-19, as evinced by overall mortality, initiation of ventilation, and duration of hospitalization (*6*). A study then analyzed a drug library of approximately 12,000 clinical-stage or Food and Drug Administration (FDA)-approved small molecules, and it identified 13 drugs, including kinase inhibitors and protease inhibitors, that were effective at concentrations commensurate with therapeutic doses (*13*). In addition, masitinib was found to influence SARS-CoV-2 replication by competitively inhibiting proteinase 3CL. Masitinib substantially inhibited several variants of concern such as B.1.1.7, B.1.351, and P.1. Animal studies indicated that the SARS-CoV-2 viral load in the lungs and nasal turbines of mice treated with masitinib decreased by more than 99% on day 6, and the extent of pulmonary pathology and levels of cytokines were significantly lower than those in the control group (*14*). More importantly, survival rates were also significantly higher in the treated group than in the control group.

In a multicenter, randomized, double-blind, placebo-controlled trial, the efficacy of nitazoxanide, an anti-parasite drug, was tested in 392 patients with COVID-19 who were hospitalized with adult-onset disease. Viral load decreased after 5 days of nitazoxanide treatment compared to a placebo (55% *vs*. 45%). However, there were no significant differences in the time to remission of symptoms (*15*). Another oral antiviral targeting mild COVID-19, molnupiravir, has been found to exhibit antiviral action in *in vitro* experiments primarily by increasing the rate of viral genomic RNA mutations

(*16,17*). Animal testing trials have also found it to be effective in reducing viral load (*18,19*). Results from a Phase IIa trial indicated a shorter time to viral RNA clearance and a greater proportion of participants achieving overall clearance with 800 mg of molnupiravir compared to a placebo (*20*). Molnupilavir was generally well tolerated, with a similar number of adverse events in all groups.

Tyrosine kinase inhibitor imatinib may reverse pulmonary capillary leak. A randomized, double-blind, placebo-controlled clinical trial was conducted in 400 hospitalized patients with COVID-19 who required supplemental oxygen to maintain a peripheral oxygen saturation greater than 94% (*21*). Imatinib did not reduce the time for discontinuation of ventilation and supplemental oxygen for more than 48 consecutive hours compared to a placebo. The observed effects on survival and the median duration of mechanical ventilation suggest that imatinib may provide a clinical benefit to patients hospitalized with COVID-19, and the Solidarity trial will confirm or reject these findings.

In addition, baricitinib, a selective Janus kinase 1 and 2 inhibitor for the treatment of rheumatoid arthritis, has been noted to have two major advantages in the treatment of COVID-19: possible anti-inflammatory and antiviral action. A randomized double-blind placebo-controlled trial noted a reduced mortality rate in patients requiring oxygen support who received baricitinib plus remdesivir compared to remdesivir alone (5.1% *vs*. 7.8%) (*22*). Another selective Janus kinase 1 and 3 inhibitor, tofacitinib, was associated with a lower risk of death or respiratory failure through day 28 than a placebo after treatment of 19 adult patients hospitalized with COVID-19 (*23*).

This strategy continues to offer promising and could be used to explore more drugs.

The third strategy is to directly target SARS-CoV-2 by designing and developing new drugs that target its genomic or biophysical structures. SARS-CoV-2 infects cells by binding to the angiotensin-converting enzyme 2 (ACE2) receptor on host cells *via* the surface spike protein and by inducing membrane fusion (*24*). Therefore, monoclonal antibodies and vaccines targeting the spike protein could be a way to treat and prevent COVID-19.

Bamlanivimab and etesevimab are monoclonal antibodies against the surface spike protein S1 subunit receptor-binding domain (RBD) of SARS-CoV-2. The antibodies were respectively isolated from the plasma of recovering patients with COVID-19 in the United States and China. A phase III trial involving 1,035 outpatients with COVID-19 of mild or moderate severity indicated that the combination of bamlanivimab and etesevimab (2,800 mg of bamlanivimab and 2,800 mg of etesevimab) reduced the incidence of hospitalization and death associated with COVID-19 compared to a placebo (2.1% *vs*. 7%) and accelerated the decline in viral load (*25*). This demonstrates the effectiveness of early administration of bamlanivimab plus etesevimab in reducing hospitalization rates. A point worth mentioning is that a previous randomized phase 2/3 trial, also in outpatients, noted a significant decrease in viral load between a combination of the two antibodies and a placebo, while there were no significant differences in viral load with bamlanivimab monotherapy, regardless of the dose (*26*). Studies in hospitalized patients have also found that bamlanivimab did not display efficacy even when combined with remdesivir (*27*). This may be because combining several types of antibodies helps to reduce the frequency of evasion of antibody-mediated neutralization due to viral variants.

REGN-COV2 is a combination of two powerful neutralizing antibodies, casirivimab and imdevimab, that bind to two distinct and non-overlapping sites on the RBD. An ongoing phase I study has indicated that REGN-COV2 enhanced viral clearance in outpatients with COVID-19, and particularly in patients who had not initiated an endogenous immune response (*i.e.*, serum antibody-negative) or who had a high baseline viral load, and it noted no serious adverse events in the high-dose group (REGN-COV2 8 g) (*28*).

In addition to the ACE2 receptor, direct interaction between CD147 and the SARS-CoV-2 spike protein can mediate viral infection of host cells (*29*). CD147 is a transmembrane glycoprotein of the immunoglobulin superfamily and is involved in several pathogenic processes such as tumor development and parasitic, bacterial, and viral infections. Therefore, meplazumab may, as a CD147 monoclonal antibody, be a new therapeutic pathway by inhibiting novel coronavirus replication through depletion of CD147 or blocking of CD147. Phase I and exploratory phase II clinical trials of meplazumab have been completed with favorable results in terms of safety and efficacy (*30*).

A vaccine to prevent SARS-CoV-2 infection is believed to be the most promising method to control the outbreak. Primate studies and human epidemiological studies have found that a SARS-CoV-2 infection causes the body to produce functional neutralizing antibodies that are protective against reinfection (*31-34*). Therefore, vaccines that elicit an adequate neutralizing response should protect against COVID-19. As of August 16, 2021, dozens of vaccines are available in different regions of the world, more than 100 vaccines are in clinical trials, and more than 180 vaccines are in preclinical trials (*35*). SARS-CoV-2 vaccine development has used a variety of different platforms, including conventional inactivated live inactivated vaccines (LIVs), live attenuated vaccines (LAVs), novel recombinant protein vaccines (RPVs), viral vector vaccines (VVVs), DNA vaccines, and RNA vaccines. Of the eight vaccines in Phase 4 clinical trials, three are non-replicating vector vaccines (ChAdOx1 nCoV-19/AZD1222, Ad26.COV2.S, Ad5-COVID-19), three are RNA vaccines (BNT162b2,

mRNA 1273, mRNA 1273.351), and the remaining two are inactivated vaccines (BBIBP-CorV, CoronaVac). After a SARS-CoV-2 vaccine becomes available and widely used, unresolved efficacy issues need to continue to be assessed in clinical trials and vaccine safety needs to be monitor. Data from mass vaccination with CoronaVac in Chile indicated that the vaccine was effective in preventing COVID-19, including severe illness and death (*36*). At the same time, the continued emergence of new variants poses a challenge for vaccine design and development, which is a fact that cannot be overlooked.

In vitro experiments recently indicated that a neutralizing antibody (named S2X259), broadly neutralizes multiple SARS-CoV-2 variants of concern (VOC), including B.1.1.7, B.1.351, P.1, and B.1.427/B.1.429 (*37*). This identification of monoclonal antibodies from memory B cells of individuals with COVID-19 who recovered may guide future efforts to develop novel vaccines that can overcome the emergence of variants.

Moreover, a randomized, placebo-controlled phase I/II trial of a human I-R-F vaccine (V-01) in 180 healthy adults has concluded. Developed in China, the vaccine was created by fusing IFN-α at the N-terminal end of the RBD after the RBDs were combined to immunoglobulin Fcs as a stable dimer. This structure increases the passage of vaccine molecules through the lymph nodes while improving the efficiency of dendritic cells in capturing and presenting antigens. Clinical trials have noted no serious adverse events and all vaccinated adults were positive for antibodies to the RBD after two doses of the V-01 vaccine (*38*). Because of its efficacy and safety, this engineered vaccine may be a next-generation candidate in the global effort to defeat COVID-19.

As new drugs are developed, combinations of neutralizing antibodies such as bamlanivimab plus etesevimab have superior efficacy and could potentially be potent agents, but further clinical trials still need to be conducted. The Solidarity clinical trial will test three new drugs in hospitalized patients with COVID-19: the anticancer drug imatinib, an antibody called infliximab for autoimmune diseases, and artesunate, an antimalarial drug (*39*). Several vaccines are now widely administered worldwide and provide good protection, but the emerging variants of SARS-CoV-2 are a serious challenge to be faced in vaccine design and development.

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References

1. World Health Organization. Numbers at a glance. *https://www.who.int/emergencies/diseases/novel-coronavirus-2019* (accessed August 15, 2021).
2. Lu H. Drug treatment options for the 2019-new coronavirus (2019-nCoV). Biosci Trends. 2020; 14:69-71.
3. Lei X, Dong X, Ma R, Wang W, Xiao X, Tian Z, Wang C, Wang Y, Li L, Ren L, Guo F, Zhao Z, Zhou Z, Xiang Z, Wang J. Activation and evasion of type I interferon responses by SARS-CoV-2. Nature Commun. 2020; 11:3810.
4. Hung IF, Lung KC, Tso EY, *et al*. Triple combination of interferon beta-1b, lopinavir-ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: An open-label, randomised, phase 2 trial. Lancet. 2020; 395:1695-1704.
5. Monk PD, Marsden RJ, Tear VJ, Brookes J, Batten TN, Mankowski M, Gabbay FJ, Davies DE, Holgate ST, Ho LP, Clark T, Djukanovic R, Wilkinson TMA; Inhaled Interferon Beta COVID-19 Study Group. Safety and efficacy of inhaled nebulised interferon beta-1a (SNG001) for treatment of SARS-CoV-2 infection: A randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Respir Med. 2021; 9:196-206.
6. Pan H, Peto R, Henao-Restrepo AM, *et al*. Repurposed antiviral drugs for Covid-19 - Interim WHO Solidarity Trial Results. N Engl J Med. 2021; 384:497-511.
7. RECOVERY Collaborative Group. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): A randomised, controlled, open-label, platform trial. Lancet. 2021; 397:1637-1645.
8. Gordon AC, Mouncey PR, Al-Beidh F, *et al*. Interleukin-6 receptor antagonists in critically ill patients with Covid-19. N Engl J Med. 2021; 384:1491-1502.
9. Ramiro S, Mostard RLM, Magro-Checa C, van Dongen CMP, Dormans T, Buijs J, Gronenschild M, de Kruif MD, van Haren EHJ, van Kraaij T, Leers MPG, Peeters R, Wong DR, Landewé RBM. Historically controlled comparison of glucocorticoids with or without tocilizumab versus supportive care only in patients with COVID-19-associated cytokine storm syndrome: Results of the CHIC study. Ann Rheum Dis. 2020; 79:1143-1151.
10. Horby P, Lim WS, Emberson JR, *et al*. Dexamethasone in hospitalized patients with Covid-19. N Engl J Med. 2021; 384:693-704.
11. Tomazini BM, Maia IS, Cavalcanti AB, *et al*. Effect of dexamethasone on days alive and ventilator-free in patients with moderate or severe acute respiratory distress syndrome and COVID-19: The CoDEX Randomized Clinical Trial. JAMA. 2020; 324:1307-1316.
12. Rashad A, Mousa S, Nafady-Hego H, Nafady A, Elgendy H. Short term survival of critically ill COVID-19 Egyptian patients on assisted ventilation treated by either dexamethasone or tocilizumab. Sci Rep. 2021; 11:8816.
13. Riva L, Yuan S, Yin X, *et al*. Discovery of SARS-CoV-2 antiviral drugs through large-scale compound repurposing. Nature. 2020; 586:113-119.
14. Drayman N, DeMarco JK, Jones KA, *et al*. Masitinib is a broad coronavirus 3CL inhibitor that blocks replication of SARS-CoV-2. Science. 2021; doi: 10.1126/science.abg5827.
15. Rocco PRM, Silva PL, Cruz FF, *et al*. Early use of nitazoxanide in mild COVID-19 disease: Randomised,

placebo-controlled trial. Eur Respir J. 2021; 58:2003725.
16. Bakowski MA, Beutler N, Wolff KC, *et al*. Drug repurposing screens identify chemical entities for the development of COVID-19 interventions. Nature Commun. 2021; 12:3309.
17. Sheahan TP, Sims AC, Zhou S, *et al*. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. Sci Transl Med. 2020; 12:eabb5883.
18. Wahl A, Gralinski LE, Johnson CE, *et al*. SARS-CoV-2 infection is effectively treated and prevented by EIDD-2801. Nature. 2021; 591:451-457.
19. Cox RM, Wolf JD, Plemper RK. Therapeutically administered ribonucleoside analogue MK-4482/EIDD-2801 blocks SARS-CoV-2 transmission in ferrets. Nat Microbiol. 2021; 6:11-18.
20. Fischer W, Eron JJ, Holman W, *et al*. Molnupiravir, an oral antiviral treatment for COVID-19. medRxiv. 2021; doi: 10.1101/2021.06.17.21258639.
21. Aman J, Duijvelaar E, Botros L, *et al*. Imatinib in patients with severe COVID-19: A randomised, double-blind, placebo-controlled, clinical trial. Lancet Respir Med. 2021; doi: 10.1016/S2213-2600(21)00237-X.
22. Kalil AC, Patterson TF, Mehta AK, *et al*. Baricitinib plus remdesivir for hospitalized adults with Covid-19. N Engl J Med. 2021; 384:795-807.
23. Guimarães PO, Quirk D, Furtado RH, *et al*. Tofacitinib in patients hospitalized with Covid-19 pneumonia. N Engl J Med. 2021; 385:406-415.
24. Zhou P, Yang XL, Wang XG, *et al*. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020; 579:270-273.
25. Dougan M, Nirula A, Azizad M, *et al*. Bamlanivimab plus etesevimab in mild or moderate Covid-19. N Engl J Med. 2021; doi: 10.1056/NEJMoa2102685.
26. Gottlieb RL, Nirula A, Chen P, *et al*. Effect of bamlanivimab as monotherapy or in combination with etesevimab on viral load in patients with mild to moderate COVID-19: A randomized clinical trial. JAMA. 2021; 325:632-644.
27. Lundgren JD, Grund B, Barkauskas CE, *et al*. A neutralizing monoclonal antibody for hospitalized patients with Covid-19. N Engl J Med. 2021; 384:905-914.
28. Weinreich DM, Sivapalasingam S, Norton T, *et al*. REGN-COV2, a neutralizing antibody cocktail, in outpatients with Covid-19. N Engl J Med. 2021; 384:238-251.
29. Wang K, Chen W, Zhang Z, *et al*. CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. Signal Transduct Target Ther. 2020; 5:283.
30. Bian H, Zheng ZH, Wei D, *et al*. Safety and efficacy of meplazumab in healthy volunteers and COVID-19 patients: A randomized phase 1 and an exploratory phase 2 trial. Signal Transduct Target Ther. 2021; 6:194.
31. Mercado NB, Zahn R, Wegmann F, *et al*. Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. Nature. 2020; 586:583-588.
32. Yu J, Tostanoski LH, Peter L, *et al*. DNA vaccine protection against SARS-CoV-2 in rhesus macaques. Science. 2020; 369:806-811.
33. Gao Q, Bao L, Mao H, *et al*. Development of an inactivated vaccine candidate for SARS-CoV-2. Science. 2020; 369:77-81.
34. Addetia A, Crawford KHD, Dingens A, Zhu H, Roychoudhury P, Huang ML, Jerome KR, Bloom JD, Greninger AL. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with a high attack rate. J Clin Microbiol. 2020; 58:e02107-20.
35. World Health Organization. COVID-19 vaccine tracker and landscape. *https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines* (accessed August 15, 2021).
36. Jara A, Undurraga EA, González C, Paredes F, Fontecilla T, Jara G, Pizarro A, Acevedo J, Leo K, Leon F, Sans C, Leighton P, Suárez P, García-Escorza H, Araos R. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. N Engl J Med. 2021; doi: 10.1056/NEJMoa2107715.
37. Tortorici MA, Czudnochowski N, Starr TN, *et al*. Broad sarbecovirus neutralization by a human monoclonal antibody. Nature. 2021; doi: 10.1038/s41586-021-03817-4.
38. Sun S, Cai Y, Song TZ, *et al*. Interferon-armed RBD dimer enhances the immunogenicity of RBD for sterilizing immunity against SARS-CoV-2. Cell Res. 2021;1-13.
39. Kupferschmidt K. WHO relaunches global drug trial with three new candidates. Science. 2021; 373:606-607.

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RETRACTED: Silence of MACC1 decreases cell migration and invasion in human malignant melanoma through inhibiting the EMT

Due to questions raised regarding siRNA sequence in the article entitled "Silence of MACC1 decreases cell migration and invasion in human malignant melanoma through inhibiting the EMT" by Yingguo Ding, Xiaomin Li, Dongsheng Hong, Li Jiang, Yong He, and Hong Fang, published in *BioScience Trends* (*https://www.biosciencetrends.com/article/1151*. DOI: 10.5582/bst.2016.01091), the editors have decided to retract this article. Authors did not respond to request for comment. The Editors regret any inconvenience to the readers.

Reference

1. Ding YG, Li XM, Hong DS, Jiang L, He Y, Fang H. Silence of MACC1 decreases cell migration and invasion in human malignant melanoma through inhibiting the EMT. *BioSci Trends.* 2016;10(4):258-264. (DOI: 10.5582/bst.2016.01091)

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