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Contents

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Contents

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REVIEW

Clinical update for the diagnosis and treatment of *Clostridium difficile* infection

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Abstract

Clostridium difficile infection (CDI) presents a rapidly evolving challenge in the battle against hospital-acquired infections. Recent advances in CDI diagnosis and management include rapid changes in diagnostic approach with the introduction of newer tests, such as detection of glutamate dehydrogenase in stool and polymerase chain reaction to detect the gene for toxin production, which will soon revolutionize the diagnostic approach to CDI. New medications and multiple medical society guidelines have introduced changing concepts in the definitions of severity of CDI and the choice of therapeutic agents, while rapid expansion of data on the efficacy of fecal microbiota transplantation heralds a revolutionary change in the management of patients suffering multiple relapses of CDI. Through a comprehensive review of current medical literature, this article aims to offer an intensive review of the current state of CDI diagnosis, discuss the strengths and limitations of available laboratory tests, compare both current and future treatments options and offer recommendations for best practice strategies.

Key words: *Clostridium difficile*; Antibiotic-associated diarrhea; Fidaxomicin; Rifaximin; Fecal transplantation; Probiotics

Core tip: This paper seeks explore the treatment and diagnosis of *Clostridium difficile* infection (CDI) through an extensive literature review of available laboratory techniques and new treatment options. For diagnosis, this includes the glutamate dehydrogenase of stool and polymerase chain reaction for gene toxin. For treatment this includes guidelines based on severity, newer antibiotics for the treatment of CDI, fecal microbiota transplantation, and several new experimental treatment options. Finally, this manuscript offers suggested clinical guidelines for how to diagnose and treat CDI.

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INTRODUCTION

Clostridium difficile infection (CDI) continues to be a significant and increasing problem. By far, CDI remains, the most common cause of hospital acquired diarrhea with the number of hospitalized patients with any CDI discharge diagnosis doubling from 139000 in 2000 to 336600 in 2009 at a cost of $1 billion annually[1]. In fact, recently CDI has surpassed methicillin-resistance Staphylococcus aureus (MRSA) as the most common hospital-onset, healthcare facility-associated infection[2]. Despite

significantly trailing MRSA in nosocomial deaths[3], the CDI death rate has dramatically increased from 3000 per year in 1999-2000 to 14000 per year in 2006-2007[4].

One of the most important developments has been the emergence of a new epidemic strain, which is resistant to quinolone antibiotics, such as ciprofloxacin. The first noted in 2001, the epidemic strain produces 16-fold more toxin A and 23-fold more toxin B than other *C. difficile* strains[5]. In addition, the organism produces more spores which results in contamination of the environment and the potential for further spread. The epidemic strain has been associated with an increased incidence of complicated cases and mortality compared to other strains[6]. Confusingly, the epidemic strain is referred to as 027 by polymerase chain reaction (PCR)-ribotyping, B1 by restriction endonuclease analysis (REA), Type 1 by pulse field gel electrophoresis (PFGE) and toxin type Ⅲ by restriction fragment length polymorphism PCR. For continuity throughout the article, the epidemic strain will be identified only as B1. The B1 epidemic stain has now spread widely throughout the United States, however, very few clinicians are aware of its presence in their hospital because culture and identification of *C. difficile* strains is rarely, if ever performed.

In this update, we will review recent advances in the diagnosis of CDI, with a focus on laboratory methods, and also new advances in the treatment of CDI and relapses, including the rapidly expanding area of fecal transplants.

DIAGNOSIS OF CDI

Risk factors for CDI

The first issue in a patient with suspected CDI is to determine if there are associated risk factors. Antibiotic use increases the risk of CDI by 8-10-fold during and for one month after administration and 3-fold for the next 2 mo[7]. Numerous studies have looked at risk factors for CDI with a consistent implication of ampicillin (or amoxicillin), clindamycin and cephalosporins (in particular the third generation cephalosporins (TGC), such as cefotaxime, ceftriaxone and ceftazidime). For the TGCs, an almost perfect correlation has been noted between increasing use and rising incidence of CDI[8] and conversely a decrease in CDI with decreased use of TGCs[9]. Increasingly, quinolones have been shown to be a significant risk factor for CDI, especially with the epidemic B1 strain[6]. The use of multiple antibiotics and > 10 d of antibiotics have also been associated with increased risk (suggesting that therapeutic use of antibiotics poses a greater use than prophylactic use)[10,11]. Antibiotics which have been less commonly associated with CDI include aminoglycosides, macrolides, sulfonamides and tetracyclines. Although the correlation with CDI is highest with certain antibiotics, all antibiotics, even vancomycin and metronidazole on rare occasion, have been reported to cause CDI. However, exposure to antibiotics is not necessary for acquisition of CDI. In one study, 24% of patients with CDI had no antibiotic exposure and 9% had received 3 d or less[12]. Of the patients without any antibiotic exposure, however, 75% were either hospitalized or had close contact with a person with diarrheal illness.

Table 1 Characteristics of tests for *Clostridium difficile* infections

	Sensitivity	Detects toxin in stool	Time to test completion	Cost	Availability	Stand alone test
EIA Toxin A/B	++	Yes	h	+	++++	Yes
GDH[1]	++++	No[5]	h	+	++++	No
PCR[2]	+++	No[6]	h	++++	+++	Yes
TC[3]	+++++	No[6]	d	+++	+[7]	Yes
CCCNA[4]	+++	Yes	d	+++	+[7]	Yes

[1]Glutamate dehydrogenase; [2]Polymerase chain reaction; [3]Toxigenic culture; [4]Cell culture cytotoxicity neutralization assay; [5]Detects presence of *Clostridium difficile* (*C. difficile* Ⅰ) only, but not toxin producing capability, requires confirmatory testing; [6]Detects toxin producing *C. difficile*, but not toxin in stool, false (+) in asymptomatic carriers; [7]Only available in research laboratory. EIA: Enzyme immunoassay; GDH: Glutamate dehydrogenase; PCR: Polymerase chain reaction; TC: Toxigenic culture; CCCNA: Cell culture cytotoxicity neutralization assay.

Antineoplastic agents have also been associated with CDI, including doxorubicin, cisplatin, cyclophosphamide, fluorouracil and chlorambucil[13], with methotrexate most commonly implicated. The proposed mechanism behind the pathogenesis of chemotherapy related CDI is twofold. First, the antineoplastic agents have been shown to alter the gut microflora in a manner similar to antibiotics, acting as the primary predisposing factor for developing CDI[14]. The second, these agents are capable of inducing mitotic arrest in intestinal epithelial cells, subsequently causing necrosis and desquamation of the mucosal membrane[15].

Immunocompromised patients may represent a special subset of CDI for which the incidence and treatment may be more challenging to approach, in particular those with solid organ transplantation. The incidence of CDI in transplant patients has been estimated at 3%-7% for liver recipients, 3.5%-16% for kidney recipients, 1.5%-7.8% in pancreas-kidney recipients, 9% in intestinal recipients, 15% in heart recipients, and 7%-31% in lung recipients[16]. Further fulminant colitis is noted to occur in up to 8% of immunocompromised patients and 13% of solid organ transplant recipients with the highest incidence within the first 3 mo[17,18]. The treatment of CDI in immunosuppressed patients should follow the same guidelines based on disease severity as those outlined in this paper. One important caveat to consider is the potential for drug interactions with metronidazole, in particular the potential for alteration in levels of tacrolimus[19] (Table 1).

Increasing age has been a consistently noted risk factor, with a > 10-fold increased risk for those 60-90 years old[11,20]. In fact, 90% of all deaths are in persons 65 and older[11]. Other associated risk factors have included enemas, stool softeners and gastrointestinal stimulants[21], and also enteral feedings (especially postpyloric), which have been associated with an 11-fold increased risk of CDI[22].

Although rates of non-CDI diarrhea with enteral feedings have been reported in up to 60% of patients[23], the increased risk of CDI would suggest that CDI is a significant problem for enterally fed patients. The significant risk related to postpyloric tube feeding may be related to the fact that gastric acidity has been shown to eliminate 99% of vegetative *C. difficile* cells[24]. Rates have also been noticed to be increased after gastrointestinal operations up to 25-fold compared to controls, probably related to impaired motility, nasogastric tubes and preoperative antibiotics[11].

Recently, several studies have found a higher risk of *C. difficile* infection in proton pump inhibitor (PPI) users. In theory, PPIs may increase the risk of *C. difficile* infection by increasing the ability of the spore to convert to the vegetative form and to survive in the lumen of the gastrointestinal tract. Several meta-analysis have found a significant relationship between PPI use and CDI with odds ratios ranging from 1.69 (95%CI: 1.395-1.974)[25] to 2.05 (95%CI: 1.47-2.85)[26]. Despite these results, the most recent studies offer conflicting viewpoints as to the association between PPI use and increased risk of *C. difficile* infection. These studies showed that while univariate analysis may show a statistically significant relationship between PPI use and CDI, multivariable analysis reveals no significant relationship[27-29]. Further, the most recent review on detection, prevention and treatment of *C. difficile* does not include restriction or avoidance of PPIs in the recommendations for prevention of *C. difficile* infection[30], nor is this recommended by multi society clinical practice guidelines[31].

Although CDI is commonly felt to be a hospital-acquired infection, with up to 87% of infections nosocomially acquired, a significant number of cases are community acquired[10]. In a prospective study of diarrheal pathogens, 20% of infections were community acquired. For an additional 15% of patients, CDI was acquired in the hospital, but diarrhea began after discharge at home for a total of 43% of cases with onset of symptoms at home[16]. As many as 25% of all cases of CDI develop in nursing home patients[1]. Suspicion should always be high for CDI whenever there is diarrhea in a resident of a long term care facility where there is a concentration of elderly, high use of antibiotics, CDI infection in other residents, or frequent exposure to hospitals. This increased risk is bidirectional: 20% of CDI with onset in the hospital are in residents of a nursing home and 67% of CDI in nursing home residents occurs in patients recently discharged from an acute care hospital[1].

Even among asymptomatic patients many of these risk factors appear to be the same. During a 2-mo period, researchers at a tertiary care hospital in Minnesota performed PCR for toxigenic *C. difficile* on all consenting asymptomatic patients, who had greater than a 24 h stay without any known or suspected CDI, diarrhea or colitis. Of the 320 stool samples collected, 31 samples (9.7%) were positive for toxigenic *C. difficile*[29]. Multivariate analysis revealed three main risk factors for *C. difficile* colonization: recent hospitalization within 3 mo (OR = 2.45, 95%CI: 1.02-5.84), chronic dialysis (OR = 8.12, 95%CI: 1.80-36.65), and corticosteroid use (OR = 3.09, 95%CI: 1.24-7.73).

Clinical presentation of CDI

There is a broad range of clinical manifestations from asymptomatic carriage (20% of culture positive patients) to colitis with or without pseudomembranes to fulminating colitis and toxic megacolon. In one series, a two-year institutional study of CDI revealed that "acute abdomen" was the presenting feature in 5% of patients with CDI, with 2 of 5 having no diarrhea prior to emergency laparotomy[32]. This acute abdomen presentation without diarrhea may be particularly confusing in the postoperative patient. Onset is usually 5-10 d after antibiotic use, but ranges from 1 d up to 10 d after antibiotics are stopped. Frankly bloody diarrhea is uncommon (5%-10%)[33]. In fact, only 26% have occult blood[10]. Fever is noted in 30%-50%, usually low grade, not to exceed 102F[28].

Leukocytosis, hypoalbuminemia and elevation of baseline serum creatinine are highly suggestive of CDI. Elevated white blood cell (WBC) count is common (50%-60%), as well as increased band forms (47%) and may be marked elevated[34]. Wanahita found a mean WBC of 15800/mm^3 with 26% of patients having a WBC > 20000/mm^3 and 6% > 30000/mm^3. In fact, for all patients without a hematologic malignancy who had a WBC > 30000/mm^3, 25% were found to have CDI[34]. The elevation of WBC may even precede the onset of diarrhea or abdominal discomfort[35] and may be responsible for up to 58% of cases of unexplained leukocytosis in hospitalized patients[36]. In a series of patient with leukocytosis who were *C. difficile* toxin negative, empiric treatment for CDI led to resolution of leukocytosis[36]. CDI results in a protein losing enteropathy with resultant hypoalbuminemia[37]. Serum albumin of < 2.5 or a fall in albumin of > 1.1 have been associated with a poor prognosis[38]. Bartlett has noted that hypoalbuminemia in persons with antibiotic associated diarrhea may be a clinical clue suggesting CDI[37,39]. Fecal leukocytes have been found in 28%-40% of cases[40]. Detection of fecal lactoferrin (typically used as an indicator of inflammatory bowel disease activity) has been shown to be almost twice as sensitive (75%) as fecal leukocyte detection by methylene blue stain[41]; however, both tests lack sensitivity and specificity and add little to the diagnostic evaluation.

Radiologic diagnosis of CDI

Radiologic studies such as acute abdominal series have been of little value with non-specific findings. Plain films of the abdomen may reveal colonic dilation, (especially cecal) , and non-obstructive related small bowel air fluid levels indicative of ileus pattern. Abdominal computed tomography (CT) has been reported to be normal in 39% of cases, but often reveals a thickened colonic wall, which may be focal or diffuse[42]. With fulminant colitis, there may be mucosal thumbprinting and an "accordion" appearance with oral contrast trapped in the thickened mucosal folds.

Table 2 Comparison of American College of Gastroenterology 2013 and Society for Healthcare Epidemiology of America/Infectious Diseases Society of America 2010 Guidelines for Treatment of *Clostridium difficile* infection (Differences between the guidelines are in bold)

	SHEA/IDSA 2010[1]		ACG 2013[2]	
Severity	**Definition**	**Treatment**	**Definition**	**Treatment**
Mild-to-Moderate	WBC < 15000 cells/μL or lower and serum Cr < 1.5 times the premorbid level	Metronidazole 500 mg 3 times/d by mouth for 10-14 d	Diarrhea plus any additional signs or symptoms not meeting severe or complicated criteria	Metronidazole 500 mg orally 3 times/d for 10 d. If no improvement in 5-7 d, consider change to vancomycin at standard dose
Severe	WBC > 15000 cells/μL or higher or a serum Cr > or equal to 1.5 times the premorbid level	Vancomycin 125 mg 4 times/d by mouth for 10-14 d	Serum albumin < 3 g/dL plus one of the following: WBC ⩾ 15000 or abdominal tenderness	Vancomycin 125 mg orally 4 times/d by mouth for 10 d
Severe, complicated	Hypotension or shock, ileus, megacolon	Vancomycin 500 mg four times/d by mouth or by nasogastric tube, plus metronidazole 500 mg every 8 h intravenously. If complete ileus, consider adding rectal installation of vancomycin	Any of the following attributable to CDI: ICU admission, hypotension with or without the need for vasopressors, fever ⩾ 38.5 ℃, ileus or significant abdominal distension, mental status changes, WBC > 35000 cells/mm^3 or < 2000 cells/mm^3, serum lactate > 2.2 mmol/L, end organ failure	Vancomycin 500 mg orally four times/d and metronidazole 500 mg IV every 8 h and vancomycin per rectum (500 mg in 500 mL saline as enema) four times a day

[1]Society for Healthcare Epidemiology of America (SHEA)/Infectious Diseases Society of America (IDSA)[31]; [2]American College of Gastroenterology (ACG)[71]. WBC: white blood cell; CDI: *Clostridium difficile* infection; ICU: Intensive care unit.

Endoscopic diagnosis of CDI

Endoscopy is usually reserved for special situations. The American College of Gastroenterology (ACG) guidelines recommend endoscopy when a rapid diagnosis is needed, when there is a delay in results of toxin assay or an initial negative toxin assay when CDI is strongly suspected, when there is an ileus and stool is not available and when other colonic diseases are in the differential[43] (Table 2). Endoscopy is frequently normal with mild disease, but often reveals multiple typical yellowish-white plaques (pseudomembranes) elevated above the surrounding mucosa[44]. The plaques vary from a few millimeters to 20 mm and may become confluent with advanced disease and may slough off leaving a denuded underlying mucosa. The intervening mucosa between the plaques may be normal or erythematous and edematous. Overall, pseudomembranes have been detected in 41% of cases of CDAD[45]. Distal involvement of the colon is most common, making flexible sigmoidoscopy a reasonable initial test although in one series, false negative rate due to proximal involvement with rectal sparing was reported in 10% of cases[46]. Histologically, the pseudomembranes, composed of fibrin, mucus, epithelial and inflammatory cells appear as "clouds" rising from points of superficial ulcerations. The lesions have been termed "volcano" lesions appearing like an eruption above underlying glandular lesions[47]. In 22% of cases, pseudomembranes were visualized on endoscopy, but not present histologically[48].

Laboratory diagnosis of CDI

The state of the art for best practice is controversial and confusing. Curry noted that "diagnosis of CDI remains one of the most vexing difficulties for hospital microbiology laboratories", because there is no single accepted gold standard[49]. For many years, cell culture cytotoxicity neutralization assay (CCCNA) was the accepted gold standard. By this method, stool filtrates are inoculated onto a monolayer of a cell culture in wells with and without *C. difficile* antitoxin. Rounding of the cells in the antitoxin-free well demonstrates a cytopathic effect and the presence of toxin. If there is no change in the antitoxin containing well, then the presence of *C. difficile* toxin in the stool is confirmed. CCCNA is quite specific for CDI and can detect toxin in the stool as low as 10 picograms. However, the assay is expensive, has a slow turnaround time (2 d minimum), lacks standardization among laboratories and is generally unavailable outside the research setting. More recently, many investigators have considered toxigenic culture (TC) as the method of choice for diagnosis of CDI. With the toxicogenic culture method, stool is cultured for *C. difficile* on a selective differential medium (cycloserine, cefoxitin, fructose agar or CCFA). In the next step, the organism is tested for ability to produce toxin. Compared to TC, CCNA has only 67%-79% sensitivity[49]. The Society for Healthcare Epidemiology of America (SHEA)/Infectious Diseases Society of America (IDSA) 2010 guidelines note that "the sensitivity and specificity of stool culture followed by identification of a toxigenic isolate (TC) as performed by an experienced laboratory provides the standard against which other clinical tests should be compared"[31]. Despite the assertions of the superiority of TC as a gold standard, there are significant issues with using TC as a gold standard. The TC identifies the ability to produce toxin, but not actual toxin in stool. This can lead to false positives due to the fact that up to 7% of asymptomatic hospitalized patients may be colonized on admission with toxigenic *C. difficile*[50]. Rates of asymptomatic colonization with toxin producing *C. difficile* can be even

higher among elderly patients in skilled nursing facilities, approaching 20%[51]. Concern about using TC as the gold standard was raised by a recent study conducted by the National Health Service (NHS) Laboratories in the United Kingdom, which evaluated 12441 diarrheal fecal samples[52]. The study showed that the presence of toxin in the fecal specimens was associated with poor clinical outcomes; however, culture of toxin producing *C. difficile* without detection of toxin in the diarrheal stool specimens was not associated with worse clinical outcomes than stools that were negative for toxigenic *C. difficile*. At best, which test should be the gold standard for diagnosis of CDI, TC or CCCNA, is currently undecided. One thing is clear if the gold standard being used is TC, then all the comparators, whether enzyme immunoassay (EIA), glutamate dehydrogenase (GDH) or polymerase chain reaction (PCR), will be less sensitive. If CCCNA is used as the gold standard, the comparators will appear more sensitive.

There is consensus that the EIA for toxin A/B, currently the primary test used in up to 90% of clinical laboratories[53] is too insensitive and non-specific and no longer recommended as a stand-alone test[54]. The EIA for toxin A/B has been adopted by most clinical laboratories because it is fast, convenient and inexpensive. Recent studies have shown however, that the sensitivity can be as low as 38%[55]. The EIA requires 100-1000 picograms of toxin as compared to the ability of the CCCNA to detect less than 10 picograms of toxin[53]. In addition to poor sensitivity, the EIA also has a positive predictive value (PPV) as low as 50% due to the low prevalence of *C. difficile* among all specimens submitted for testing from symptomatic patients[54]. Historically, 15%-25% of antibiotic associated diarrhea has been felt to be due to *C. difficile*. However, most recent studies suggest a decreasing rate of positivity with only 5%-10% of samples testing positive[54]. In 2001, 22% of samples tested were positive for toxin by EIA *vs* only 11% in 2007[56]. If the prevalence of positive stools is 10%, then the PPV of a positive toxin EIA varies from less than 50%-90%. Falsely diagnosing a patient with CDI can lead to isolation of patients who are not infected. Isolation has been shown to have negative consequences, with a doubling of adverse events and days without a physician note and an increase in formal complaints by 8-fold[57]. A false diagnosis of CDI can also lead to cohorting of uninfected patients with patients who have active CDI, particularly in skilled nursing facilities, as well as delay in finding the true etiology of the diarrhea and the unnecessary use of antibiotics. A systematic review of toxin detection kits concluded that the sensitivity and specificity of the different test kits were sufficiently heterogenous between studies of the same test, such that meta-analytic methods could not be used to pool studies on a particular toxin EIA assay[56]. They concluded that differences in test characteristics were most likely related to the threshold cutoff chosen for each test. Choosing a low threshold increased the sensitivity, but at the same time decreased specificity and vice versa. Overall, the authors concluded that none of the EIA toxin assays had an acceptable predictive value and that a two-step testing strategy should be used.

The lack of sensitivity and specificity of the toxin A/B EIA assay has led to a search for more accurate test methods. The detection of GDH in stool has shown significant promise. The test is fast (15-45 min), convenient, inexpensive, and sensitive. The GDH is a common antigen expressed at high levels by all *C. difficile* strains. However, the test only documents the presence of *C. difficile*, but not the presence of a toxigenic strain (20% of *C. difficile* strains do not produce toxin) or the presence of toxin in stool[58]. Therefore, GDH (+) stool requires confirmation of toxin production with a second test. Early studies reported sensitivities as high as 100% for detection of *C. difficile*[59]. However, more recent studies have raised concern about the sensitivity of the GDH assay for non-epidemic B1 strains. For non-epidemic B1 strains, the sensitivity may be as low as 69%[60].

The use of PCR to detect the gene for toxin production (*tcdB* gene) is promising as a stand-alone test for CDI. The PCR for the toxin gene is fast (2 h) and sensitive with a minimum detection limit of 105 per gram of stool[61]. However, the cost can be 5-10 times greater than EIA for toxin A/B. Sensitivity has been 91% as compared to enzyme immunoassay at 67%[62]. Overall, sensitivity has been 84%-94% in comparison to TC, similar to the CCCN[60]. Many hospital laboratories will be able to offer ready availability of PCR testing with rapid turnaround.

There are currently four Food and Drug Administration (FDA) approved PCR assays, Gene Ohm (Becton Dickinson, San Diego), Gene Xpert (Cephid, Sunnyvale, Ca.), which not only can identify the *toxin* gene but also the epidemic B1 strain, Progastro (Prodesse, Waukesha, Wi) and Simplexa (Quest Diagnostics, Madison NJ). In a meta-analysis of PCR *vs* TC, a pooled sensitivity of 92% and specificity of 94% was reported[63]. However, as with TC mentioned earlier, the PCR detects the *toxin* gene, but does not detect toxin in stool raising concerns about over diagnosis by detecting asymptomatic carriers. In addition, the use of the PCR may increase CDI incidence rates by greater than 50%[64]. This raises concern with mandatory reporting programs and inter-hospital comparisons. Some authors have noted an increase from 6.5% positive samples before the use of PCR to 15% after their laboratory changed to PCR for *C. difficile* detection[65]. In addition, the PCR cannot be used for suspected relapse as up to 56% of patients will be positive by PCR at 1-4 wk after completion of therapy[66]. However, despite its high sensitivity and specificity, at the recently noted prevalence of 10% of CDI among tested specimens, the positive predictive value may be only 63%[65]. Despite these issues, some laboratories have now adopted PCR as a stand-alone diagnostic test for *C. difficile* [67].

Another promising method for CDI diagnosis is detection of the *toxin* gene by loop-mediated isothermal amplification (LAMP), which does not require a large capital outlay for PCR[63]. This non-PCR based gene amplification method detects the pathogenicity locus of

toxigenic *C. difficile*. The test is simple, rapid (1 h) and significantly less expensive than PCR based methods. The Illumigene (Meridian Bioscience, Europe) assay was found to have a 92% sensitivity, 98% specificity, 99% negative predictive value and 84% positive predictive value, respectfully[68]. However, the same issues that raise concerns about TC and PCR, *i.e.*, detection of toxigenic *C. difficile*, but not toxin in stool, are true for LAMP.

The concerns with EIA for toxin A/B, PCR and GDH as stand-alone tests has led to the study of an algorithmic approach to the diagnosis of CDI, similar to HIV and syphilis testing. Larson *et al*[69] studied a 3 step algorithm with the initial test being a glutamate dehydrogenase. If the GDH is positive, this was followed by confirmation of toxin in stool with an EIA for toxin A/B. If both are positive, the test is reported as positive for CDI. If the EIA toxin A/B is negative, the final result is determined with a PCR. Using this algorithm, they found a sensitivity of 84% and specificity of 99.7% with very high PPV of 97.5% and NPV of 99.7% compared to a modified gold standard using CCCNA and PCR. In the previously mentioned United Kingdom NHS study using 12441 diarrheal fecal specimens, Wilcox concluded that a two-step protocol with an EIA for GDH or a nucleic acid amplification test (NAAT), such as a PCR for *toxin* gene, followed by confirmation of stool toxin by a EIA for toxin A/B was the most effective testing algorithm in distinguishing patients with *C. difficile* infection from those who did not have CDI[53]. This two-step algorithm has now become the standard in NHS laboratories in England as of April 2012[70]. The combination optimizes specificity and positive predictive value (90%)[52]. This same study found that using an algorithm that optimized for sensitivity such as a GDH followed by a PCR resulted in a 95% sensitivity, but a PPV that was only 60%. In other words, 4 of 10 positive tests did not really have CDI. This would be an optimal method for excluding CDI, but not a very good method for determining if CDI was really present. The American Society of Microbiology (ASM) recommends that if the toxin A/B EIA or CCCNA is used and is negative, specimens should be further tested by PCR or TC[54]. The ASM noted that utilizing toxin A/B EIA for *C. difficile* diagnosis is insensitive and no longer recommended as a stand-alone test. The ASM also noted that laboratories can also use a PCR to detect *C. difficile toxin* genes as a stand-alone diagnostic test. The SHEA/IDSA guidelines suggested that an initial GDH test followed by confirmation with either TC or CCCN was an option[31]. However, as previously noted, the last two tests are rarely available in clinical laboratories and results would not be available in time for clinical use. The 2013 ACG guidelines recommend a NAAT such as PCR as a standard diagnostic test for CDI. The guidelines also suggest that a GDH EIA can be used an initial screening test in a two-or three-step algorithm with subsequent confirmation of positive results with an EIA for toxin A/B. If the EIA for toxin A/B is negative, then a NAAT test should follow. However, the ACG guideline notes that the sensitivity is lower than a strategy based on an initial PCR[71].

Repeat testing for CDI

One aspect of testing about which there is broad agreement is that there are limited indications for repeat testing. Yassin *et al*[72] have suggested that performing the EIA for toxin on two or three samples can increase sensitivity to about 90%. However, Renshaw *et al*[73] suggested that repeated assays accounted for 36% of all toxin assays ordered, but provided clinically useful information in only 1% of the cases and significantly increased cost. Aichinger *et al*[74] found that repeat testing within 7 d by EIA for toxin A/B or by PCR for *C. difficile* toxin resulted in < 2% positive tests. In another study, repeat testing accounted for 17% of all tests ordered, but only 1% were positive[75]. Peterson *et al*[59] noted that with a sensitivity of 73% and a specificity of 97.6% that if the *C. difficile* EIA was negative on the first two tests, a positive result on the third test was three times more likely to be a false positive than a true positive due to decreasing pretest probability with consecutive negative tests. In fact, even on the second test after an initial negative, the positive predictive value is less than 50%, about as good as flipping a coin. The 2013 ACG guidelines make a strong recommendation that repeat testing not be performed.

There is clearly no indication for serial monitoring of stools or an end of treatment "test of cure" as 1/3 of patients will still have a positive assay at the end of successful treatment[40]. Stool carriage has been noted to persist for 3-6 wk after successful treatment and has not been found to predict who will relapse[76]. Requiring a negative test to come out of isolation or before transfer to a long term care facility is inappropriate. Again, the 2013 ACG guidelines make a strong recommendation that testing for cure should not be done.

Given the limitations of the available laboratory tests for CDI, a reasonable approach is: (1) if CDI is suspected on clinical grounds, perform *C. difficile* testing according to your hospital laboratory protocol. Be aware of the test or algorithm they are using. Many clinical laboratories are in the process of changing testing protocols; (2) if the test is positive, continue or initiate treatment, if not started empirically; and (3) if the test is negative, make a clinical decision on whether to treat based on the likelihood of CDI (recent exposure to antibiotics or prior CDI, elevated white blood count or elevated creatinine or decreased albumin, age or other risk factors). If CDI is still suspected after a negative test, empiric treatment is reasonable. Repeat testing yields minimal additional true positives and increases cost. The ACG Guidelines make a strong recommendation that "Repeat testing should be discouraged"[71].

In summary, testing for CDI is in flux, confusing and controversial. As noted by Fang, "the clinical laboratory can place the perpetrator (*C. difficile*) at the scene of the crime, but only the clinician can establish whether a crime (CDI) has taken place"[77].

THE CONTROVERSY OVER BASIC TREATMENT CHOICES

Despite numerous treatment trials for *C. difficile* infection, dating back to 1978, the drug of choice for CDI remains controversial. In fact, Pepin noted that "there are few common infectious diseases in developed countries for which the treatments used in 2006 are essentially the same as those recommended one-quarter of a century ago"[78]. The same can be said for 2013 and the foreseeable future. The recent Cochrane Collaboration review of antibiotic treatment for CDI vividly illustrates the ongoing problems related to treatment decisions[79,80]. The authors reviewed randomized, controlled trials of antibiotic therapy for CDI. There were 15 studies considered evaluable with 1152 patients involved. There was only one placebo controlled trial, which was considered to be of small size with poor methodological quality. The authors concluded that even the most basic question of whether any antibiotic is effective, much less which one, has not yet been answered. The authors stated, "this review cannot establish the efficacy of antibiotic therapy for CDI as the only placebo controlled trial is inadequate". In fact, they noted that there is "uncertainty whether mild CDI needs to be treated". Further, they noted that "this review cannot definitively make a specific antibiotic recommendation for the treatment of CDI". When looking at particular antibiotics, they concluded that "no single antibiotic is clearly superior to others". Although, they did note that teicoplanin was superior to vancomycin. Unfortunately, teicoplanin is not available in the United States.

Part of the reason that there have been so few changes in our treatment of CDI over the last 30 years may be due to the lack of development of significant resistance. Fortunately, a number of recent studies have not revealed resistance to the main standbys for treatment of CDI: metronidazole and vancomycin. Aspevall *et al*[81] studied 238 isolates of *C. difficile* collected from 2000 to 2001 and found no evidence of resistance to metronidazole or vancomycin. Hecht *et al*[82] studied 110 strains collected between 1983 and 2004. All strains were sensitive to metronidazole at less than or equal to 0.5 μg/mL. Bourgault *et al*[83] looked at 251 isolates collected during the outbreak in Quebec, Canada, which started in 2003. Of these, 69% were the B1 epidemic strain, while 11% were the NAP2 strain by PFGE. All isolates were sensitive to metronidazole and vancomycin. There was no increase in MIC's compared to historical isolates.

Unfortunately, the same cannot be said for other antibiotics. Recently, the complete genome of *C. difficile* has been sequenced revealing a significant potential for development of antibiotic resistance[84]. Significant portion of the genome (11%) consists of mobile genetic elements, mainly conjugative transposons, which can be used to transfer genetic material between bacteria. These mobile genetic elements are often involved in the transfer of antimicrobial resistance and virulence factors. Bourgault *et al*[83] found that for the B1 epidemic strain the quinolones, macrolides and other commonly used antibiotics have succumbed to the antibiotic resistance mechanisms of *C. difficile*. All strains were resistant to bacitracin, ciprofloxacin, levofloxacin and clarithromycin, while 80% were resistant to gatifloxacin, moxifloxacin and ceftriaxone. All historical NAP1 isolates were resistant to quinolones, suggesting that the epidemic may be more associated with the increased use of fluoroquinolones, as opposed to the recent development of quinolone resistance by the epidemic strain. Of note, 69% of the B1 epidemic strains were sensitive to clindamycin, while only 11% of the non-epidemic strain strains were sensitive to clindamycin.

METRONIDAZOLE OR VANCOMYCIN

Having summarized the murky state of the evidence based treatment of CDI, it would be reasonable to look at the pros and cons of metronidazole and vancomycin. The oft-quoted reasons for metronidazole assuming the status of preferred agent for treating CDI has been the potential for development of vancomycin-resistant enterococci (VRE) and the higher cost of oral vancomycin. In contrast to this notion, a small study looking specifically at the issue of developing VRE found no patients developed VRE while being treated with oral vancomycin[85]. Unfortunately, vancomycin capsules (Vancocin HCl Pulvules) are extraordinarily expensive, with an average wholesale price of $31.83 per capsule or $127.32 per day for a dose of 125 mg *qid vs* $2.19 per day for generic metronidazole 500 mg *tid*[86]. Further, retail costs are much higher. Most hospitals avoid the extraordinary cost of vancomycin capsules by using the generic intravenous formulation and compounding it in water as a liquid vancomycin solution. One pharmacy, close to our clinic, sells vancomycin intravenous formulation for $5.85 per 500 mg *vial*. If this 500 mg of vancomycin powder is reconstituted in 20 cc of water (often with flavoring to hide the bitter taste of vancomycin), the cost of vancomycin approaches $1.50 per dose. Stability of the vancomycin solution in the refrigerator (4 degrees C) is at least 75 d and at least 26 d at room temperature (25 degrees C)[87].

Despite issues related to fostering VRE and cost, prior comparative studies of metronidazole and vancomycin have not revealed a statistically significant difference between the two antibiotics[88,89]. In one study, 95% were cured with metronidazole *vs* 100% with vancomycin[88]. In the second study, the cure rates were identical at 94% in each group[89]. However, the number of patients was small and neither study was stratified by severity of disease.

Despite similar response rates, there are significant pharmacologic concerns related to metronidazole, which tilt the balance in favor of vancomycin. Metronidazole is rapidly absorbed from the gastrointestinal tract and excreted through the biliary system, with only about 14% of the drug excreted in the stool[90]. Fecal metronidazole

levels have been noted to increase with colonic inflammation, probably from transudation into the lumen, but these levels decrease as inflammation subsides and are undetectable upon recovery[37,91]. More recently, Musher noted a failure rate of 22% with standard doses of metronidazole[92]. This was not due to resistance, as those strains tested, were all sensitive to metronidazole. Interestingly, in this study there was no difference in outcomes between those who were continued on metronidazole despite clinical failure compared to those who were changed to vancomycin. Musher *et al*[92] suggested that patients with severe disease could have decreased blood flow to the colon, which would result in less transudation of metronidazole into the lumen and either a slower response or clinical failure[93]. Despite this potential for low metronidazole levels, *in vitro* the drug has been shown to be very rapidly bactericidal at 8-times the minimum inhibitory concentration (MIC), a level which is usually reached in the colon. This rapid bactericidal effect can be compared to vancomycin, which has been shown to be only inhibitory of bacterial growth[40]. As opposed to the poor pharmacokinetics of metronidazole, vancomycin has near perfect characteristics for a drug used to treat an infection limited to the lumen of the colon. Vancomycin achieves levels in the colon of about 1000 μg/mL in stool due to the fact that there is limited or no absorption from the colon. Al-Nassir *et al*[94] have shown that vancomycin is much more effective than metronidazole in removing *C. difficile* from the stool as measured by *C. difficile* density cultures[94]. By day 5 of treatment, patients treated with vancomycin were 3.3 times more likely to have undetectable *C. difficile* than metronidazole (P = 0.015). In this study, 10 of 34 patients were switched from metronidazole to oral vancomycin between days 2 and 10 due to suboptimal clinical response, of whom 8 of the 10 had less than a one log decrease in *C. difficile*. Once they were switched to oral vancomycin, 7 of these 8 patients had undetectable *C. difficile* by culture. Freeman *et al*[95,96] confirmed the favorable characteristics of oral vancomycin in a human gut model composed of three vessels operating in a weir cascade system in an oxygen free nitrogen atmosphere. They found that cytotoxin titers were unaffected by metronidazole, while vancomycin resulted in a marked decrease in toxin and the *C. difficile* vegetative form, leaving only spores which do not produce toxin. Another issue which may decrease the effectiveness of metronidazole is inactivation by Enterococcus faecalis, which has been shown to allow protection of organisms which would normally be killed by metronidazole[97]. There also appears to be a higher failure rate with metronidazole when the physician is forced to continue the offending antibiotics, which is often the case. In one series, all patients who could have the offending antibiotic discontinued had resolution of diarrhea by 14 d when treated with metronidazole[98]. However, 41% of the patients who had antibiotics continued failed to have symptomatic resolution of diarrhea by day 14 (P = 0.02).

Because rifampin has been shown to have markedly superior *in vitro* activity in comparison with other antimicrobials against *C. difficile*[99] combination therapy has been studied as a means to improve outcomes with metronidazole therapy. Lagrotteria *et al*[100] conducted a prospective, randomized, single-blind study of metronidazole alone *vs* metronidazole plus rifampin[100]. There was a similar time to improvement, similar proportion of relapses, but significantly more deaths in the combination group as compared to metronidazole alone (32% *vs* 5%, P = 0.04). The authors concluded: "there is no role for rifampin as an adjunct to treatment with metronidazole."

TREATMENT DECISIONS BASED UPON STRATIFICATION BY DISEASE SEVERITY

A concern with all of the preceding comparative studies of vancomycin with metronidazole has been that there was no stratification by disease severity. One of the most important recent advances in the treatment of CDI has been the development of scoring systems, which allow the physician to determine which patients are at highest risk for severe CDI. The development of scoring systems was started by Pepin *et al*[78] who developed local recommendations, because of the overwhelming epidemic in Quebec caused by the new epidemic B1 strain. In January of 2004, they developed local recommendations for the use of oral vancomycin: a WBC greater than 20000 cells/mm^3 and a serum creatinine greater than or equal to 200 μmol/L. This recommendation was based upon a reduction of complicated CDI by 79% if vancomycin was the initial treatment compared to metronidazole[101].

Zar *et al*[102] conducted the first randomized, double-blind, placebo controlled trial comparing metronidazole and vancomycin in the treatment of CDI that stratified patients at study entry based upon severity of disease. The authors developed a scoring system giving 1 point each for the presence of age greater than 60 years, temperature greater than 38.3 degrees centigrade, albumin less than 2.5 mg per deciliter, or a WBC count greater than 15000 cells per mm^3. They also gave 2 points for endoscopic evidence of pseudomembranous colitis or treatment in an intensive care unit setting. Mild disease was defined as 0 or 1 points and severe CDI was defined as greater than or equal to 2 points. Clinical cure was noted in 90% of those with mild CDI randomized to metronidazole and 90% of those randomized to vancomycin. For those with severe CDI, clinical cure was noted in 76% who received metronidazole *vs* 97% who received vancomycin (P = 0.02). Recurrences were similar for both groups at 15% and 14% for the metronidazole and vancomycin groups, respectively. The authors concluded that metronidazole and vancomycin are equally effective for the treatment of mild CDI; however, vancomycin is superior for treating patients with severe CDI. Critiques of the Zar *et al*[102] article were that one of the criteria for failure was persistent toxin positivity at day 6 and 10 of therapy. In addition, there was exclusion of 8 patients

Table 3 Comparative average wholesale price for antibiotics used in the treatment of *Clostridium difficile* infection

Antibiotic	Cost per dose[1]	Usual regimen	Cost per treatment[1]
Metronidazole	$0.73		
Vancomycin capsules (Vancocin HCL pulvules)	$31.83	125 mg 4 times/d × 10 d	$1273.20
Vancomycin intravenous formulation (generic)	$5.00/g ($0.62 per 125 mg dose)	125 mg 4 times/d × 10 d	$25.00
Fidaxomicin (Dificid)	$168.00	200 mg 2 times/d × 10 d	$3360.00
Rifaximin (Xifaxan)	$19.02 400 mg	400 mg 3 times/d × 20 d[2]	$1141.20

[1]Average Wholesale Price (AWP); Anon, edition. Red Book online. [2]Dose as a "chaser" after a course of oral vancomycin for recurrent CDI[151]. *Via* Drugdex System (internet database) Greenwood Village, CO: Thompson Healthcare, 2011[86]. CDI: *Clostridium difficile* infection.

with early death[103]. When the 2 patients who were judged as having failed therapy solely on the basis of persistent toxin positivity and the 8 early deaths were included, vancomycin was still superior to metronidazole for those with severe disease with a 90% cure rate for vancomycin *vs* 71% for metronidazole ($P = 0.04$).

The 2010 IDSA guidelines recommend oral metronidazole for CDI with a WBC 15000 cells/mm^3 and < 50% increase in serum Cr from baseline. The guidelines define severe disease as CDI with a WBC 15000 cells/mm^3 or a 50% increase of serum Cr from baseline. For severe CDI, they recommend starting therapy with oral vancomycin 125 mg *qid*[50]. Most recently, the ACG has updated its practice guidelines to include summary recommendations based on CDI severity[71]. Mild-to-moderate disease is defined as diarrhea plus any additional signs or symptoms not meeting severe or complicated criteria. Notably, the ACG classification for severe disease has been redefined from the IDSA guidelines to use only three criteria: a serum albumin < 3 g/dL plus one of either WBC 15000 cells/mm^3 or abdominal tenderness. The choice to limit the guidelines to these three criteria was based on a prospective observational study by Fujitani *et al*[104] which found that the only independent risk factors for severe CDI were abdominal distention, fever, WBC > 20000 cells/mm^3, and serum albumin < 3 mg/dL. The ACG guidelines recommend the same initial treatments of metronidazole 500 mg orally three times daily for 10 d for mild-to-moderate disease and vancomycin 125 mg orally four times daily for severe disease.

NEWER ANTIBIOTICS FOR CDI

Rifaximin

Rifaximin (Xifaxan, Salix Pharmaceuticals, Inc. Raleigh, NC) is a broad spectrum, non-absorbable antibiotic used for the treatment and prevention of traveler's diarrhea. The drug is not inactivated by gastric fluids and is also poorly absorbed, thereby largely excreted unchanged in the feces reaching concentrations up to 8000 g/gm of stool after 3 d of therapy[105]. Rifaximin treatment has demonstrated survival rates in animal models equivalent to vancomycin. Rubin *et al*[106] conducted an open label pilot study to assess the efficacy of rifaximin as an initial treatment option in patients without recurrent CDI. Of the 8 patients who completed the 10-d course of rifaximin 400 mg three times daily, 7 (88%) had symptom resolution with 10 d of rifaximin treatment with no relapse within 2 wk. Additionally, Boero *et al*[107] compared the efficacy of rifaximin 200 mg *tid* and vancomycin in a study of 20 patients. Response rates were 90% and 100% for rifaximin and vancomycin, respectively. One concern about rifaximin is the potential for resistance, especially given the lack of sensitivity testing outside of a research laboratory. A study of rifaximin susceptibility of 80 different *C. difficile* isolates found resistance among 14 isolates, of which 64% were the epidemic B1 strain[108]. At this point, it is difficult to ascertain the clinical impact of these findings, especially given the extremely high fecal concentrations achieved with rifaximin. While these small studies suggest a potential application for rifaximin for the initial treatment of CDI, more attention has been placed on a rifaximin "chaser" in the treatment or prevention of recurrent CDI (see section on Recurrent CDI) (Table 3).

Nitazoxanide

Nitazoxanide (Alinia, Romark Laboratories, Tampa, Florida) is a broad-spectrum antiparasitic agent currently approved for the treatment of giardiasis and cryptosporidiosis[109]. Nitazoxanide is highly active *in vitro* against *C. difficile*. Studies have shown that two-thirds of the drug is excreted in the stool as an active metabolite with activity against *C. difficile* comparable to the parent compound[110]. Nitazoxanide has been shown to prevent colitis in the hamster model[111]. Further, nitazoxanide has been shown to very active against a panel of 127 *C. difficile* isolates from the United Kingdom's *C. difficile* Ribotyping Network at an MIC range of 0.03-0.5 mg/L[112]. A recent prospective, randomized, double blind study by Musher *et al*[113] compared metronidazole 250 mg *qid* for 10 d to nitazoxanide 500 mg *bid* for 7 or 10 d[113]. After 7 d of treatment, the metronidazole response was 82% compared to 90% for nitazoxanide. At 31 d after starting treatment, a sustained response was noted for 58% of patients treated with metronidazole *vs* 66% for the 7-d course of nitazoxanide and 74% for the 10-d course ($P = 0.34$). Musher *et al*[114] also reported the use of nitazoxanide in 35 patients that failed to respond to metronidazole after 14 d of therapy or who had prompt recurrence on at least two occasions after an initial response. They noted that 74% of patients responded, however, 7 of the 26 recurred, leaving an overall cure rate of 54%.

Most recently, Musher *et al*[115] completed a randomized, double-blind study of nitazoxanide *vs* vancomycin. After 10 d of treatment, resolution of CDI occurred in 20 of 27 vancomycin patients (74%) and 17 of 22 nitazoxanide patients (77%). For those completing therapy, both treatments had similar times to resolution with response rates of 87% for vancomycin and 94% for nitazoxanide. Subsequently, 2 vancomycin patients and 1 nitazoxanide patients relapsed, leaving a sustained response rate of 78% for vancomycin and 89% for nitazoxanide. The authors noted that, while the small sample size may not have the power to prove noninferiority *vs* vancomycin, as the first randomized control trial their results suggest nitazoxanide may be equally effective.

Fidaxomicin

Fidaxomicin (Dificid®, Optimer Pharmaceuticals, San Diego, CA) is a macrocyclic antibiotic with a narrow spectrum of activity against gram-positive cocci. Fidaxomicin has been 100% protective in a hamster model of CDI[116]. Importantly, fidaxomicin has been shown to have a comparable safety profile to vancomycin[117], have undetectable serum levels while achieving high fecal concentrations, averaging greater than 10000 times the MIC for *C. difficile*[118], a bactericidal mechanism of action[119], preserve the intestinal microbiome (by sparing of *Bacteroides* sp.), reduce both toxin reexpression and CDI recurrence[120], and reducing the acquisition of VRE and Candida species during CDI treatment[121,122].

Much of the attention centered on fidaxomicin has been based on findings from two prospective, multicenter, double-blind, randomized Phase Ⅲ trials demonstrating non-inferiority to vancomycin. The first trial (003 in the United States and Canada) of 629 patients randomized to receive either fidaxomicin 200 mg twice daily (with intervening placebo) (n = 302) or vancomycin 125 mg four times daily (n = 327), revealed no significant difference in the clinical cure rates: 88.2% for fidaxomicin and 85.8% for vancomycin[123]. Another interesting observation that arose from the 003 trial was that overall recurrence rates, as defined by the reappearance of more than three diarrheal stools per 24-h period within 4 wk after cessation of therapy, were lower in the fidaxomicin group at 15.4% compared to 25.3% in the vancomycin group (P = 0.005). However, recurrence rates with the epidemic B1 strain were similar between fidaxomicin and vancomycin with 24.4% and 23.6% recurrences, respectively. The second Phase Ⅲ trial (004 conducted at 45 sites in Europe and 41 sites in the United States and Canada) also found fidaxomicin to be non-inferior with cure rates of 91.7% *vs* 90.6% for vancomycin (one sided 95%CI: -4.3)[124].

Most recently, a post-hoc intent to treat meta-analysis was performed on the results of the combined 003/004 Phase Ⅲ trials. Of the 1164 patients included, fidaxomicin when compared to vancomycin was associated with a 40% reduction in persistent diarrhea, recurrence, or death through day 40 (95%CI: 26%-51%; $P < 0.0001$)[125]. Subgroup analysis limited to the epidemic B1 strain, revealed a 22% non-significant reduction in persistent/recurrent diarrhea (95%CI: 44% reduction to 8% increase, P = 0.14). The authors point out that with only 292 of 814 strains testing positive for B1, the results from this analysis are too underpowered to conclude fidaxomicin lacks beneficial effect for the B1 strain.

One important aspect of fidaxomicin remains, cost. At $168 per 200 mg tablet, a twice-daily 10-d treatment course costs $3360 for a 10 d course[86]. The pharmaceutical company selling this medication has recently developed several strategies to help reduce the patient cost if the medication is needed.

COMPLICATED CDI

Complicated CDI is defined in the 2010 IDSA guidelines as severe CDI plus intensive care unit (ICU) admission, need for colectomy, ileus, toxic megacolon, hypotension or colonic perforation[31]. The 2013 ACG guidelines define severe complicated CDI as any of the following: admission to the ICU, hypotension with or without the need for pressors, fevers > 38.5 ℃, ileus or significant abdominal distension, mental status changes, WBC ⩾ 35000 cells/mm^3 or < 2000 cells/mm^3, serum lactate > 2.2 mmol/L, or end organ failure[71].

For severe complicated CDI, the IDSA guidelines recommend high dose oral vancomycin 500 mg *qid* (by nasogastric tube, if necessary) and/or metronidazole 500-750 mg q8h intravenously. Metronidazole and vancomycin combination has been shown to be synergistic *in vitro* for 68% of *C. difficile* isolates[99]. Apparently, the increased dose of vancomycin for complicated CDI is related to a delay in attaining adequate fecal levels with 125 mg *vs* a higher dose when given orally[126]. For complete ileus, metronidazole intravenously plus vancomycin administered by retention enema is recommended. The critical point is that the vancomycin, needs to be retained and distributed in the colon to be effective. Specific orders should detail the administration lest the vancomycin be administered as a plain enema, providing no benefit for the patient and creating a hazard for nursing staff. The vancomycin should be administered using a # 18 French Foley catheter with a 30 mL balloon. The Foley catheter should be inserted into the rectum, the balloon inflated and the vancomycin instilled. The catheter is then clamped; some authors recommend turning the patient on their right side to assist distribution of the vancomycin solution throughout the colon. After 60 min, the balloon is deflated and the catheter is removed[127]. Because there have been no controlled trials of vancomycin by intracolonic installation, the optimal dose and interval are unclear. Apisarnthanarak *et al*[128] reported a descriptive case series of nine consecutive patients treated with intracolonic vancomycin as adjunctive therapy for severe CDI. Eight of nine patients had failed five to 7 d of standard therapy for CDI and had evidence of a severe ileus with resultant cessation of diarrhea. Further evidence of the severity of the colitis was suggested by the fact that six of the nine patients were hypotensive at the time CDI

was diagnosed. They administered intracolonic vancomycin 0.5-1.0 g in one to two liters of normal saline as a retention enema. Because this was a retrospective collection of cases, the dosing interval and duration of therapy were variable. Two patients received intra-colonic vancomycin at 4 h intervals, two at 6 h, two at 8 h and three at 12 h. The authors noted complete resolution of colitis in eight of nine patients with no relapses and no surgical interventions. As a note of caution, four patients were colonized with VRE prior to the intracolonic vancomycin and two of these 4 developed VRE bacteremia. However, none of five patients who were not colonized with VRE before therapy developed subsequent colonization.

The 2013 ACG guidelines offer slight changes in recommend therapy for severe and complicated CDI[71]. Initial therapy for severe and complicated CDI without any significant abdominal distention is vancomycin orally 125 mg *qid* plus intravenous metronidazole 500 mg *tid*. For severe and complicated CDI with ileus, toxic colitis, or significant abdominal distention, the recommended therapy is vancomycin delivered both orally 500 mg *tid* and per rectum 500 mg in volume of 500 mL *qid* plus intravenous metronidazole 500 mg *tid*. Of note, the author's discuss the potential for development of electrolyte imbalances with the use of saline for delivery of the vancomycin enema, in particular hyperchloremia. In such a situation, the authors propose the use of Ringer's Lactate, which contains a lower concentration of chloride[71].

Tigecycline is a broad-spectrum glycylcycline antibiotic with reportedly low MIC values against *C. difficile*, along with evidence that it does not promote growth or toxin production in both a mouse and human model[82,129,130]. To date no clinical trials have been performed on the use of tigecycline; however, several case reports have reported the successful use of Ⅳ tigecycline in severe or severe complicated CDI in which patients failed prior treatment with metronidazole and vancomycin[131]. There has also been a case report noting the successful treatment of severe refractory CDI using a combination of tigecycline (50 mg Ⅳ every 12 h for 10 d) and rifaximin (400 mg twice daily for 17 d)[132].

SURGICAL INTERVENTION

Failure to respond to maximal medical management, including unrelenting sepsis, cecal dilatation greater than 10 cm and bowel perforation have been considered indications for surgical intervention. In large series, 0.4%-3.6% of patients have required surgery, with an overall mortality of 30%-80%[133-135]. Series of severe CDI repeatedly emphasize how difficult the diagnosis may be. In a report of 14 patients requiring surgical intervention, only 50% had a preoperative diagnosis of CDI, because they required laparotomy before results of *C. difficile* testing became available[136]. Of note, the survival was better (86% *vs* 33%) in those with a preoperative diagnosis of CDI, which may have been due to the surgeon being more aware of the need for a total colectomy. Longo *et al*[137] noted some of the difficulties in the diagnosis of severe CDI in a series of 67 patients who required colectomy; 37% of the patients had no history of diarrhea, 45% presented in shock and 64% presented as an acute surgical abdomen[137]. Dallal *et al*[17] in a review of 64 patients who required a colectomy or died directly from CDI noted that 20% of the patients presented without diarrhea due to ileus. Of note, in this study 35% of diagnoses of severe CDI were found only at autopsy and the author suggested that a significant number of ICU deaths from "sepsis" may actually be CDI. Overall, 13% were *C. difficile* EIA toxin assay negative. Longo *et al*[137] found false-negative *C. difficile* cytotoxin assay in 18% of CDI severe enough to require colectomy[17]. Better diagnostic accuracy for severe CDI has been reported for the abdominal CT (89%-100% positive) and colonoscopy (100% positive)[17,136,137]. Of note, intravenous and oral contract were not required for a correct diagnosis with CT of the abdomen. Flexible sigmoidoscopy was falsely negative in 25% (2 of 8), one due to poor prep and one due to right sided colitis[17].

Lamontagne conducted a retrospective review of 165 cases of CDI which required ICU admission during the epidemic in Quebec between January 2003 to June 2005[138]. Of note, 24% of these ICU admissions resulted from relapse of previously diagnosed CDI, confirming how serious relapses can be. Predictors of 30 d mortality included a WBC of greater than 50000, age greater than 75-year-old, requirement for vasopressors and immunosuppression. Thirty eight patients underwent colectomies, 15 because of shock despite vasopressors, 11 with toxic megacolon, 10 with a lack of response to medical therapy and 2 because of perforation. The authors noted a significant decrease in mortality in those who had a colectomy *vs* those who were treated medically, with an adjusted odds ratio of 0.22, suggesting a 78% reduction in mortality. The major surgical benefit was found in those patients greater than 65 years of age who were immunocompetent with a WBC greater than 20000 and a lactate between 2.2 and 4.9 mm per liter. No surgical benefit was found in those with a white blood cell count less than 20000, less than 65 years of age and those with a normal lactate.

Recent surgical series have revealed conflicting data on which surgical procedure is preferred. Koss *et al*[136] presented a retrospective review of 14 patients who required surgery. The indications were systemic toxicity (n = 10), progressive toxic colonic dilatation (n = 4), and one with both colonic dilation and bowel perforation. Overall, mortality was 36%. Of those who underwent a total colectomy, mortality was 11% compared to 100% mortality in those whose surgical procedure was limited to a left hemicolectomy. Of note, at the time of surgery the exterior surface of the colon frequently was noted to be unremarkable, but all were distended and edematous. Longo *et al*[137] conducted a population based study from all 159 Department of Veterans Affairs Hospitals of patients who required colectomy for fulminant CDI between 1997 and 2001. For the 67 patients, the postoperative 30 d mortality was 48%. Of those who underwent

segmental colectomy, the mortality was 14%, while the mortality was 57% for those who underwent total colectomy (80% of the cases). At surgery, 58% of the patients were noted to have perforation or colonic infarction. As opposed to the Koss study, 12 of 14 patients who underwent hemicolectomy survived, probably because the colitis was restricted to the involved segment. A study by Dallal *et al*[17] confirms the possibility of segmental colectomy. This study was a retrospective review of 64 patients who died or underwent colectomy for pathologically proven CDI drawn from 2334 hospitalized patients with CDI, who were hospitalized between January 1989 and December 2000. There were 44 patients who required surgical intervention. Of those undergoing a right hemicolectomy, 100% survived. This was a select subgroup of 4 patients, all of whom had intraoperative colonoscopy confirming the fact that the colitis was restricted to the right hemicolon. Overall, in this study 89% of patients underwent a total colectomy, with a mortality of 63%. Most predictive of perioperative mortality was vasopressor requirement preoperatively, which increased postoperative mortality by four-fold. The authors suggested that hypotension requiring vasopressors may be too late a point for successful intervention. They noted that a white blood cell count greater than 30000 with a left shift almost always preceded the onset of shock and may be used as an early indicator of fulminant CDI, which may require surgical intervention.

Most recently, Neal *et al*[139] have studied an alternative to total colectomy advocating a diverting loop ileostomy with colonic lavage. They studied 42 patients with severe, complicated CDI. Their surgical approach was creation of a laparoscopic loop ileostomy followed by intraoperative colonic lavage with a warmed polyethylene glycol/electrolyte solution thru the ileostomy. They also performed postoperative antegrade instillation of vancomycin solution through the ileostomy. Compared to well matched historical controls mortality was reduced from 50% to 19%. Delayed reversal of the ileostomy, after recovery from the acute episode, resulted in preservation of the colon in 93% of cases. Based on these improved outcomes, they suggested that all patients with severe CDI should be considered for surgical management.

The 2013 ACG guidelines also defined signs and symptoms in complicated CDI which warrant surgical consultation, including: hypotension requiring vasopressor therapy, clinical signs of sepsis and organ dysfunction, mental status changes, WBC 50000 cells/mm^3, lactates 5 mmol/L, or complicated CDI with failure to improve on medical therapy after 5 d[71]. The suggested operative management is subtotal colectomy and end-ileostomy, which has been associated with reduced mortality in fulminant CDI[140].

RECURRENT CDI

Recurrence of CDI after initial successful treatment has been a significant problem. On average, recurrence can be expected in 20%-30% of cases. Once there has been an initial recurrence, relapse may occur in up to 65% of patients[141]. Risk factors associated with recurrence include older age (greater than 65), longer hospital stays (greater than 16 d), the presence of comorbidities and another course of antibiotics[142,143]. The new epidemic strain has been associated with an even higher rate of recurrence; rates may be as high as 47%[143]. Some authors have postulated that recurrence may be related to inability to mount an adequate antibody response as manifested by low IgG directed against toxin A[50].

The severity of recurrent episodes of CDI should not be underestimated. Pépin *et al*[144] reviewed the outcomes of a first recurrence of CDI with the epidemic strain during the Quebec outbreak[144]. They noted that 11% of patients with a first recurrence had at least one severe complication of CDI, including shock, colectomy, megacolon, perforation or death within 30 d. Complicated recurrent CDI was strongly associated with three factors: older age, elevated white blood cell count and renal failure. For those patients greater than 65 years of age, 13% developed recurrent CDI that was severe *vs* 7.5% for those 18-64 years of age. Subgroup analysis revealed recurrent CDI with a white blood cell count > 20000 was associated with a 38.9% incidence of complicated CDI *vs* only 10.6% when the white blood cell count was 10000-19000[141]. The long term negative impact of CDI was also explored by Musher *et al*[145], who reviewed outcomes for 103 patients who were considered to be cured without recurrence at 90 d after completion of therapy. They found that 22% of these patients developed recurrent diarrheal disease more than 90 d after the initial episode, 83% of whom were toxin positive.

Clinical approach to recurrent CDI

Most authors have recommended, repeating a course of the antibiotic used in the initial treatment, usually metronidazole, as the first step in the treatment of a recurrence. This sentiment is backed by the 2013 ACG guidelines[71]. For additional recurrences, a combination of a prolonged taper of the antibiotic with oral vancomycin, followed by pulsed dosing is often used. The original reports of tapered dosing utilized oral vancomycin as the preferred drug, since levels in stool are high, over 1000-fold higher than the level needed to inhibit *C. difficile* and do not decrease as diarrhea resolves[146]. Early suggested courses were vancomycin 125 mg *qid* for 7 d, tapering to 125 mg *bid* for 7 d, then daily for 7 d[147]. After the taper has been completed, pulsed dosing can begin. The pulsed dosing of vancomycin is thought to allow time for germination of residual spores during the days off antibiotics, with killing of the vegetative form when the antibiotic is given again. Although there is no standard well studied pulsing regimen, one suggestion has been to give vancomycin 250 mg every 2 or 3 d for 3 wk[148]. Bartlett has noted that he always utilizes a 6 wk course as this is the approximate time for return of normal flora[149]. More recently, some authors have recommended continued lengthening of the pulsing interval until the vancomycin is given only once every

10 d[150]. Rare patients may require chronic pulsed dosing every 3-4 d, relapsing each time they try to lengthen the interval or discontinue the vancomycin. The 2013 ACG guidelines recommend a simplified pulsed dosing only regimen with vancomycin 125 mg orally every 3 d for 10 doses without tapering of the vancomycin (Conditional recommendation, low-quality evidence). For patients with more than 3 recurrences, the ACG guidelines now suggest considering fecal microbiota transplant (FMT)[71].

A new approach to relapsing CDI using a rifaximin "chaser" has been described. Johnson *et al*[151] conducted an empirical trial of a 14-d course of rifaximin following a variety of different treatments, mainly using vancomycin, for the treatment of recurrent CDI. The authors studied eight women from their clinical practices, who had suffered from 4-8 episodes of CDI. The patients ranged in age from 43-88 years of age, with six of the eight being greater than 65 years old. The onset of recurrences varied from 1-59 d (mean of 10.5 d) after completion of treatment for CDI. For five of the patients, recurrences were as early as 1 d after treatment ended. The patients had been treated with 79-372 d with a variety of different treatments including metronidazole, vancomycin tapered and/or pulsed, probiotics and vancomycin plus rifaximin. Rifaximin was used as a "chaser" when the patients were asymptomatic, immediately at the end of the vancomycin treatment. Six of the patients received 400 mg *bid* for 14 d. Rifaximin was well tolerated without side effects. Seven of the eight patients had no further recurrence, with follow up that varied from 51-431 d. The one patient who was noted to have a recurrence was immediately retreated while symptomatic (the only deviation from their basic protocol) for 14 d. This patient was noted to develop resistance to rifaximin. More recently, a randomized, double-blind, placebo controlled trial was conducted on the efficacy of the rifaximin "chaser". Patients completing a standard antibiotic regimen for CDI were assigned to receive either placebo or 400 rifaximin mg 3 times daily for 20 d. Recurrent diarrhea occurred in 49% of placebo patients and 21% of rifaximin patients (P = 0.018). Actual CDI recurrence rates, as assessed by positive toxin assay, were 31% (11 of 35) in the placebo group and 15% (5 of 33) in the rifaximin group (P = 0.11)[152]. Although the difference between rifaximin and placebo was not significant, the study was underpowered to exclude a statistically significant difference.

Rifaximin as a stand-alone treatment for recurrent CDI has also been a focus of interest. A retrospective study examining 32 patients with recurrent CDI who had undergone an average of 4.4 antimicrobial treatment courses for CDI, found treatment with 400 mg twice-daily rifaximin for 14 d was successful in preventing relapse in 53% (17 of 32) of cases[153]. Interestingly, the authors empirically noted the success of rifaximin treatment appeared to be related to the MIC of the particular isolate, and that B1 isolates (30% in the study) had the highest MICs among those tested. There was, however, no statistically significant difference (P = 0.11) in relapse rates among those with the B1 strain, 42% (8 of 19) compared to 53% overall. Among the proposed mechanisms for this increased efficacy in treatment and prevention of recurrent CDI are rifaximin's anti-inflammatory properties; rifaximin has been shown to induce epithelial cell changes that alter bacterial attachment and internalization, while also reducing the release of inflammatory cytokines[154]. Lastly, with the increasing prevalence of the B1 strain, clinicians should be aware of the potential for rifaximin resistance given the lack of commercial testing availability. At this point, however, it is difficult to ascertain the clinical impact of these findings, in particular when rifaximin has been noted to achieve such high fecal concentrations. The most recent consensus from the ACG notes that there is no convincing evidence at this point in time for the use of rifampin or rifaximin in the treatment of recurrent CDI[71].

Fecal Transplantation

Rapidly emerging onto the scene, FMT represents the most promising candidate among non-antibiotic treatment options for patients suffering from multiple relapses or recurrences. Borody *et al*[155] in an article subtitled "Toying with Human Motions", reviewed the use of the ultimate natural probiotic, transplanted human stool. Although noted to be "aesthetically unpleasing", the use of stool transplant from one individual, usually a close relative, to the patient with relapsing CDI has had a high success rate. They reviewed the published literature of the use of fecal transplantation in 84 patients, noting a rapid response without recurrence in 86%. The authors also reviewed the use of stool transplantation for inflammatory bowel disease and irritable bowel syndrome and provide a detailed method for donor screening, preparation and administration.

Since then, the potential impact of FMT in the treatment of recurrent CDI has been more clearly elucidated and now represents a focal point of ongoing research. A systematic review of published studies between 2000-2011 identified 124 patients in seven studies with recurrent or refractory CDI who underwent FMT[156]. Among these patients, 83% reported immediate improvement following the procedure and further remained diarrhea free for months to years. The results from early studies all varied in protocol for pre-transplant antibiotic use, methods of delivery, amount of material delivered, long-term follow up, and none were controlled trials. Nonetheless, this systematic review of the early studies highlights the potential impact of fecal transplant for the treatment of recurrent or refractory CDI.

Brandt *et al*[157] in a multicenter long-term follow up study of 77 patients undergoing colonic FMT for recurrent CDI monitored both primary and secondary cure rates for individuals undergoing the procedure. A primary cure was defined as resolution of symptoms without recurrence within 90 d of treatment, while a secondary cure was resolution of symptoms with one further course of vancomycin. Follow up revealed a primary cure rate of 91% and a secondary cure rate of 98%. Of interest, the study addressed, through patient surveys, one of the

major drawbacks to FMT: the fact that the procedure is inherently aesthetically unpleasing. The survey results of these 77 patients revealed that 97% of patients would undergo another FMT for a CDI recurrence and that 53% of the patients would choose FMT as their first treatment option. This represents a promising finding that the unappealing nature of FMT may eventually be overcome by the predictable efficacy of FMT for patients facing the debilitating consequences of multiple CDI recurrences.

One of the most pressing question that has not been fully elucidated about FMT remains, how does it compare with other treatment options? While no double-blind randomized controlled trials have been completed to this date, new evidence has emerged from an interim analysis of an open-label, randomized, controlled trial in the Netherlands[158]. This study of recurrent CDI infection assigned patients to receive one of three treatments: initial vancomycin regimen (500 mg four times daily for 4 d) followed by bowel lavage and subsequent nasoduodenal infusion of donor feces, standard vancomycin regimen (500 mg four times daily for 14 d) with bowel lavage, or standard vancomycin regimen alone. With a primary endpoint measured as cure without relapse within 10 wk, the overall cure rate with FMT was 94% (15 of 16). Of these 16, 13 achieved cure on their initial treatment, with 2 more achieving cure after treatment with a different donor stool. This was compared to 31% (4 of 13) in the vancomycin alone group and 23% (3 of 13) in the vancomycin and lavage group. Lastly, post-FMT analysis of patient feces showed increased bacterial diversity, similar to that of the healthy donors.

Overall, the current literature suggests a promising future for the application of FMT in the treatment of recurrent CDI, however, some issue still remain, namely, the lack of a consensus protocol and viable sources of the donor feces. The majority of early FMT procedures utilized donor feces from spouses, intimate partners, or close family members, while potentially safer, also possesses many practical challenges in gathering the sample and administration. New evidence suggests that there may be equally efficacious alternatives to these close family donors. Between 2004-2010, a group of 32 patients with relapsing CDI at the Stockholm South General Hospital underwent FMT by either enema or colonoscopy using a fecal transplant suspension reconstituted from a single donor specimen obtained in 1994[159]. Among the patients, 69% (22 of 32) had a durable cure. These findings suggest that, in the future, it may be possible to establish a donor bank of prescreened individuals or specimens, thereby improving the ease, efficiency and safety of the process. Perhaps even more promising are results from a proof-of-principle study demonstrating that a stool substitute was capable of curing an antibiotic-resistant hypervirulent strain of *C. difficile*, ribotype 078. Researchers in this "RePOOPulating" study extensively cultured a stool sample from a 41-year-old healthy female donor to make a synthetic sample consisting of 33 different purified isolates, which was then used to treat 2 patients who had failed traditional therapy[160]. Both patients returned to normal bowel patterns within 2-3 d and remained symptom free at 6 mo. The authors of the study highlight numerous potential benefits of synthetic stool over donor stool including, the ability to control and alter the exact bacterial composition, the ability to replicate the procedure with an identical specimen, increased stability of donor stool sample, improved safety from knowledge of the exact sample composition, and the ability to adjust the sample for antimicrobial sensitivity.

A systematic review and meta-analysis by Kassam *et al*[161] provides new insight into variation between methods of FMT delivery and from donor type. This review of 11 studies including 273 CDI patients treated with FMT, performed a subgroup analysis comparing lower gastrointestinal delivery with upper gastrointestinal delivery. Lower gastrointestinal delivery (colonoscopy or enema) had clinical resolution rates of 91.4% (203/222) compared to upper gastrointestinal delivery (nasogastric/nasojejunal tube and gastroscopy) resolution rates of 82.3% (42/51). Further comparison between anonymous *vs* patient selected donors did not reveal a significant difference in clinical outcomes regardless of the follow-up time.

In April 2013, the United States FDA determined that FMT is a biologic product and drug that is regulated by the FDA. The FDA ruled that an investigational new drug (IND) application, a cumbersome and time consuming process, was needed for the use of FMT for any indication. In response to vocal and unified opposition by the gastrointestinal specialty societies, the FDA rapidly reversed this requirement and provided that the "treating physician obtains adequate informed consent from the patient or his or her legally authorized representative for the use of FMT products. Informed consent should include at a minimum, a statement that the use of FMT products to treat *C. difficile* is investigational and a discussion of its potential risks"[162].

In conclusion, as more evidence continues to become available, fecal transplantation is becoming an increasingly viable option for the treatment of recurrent or relapsing CDI, in particular given the recent recommendation for FMT to treat 3 CDI recurrences in the 2013 ACG guidelines[71]. While there remains no optimal protocol for administration or consensus on the ideal source of the transplant sample, future studies, including an NIH-funded blinded RCT and the pending FDA IND process, may provide valuable insight for these questions.

ADJUNCTIVE THERAPY: INTRAVENOUS IMMUNOGLOBULIN AND ANIONIC BINDING RESINS

There has been significant interest in the use of intravenous immunoglobulin (IVIG) to treat severe refractory and recurrent CDI. This interest is based upon the fact that development of *C. difficile* antitoxin antibody has been associated with protection from the development of CDI after colonization with *C. difficile*[50,163]. Small se-

ries and case reports have suggested a possible response to IVIG[164,165]. Of note, all immunoglobulin lots tested contained IgG against toxins A and B and were capable of neutralizing cytotoxicity in one series[166]. However, with the cost of IVIG approaching $10000 for an individual treatment course, proof of efficacy is important. McPherson conducted a retrospective review of 14 patients with either severe, refractory or recurrent CDI[167]. They used an IVIG dose of 150-400 mg/kg. Nine of these 14 patients responded in a median of 10 d, a relatively slow response, and 3 of these 9 patients had recurrent CDI after initial resolution. The most instructive study on the use of IVIG for severe CDI was conducted by Juang *et al*[168] at the University of Pittsburgh Medical Center. Because of the severity of CDI at their institution, a committee developed eligibility criteria for IVIG which was then used in a prospective manner to choose patients eligible for IVIG. Eighteen patients received IVIG at a dose of 200-300 mg/kg and these patients were pair matched by propensity scoring with other patients with severe CDI. There was no difference in mortality (3 patients in each group), colectomy (3 patients in each group) or length of stay. Although this study is not definitive, the results do not support the use of IVIG for severe CDI. The 2013 ACG guidelines addressed the use of IVIG in the treatment of recurrent CDI, and concluded that it does not have a role as sole therapy; however, they noted that it may my helpful in patients with hypogammaglobulinemia. This recommendation is based on the predisposition for CDI in patients following solid organ transplantation.

Anion binding resins, like cholestyramine and colestipol, have been used to treat CDI. The non-absorbable resin binds to *C. difficile* toxin removing 99% of the cytotoxic activity[169]. However; concerns have been raised about the use of these toxin-binding agents, because they also bind to vancomycin[170]. Thus, combination therapy should be used carefully, if at all, with separation of the anion binding resin and vancomycin by at least 2-3 h. Other sources have recommended giving the vancomycin either 1 h before or 4-6 h after the cholestyramine dose[171].

PREVENTATIVE THERAPY

One of the most important issues related to CDI from the perspective of the practicing clinician is the approach to the patient with a known history of *C. difficile*, who requires a subsequent course of antibiotics for an infection such as urinary tract infection or pneumonia or who cannot stop the antibiotics which induced the original episode of CDI. The use of metronidazole or vancomycin in this setting can be referred to as preventative therapy. Unfortunately, there is no data from systematic studies of the use of preventative therapy. However, Miller noted that "on the basis of no prospective evidence but, often, a large body of clinical experience, some clinicians now start a parallel course of oral metronidazole or vancomycin along with treatment with the potentially CDI-inducing antimicrobial, to prevent the appearance of symptomatic CDI"[172]. He goes on further to note "that despite absence of guidelines for this approach, there is remarkable homogeneity in the approaches used by most clinicians, in that clinicians who practice this prophylactic strategy use oral metronidazole or vancomycin during the entire course of antimicrobial therapy and for an additional 7 d after the end of the administration period". This preventative approach to *C. difficile* seems an intuitively reasonable approach, which can be utilized pending results of future clinical trials that would validate its effectiveness.

Probiotics

Probiotics, defined by the World Health Organization as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host," have seen a recent surge in interest and use[173]. Current estimates for sales of probiotics, as both supplements and foods, was estimated to be $770 million in the US alone for 2012, with worldwide sales at $2.25 billion, a 79% increase since 2010[174,175]. Further, recent estimates have projected worldwide spending on probiotics to reach $4 billion annually by 2016[176]. Despite all the interest and sales of probiotics, their utilization for the prevention or treatment of CDI remains controversial and unproven.

Heavy marketing campaigns and choice labeling of products have helped fuel the dramatic growth of the probiotics markets. Further helping to shape the consumer image, clinical evidence for the use of probiotics in the prevention of antibiotic-associated diarrhea (AAD) appears promising. Systematic reviews and meta-analyses were completed for the use of probiotics in any AAD. In one analysis, the pooled results from 63 randomized control trials revealed a RR of 0.58 (95%CI: 0.50-0.68, $P < 0.001$) with an number needed to treat (NNT) of 13[177]. Among those studies, a subset of 14 were randomized controlled trials for the prevention of CDI and pooled analysis revealed a RR of 0.29 (95%CI: 0.17-0.48, $P < 0.001$) with an NNT of 25. However, it was noted that poor adherence and limited reporting of the number of samples tested may have skewed the results. Another meta-analysis of 34 studies including 4138 patients showed a 0.53 relative risk for the development of antibiotic-associated diarrhea in the probiotics *vs* the placebo group (95%CI: 0.44-0.63), with an NNT of 8[178]. Importantly, the authors of this study chose to omit any trials involving the use of probiotics for the prevention or treatment of CDI.

Although some may wonder why a variant of baker's yeast, which is not a part of the normal microflora of the gut, would be effective in preventing or treating CDI, there is some theoretical support for the use of Saccharomyces boulardii, which has been shown to prevent toxin A binding and also to inactivate toxins A and B by proteolytic digestion[179,180]. Further, in the hamster model, *S. boulardii* has been shown to be effective in preventing deaths from acute disease[181]. Other mechanisms

by which Saccharomyces may prevent CDI include inhibition of *C. difficile* adhesion, cellular protection from histologic damage and inhibition of pro-inflammatory cytokine gene expression[182-184]. In fact, Czerucka used the term "immunobiotic" to describe *S. boulardii*[182].

The clinical efficacy of *S. boulardii* has shown mixed results in a number of reviews and meta-analyses of randomized, controlled trials of CDI. Dendukuri *et al*[185] concluded that the "studies conducted to date provide insufficient evidence for the routine clinical use of probiotics to prevent or treat CAD". Szajewska *et al*[186] found a reduction in antibiotic associated diarrhea of 57%, but no reduction in CDI. Katz in reviewing the use of probiotics for the prevention of CDI developed a proposed guideline which noted no evidence to support efficacy in the primary prevention of *C. difficile*, but suggested that "*S. boulardii* can be used to decrease recurrences of *C. difficile*"[187]. McFarland *et al*[188] found that *S. boulardii* was not effective in preventing recurrence after an initial episode of CDI. The authors did find, however, a 50% reduction among patients who had had a previous recurrence. A second study of the use of *S. boulardii* in patients with recurrent CDI confirmed a decrease in recurrences, but only when combined with a high dose of oral Vancomycin (500 mg *qid*). There was no reduction in recurrent CDI with lower doses of vancomycin or metronidazole[189]. McFarland later conducted a meta-analysis and noted that "from six randomized trials, probiotics had significant efficacy for CDD"[190]. Unfortunately, he combined the 2 studies using *S. boulardii* with studies using a variety of *Lactobacillus* preparations, which could lead to significant misinterpretation of the data. As noted by Gerding; "A recent meta-analysis suggested that probiotics are effective; nevertheless, because of the heterogeneity of study methods and patient populations, it is not scientifically possible to conduct a meta-analysis of findings in the probiotic literature"[191].

Another aspect of *S. boulardii* in the treatment or prevention of CDI is the risk for adverse events. While generally considered safe, there have been increasing reports of fungemia due to *S. boulardii*, especially in those with intravascular catheters and antibiotic therapy. Of the 37 patients with *S. boulardii* fungemia in one report, use of *S. boulardii* as a probiotic was considered to be the source of infection in 64%[192]. Of note, an additional five cases were reported in patients who were not receiving a probiotic. In these cases, there was evidence of healthcare associated acquisition from other patients who were being treated with *S. boulardii*. The authors suggested that special caution should be taken with probiotics in critically ill and immunocompromised patients. Segarra-Newnham in a review of the use of probiotics for CDI concluded that "there were numerous unanswered questions"[193]. She also noted that "given the potential for complications in debilitated immunosuppressed patients, the risk may outweigh the benefits". Czerucka went even further suggesting "the presence of indwelling catheters is a contraindication for the administration of *S. boulardii*" [182]. Further evidence that the safety of probiotics cannot be assumed comes from a recent double blind, placebo controlled trial of a multispecies probiotic (mostly *Lactobacillus* sp. and *Bifidobacterium* sp.) in the treatment of severe acute pancreatitis[194]. In the probiotic group, 16% of patients died *vs* 6% in the placebo group. Nine patients (8 with fatal outcomes) developed bowel ischemia. Eight involved the small bowel. The authors concluded "probiotics can no longer be considered to be harmless adjuncts to enteral alimentation, especially in critically ill patients".

While the early literature focused primarily on the application of *S. boulardii* for prevention and treatment of CDI, more recently there has been a shift towards to the use of *Lactobacillus* sp. preparations, such as Lactinex (Becton Dickinson, San Diego, Ca) or *Lactobacillus* GG (Culturelle, Bloomfield, Ct.). Early support for *Lactobacillus* came from a randomized, double-blind, placebo controlled trial, published by Hickson *et al*[195] reporting the use of Actimel (Danone, France) in the prevention of CDI. In the United States, a similar product would be DanActive by Dannon. No patients in the probiotic group developed CDI, while 17% (9 of 53) in the placebo group developed CDI (P = 0.001). The authors concluded that "this has the potential to decrease morbidity, health care cost, and mortality if used routinely in patients aged over 50". Unfortunately, the article by Hickson *et al*[195] adds little substantive new data to the argument, because of its very poor generalizability. The extraordinarily high exclusion rate resulted in only 6.4% of screened patients being evaluable in the efficacy analysis. Of the 1760 patients assessed for eligibility, 1625 (92%) were excluded and a further 148 refused to participate, leaving only 135 patients to be entered in the study. Of these, 16% were lost to follow up, leaving only 6.4% of the patients eligible for analysis. As noted in a Letter to the Editor "I was astounded to read in the study method that Hickson *et al*[195] had excluded high risk antibiotics (as well as some misclassified low risk antibiotics). To do so is akin to performing a trial of an agent that claims to prevent type 2 diabetes, but excluding obese patients" [196].

Since that time a significant number of trials have been conducted with varying levels of support for probiotics. A 2008 Cochrane Review of the use of probiotics in the treatment of CDI in adults identified 4 randomized control trials meeting inclusion criteria, all of which were noted to be small in size and have methodological problems[197]. Of these studies, only one was found to have a statistically significant benefit for probiotics, the previously mentioned study by MacFarland et al on *S. boulardii*. The most promising evidence to date for probiotics comes from a systematic review and meta-analysis involving pooled data from 20 studies and 3818 patients, which revealed a pooled RR of 0.34 (95%CI: 0.24-0.49), in other words a reduction in the incidence of CDI of 66%[198]. Calculating the optimal information size, which is the number of patients required for an adequately powered study, using the worst-plausible-assumption and applying a 5% population incidence of antibiotic-associated CDI, the authors suggest this moderate-quality

evidence predicts probiotics prophylaxis would prevent 33 episodes of CDI per 1000 persons. Additionally, while their study indicated a larger risk reduction in the use of multiple species preparation over single species, this was likely accounted for by heterogeneity between studies.

The newest evidence surrounding the use of probiotics for prevention of CDI comes from the PLACIDE trial, a multi-center, randomized, double-blind, placebo controlled trial for the use of lactobacilli and bifidobacteria in the prevention of AAD and CDD, for which inpatients over the age of 65 were randomized to either a microbial preparation or placebo. Relative risks between the groups were RR 1.04 for AAD (95%CI: 0.84-1.28) and RR 0.71 for CDD (95%CI: 0.34-1.57)[199]. The authors concluded no evidence that multi strain preparation of lactobacilli and bifidobacteria was effective in the prevention of AAD or CDD.

Overall, interpretation of results from probiotic studies present many challenges. Lawrence, who conducted a study of a *Lactobacillus* preparation to prevent recurrent CDI noted that a number of problems were faced in attempting to determine the efficacy of probiotics[200]. He noted the high percentage of patients receiving systematic antibiotics (66.7%) and a high number of patients receiving gastric acid suppression, both of which might interfere with the efficacy of a probiotic. Other problems with studies of probiotics for the prevention of recurrences of CDI, include the lack of randomization of the type or dose of the antibiotic used with the probiotics, which may have altered the outcomes. Doses of probiotics were not standardized and may have been too small or the preparations may have become nonviable after manufacture or may have a different strain than advertised. A number of authors have found that the number of colony forming units can be much lower than what is advertised on the label[201]. The 2013 ACG guidelines concluded that there is insufficient evidence that probiotics prevent CDI (Strong recommendation, low quality evidence)[71]. In summary, there is much more enthusiasm than data for the use of probiotics in the prevention or treatment of CDI.

The newest approach in the prevention of CDI focuses on targeting the infective spore to prevent germination into the vegetative toxin producing form. Since only the vegetative form produces toxin, theoretically prevention of spore germination would prevent symptomatic infection. Howerton *et al*[202] demonstrated that a cholate meta-benzene sulfonic derivative (CamSA) is a strong competitive inhibitor of taurocholate-mediated *C. difficile* spore germination. Subsequently, they administered a single 50 mg/kg dose of CamSA to mice infected with *C. difficile* spores and were able to prevent any signs of CDI[203]. The authors also noted that CamSA gave complete protection against an "unnaturally massive" *C. difficile* spore infection, equivalent to human ingestion of hundreds of grams of infective spores. While still early in the investigative process, CamSA represents an entirely new approach to preventing CDI.

FUTURE TREATMENT OPTIONS FOR CDI

With the increased virulence and decreased response to standard treatment, combined with an increase in recurrences, both due to relapse and acquisition of epidemic strains in hospitals, the need for newer approaches to the treatment of CDI becomes even more important. There are a number of exciting new antibiotics being studied for treatment of CDI, including rifalazil, ramoplanin and non-antibiotic based approaches, such as tolevamer, monoclonal antibodies against toxin A, and a vaccine.

Rifalazil

Rifalazil is an experimental, absorbable antibiotic in the rifamycin class, related to rifampin with a broad spectrum of activity against a wide range of organisms, including Mycobacterium tuberculosis, Chlamydia, Helicobacter pylori and *C. difficile*[204]. Testing of rifalazil against 110 isolates of *C. difficile* collected from 1983-2004 revealed excellent activity with an MIC 90 of 0.03 μg/mL, with only one isolate from the United States found to be resistant[82]. In a study of *C. difficile* in the hamster model, all animals treated with rifalazil or vancomycin were protected from disease. Histologically, the rifalazil treated animals had less edema and neutrophil infiltration than the vancomycin treated animals. When vancomycin was discontinued, 65% of the animals developed disease, while none of the rifalazil treated animals had positive toxin assays or disease[202]. Future trials of rifalazil in humans with CDI are eagerly anticipated, especially given the low relapse rate in animal models.

Ramoplanin

Ramoplanin is an experimental broad spectrum, non-absorbable glycolipodepsipeptide. In the same study mentioned above, all isolates of *C. difficile* were sensitive to ramoplanin with an MIC 90 of 0.5 μg/mL[204]. In another study, which included *C. difficile* isolates with reduced susceptibility to vancomycin and resistance to metronidazole, no resistance was found to ramoplanin[205]. In a hamster model of CDI, both ramoplanin and vancomycin were uniformly effective in resolution of symptoms[96]. In the vancomycin group, 100% of animals had spores detected *vs* only 30% treated with ramoplanin after 2 d of treatment. Ramoplanin was noted to have a profound effect on both the vegetative and spore forms of *C. difficile* with complete eradication of both forms of the organism by 24 h. Vancomycin, on the other hand, had no effect on spores. The authors hypothesized that the efficacy against spores may be related to the binding of lipid Ⅱ. A related antibiotic, nisin, which has been used as a food preservative for decades, had been note to inhibit transformation from spore to the vegetative form in Bacillus and other Clostridial species[206].

REP3123

REP3123 is a novel inhibitor of methionyl tRNA synthetase, which is required for bacterial growth. REP3123 inhibits toxin formation, is active in animal models,

prevents death of human cells exposed to C. *difficle* toxin and decreases spore formation. REP3123 has shown activity against 108 different *C. difficile* isolates, including the B1 strain, with an affinity for bacterial MetRS over 1000 times that of human mitochondrial or cytoplasmic MetRS[207]. In addition, REP3123 is highly selective for gram positive bacteria which may spare much of the normal colonic flora[208]. Clinical trials are eagerly awaited.

Tolevamer

Tolevamer (Genzyme Corp. Cambridge, MA) is a high molecular mass, non-absorbable polymer that has been shown to be a potent neutralizer of *C. difficlie* toxins A and B, with each polymer molecule irreversibly binding 3-4 toxin molecules[209]. A proposed advantage of non-antibiotic approaches for the treatment of CDI is the fact that there is no disturbance of the normal intestinal flora, potentially decreasing the risk of recurrent disease. Louie, et al. reported a randomized, double blind trial of tolevamer in patients with mild to moderate CDI[210]. The patients were randomized to 3 or 6 g of tolevamer for 14 d or vancomycin 125 mg *qid*. If the 6 patients who had recurrence of diarrhea while still on treatment with tolevamer (4 in the 3 g/d group and 2 in the 6 g/d group) are included in the efficacy analysis, resolution of diarrhea was found in 60% of the tolevamer 3 g group, 79% in the 6 g group and 91% with vancomycin. Recurrence rates were 10% in the tolevamer 6 g group *vs* 19% in the vancomycin group. The major side effect of tolevamer was noted be hypokalemia, found in 23% of those in the 6 g group *vs* 7% of those treated with vancomycin. Because tolevamer is an anionic polymer capable of binding cations in colonic fluid, the hypokalemia is not surprising. Addressing this issue, the next study on tolevamer utilized a modified product, which is liquid with potassium added, to allow net-neutral potassium balance. This randomized Phase I trial tested tolevamer at 6, 9, 12 and 15 g/d, normal potassium was maintained with the new product and researchers reported that tolevamer was generally safe and well tolerated in patients at does up to 15 g/d[211].

Despite its demonstrated safety with the reformulated drug, two subsequent studies challenged the efficacy of tolevamer for the treatment of CDI. The first was a Phase III randomized trial of 544 patients on either tolevamer (3 g, 3 times a day for 14 d), vancomycin (125 mg, 4 times a day for 10 d), or metronidazole (375 mg, 4 times a day for 10 d)[212]. Of the 278 patients on tolevamer only 42% achieved clinical success, thereby failing to demonstrate noninferiority to the 73% success rate of vancomycin. One interesting finding, however, was the patients on Tolevamer had a decreased rate of recurrence (6%) when compared to the vancomycin group (18%; $P = 0.009$) and metronidazole group (19%; $P = 0.006$). The authors attributed the decreased rate of recurrence to the flora-sparing activity of tolevamer. As a follow-up to the findings of this Phase Ⅲ trial, researchers in the UK studied the neutralizing effects of tolevamer on the *C. difficile* cytotoxins in an *in vitro* human gut model[213]. In contrast to previous studies, these researchers found that tolevamer was not associated with loss of the *C. difficile* cytotoxic effect. These results support and may explain the poor results for the primary endpoint in the previously described Phase Ⅲ trial.

Monoclonal antibodies

The proposed mechanism behind the use of monoclonal antibodies (MAbs) in CDI is the potential ability to directly modulate the effects of *C. difficile* cytotoxins A and B. In animal models, MAbs have been shown to reduce the severity and duration of diarrhea, death rate, and rate of recurrence[214]. Literature concerning the administration of a single MAb against either toxin A or toxin B seems to be conflicting; one early study reports that a MAb against toxin A was sufficient to protect form death, while a MAb against toxin B had no effect[215]. In contrast, it was more recently suggested that the MAb against toxin B was protective against CDI[216]. Given these conflicting reports, clinical application of MAb therapy appears to be adopting a dual administration of MAbs for both toxin A and toxin B. A randomized, double-blind Phase II placebo controlled trial of MAbs against toxin A (CDA1) and toxin B (CDB1) was able to demonstrate a lower recurrence rate with the administration of a single infusion of 10 mg/kg of MAb compared to placebo in patients also receiving either metronidazole or vancomycin[217]. Overall, recurrence rates were 7% for the MAb group *vs* 25% for the placebo group (95%CI: 7-29, $P < 0.001$), while for patients with more than one previous episode of CDI the recurrence rates were 7% for the MAb group compared to 38% for the placebo group ($P = 0.006$).

Many questions remain about the application of MAb therapy in the treatment of CDI. Concern has been raised that MAb therapy does not decrease the severity of diarrhea, duration of hospitalization, or time to resolution[218]. Additionally, the clinical applications of the current studies may not be appropriate given differences in course of illness between different patient populations, in particular the elderly[219]. Some of these questions may be answered by two Phase Ⅲ trials currently underway[220,221]. Also likely to emerge in the future is the application of new MAbs that specifically bind to epitopes in the neutralizing regions of toxins A and B. These MAbs, known as PA-50 and PA-41, were shown to confer a dramatically increased survival rate in a hamster model, where the administration of a dual PA-50/PA-41 MAb revealed long term survival rate of 95% *vs* 0% for placebo[222].

Vaccine

Interest in a vaccine is based upon the fact that development of *C. difficile* antitoxin antibody has been associated with protection from the development of CDI after colonization with *C. difficile*. A vaccine against toxins A and B, that has been efficacious in animal models as

well as humans, and demonstrated successful prevention of recurrence in 3 case reports[223]. More recently, six Phase Ⅰ trials on 200 individuals have been completed by Sanofi Pasteur with a bivalent formalin-inactivated vaccines against toxins A and B showing serconversion of 75% of participants by day 70[224]. A Phase Ⅱ trial of this vaccine, currently underway in the US, is being conducted to assess primary CDI prevention in 650 at risk adults[225]. Also in development is a chimeric antitoxin vaccine using an endotoxin free expression system from Bacillus metaerium, which was capable of producing neutralizing antitoxins and preventing spore-induced relapse in CDI[226].

CONCLUSION

The impact of CDI infection is significant. This infection places a tremendously onerous burden on the health care system worldwide and has major adverse clinical and economic impact. This topic will be a continued high priority for national guidelines and clinicians will need to pay close attention to any forthcoming revisions for diagnosis and management. Presently, best practice recommendations would be as follows: (1) only patients with diarrhea (a stool that takes the shape of the container) should be tested for CDI; (2) initial testing should be done with glutamate dehydrogenase or nucleic acid amplification test for CDI, without repeat testing unless high suspicion for infection and initial GDH testing is done; (3)patients with resolution of diarrhea should not be rested to document cure of CDI; (4) initial antibiotic treatment for patients with mild/moderate CDI infection should be metronidazole 500 mg *tid* orally (provided no drug allergy contraindication); (5) initial treatment for severe CDI or failure to respond to 5-7 d of metronidazole should be vancomycin 125 mg *qid* orally. If severe or complicated CDI, intravenous metronidazole 500 mg *tid* should be added; (6) in patients with severe ileus or complicated CDI, best antibiotic plan is intravenous metronidazole 500 mg *tid* plus vancomycin 500 mg *qid* (oral) plus vancomycin 500 mg in 500 cc fluid *qid* (rectal by retention enema); (7) use of intravenous formulation compounded by pharmacy into oral solution offers significant cost advantage; (8) the first recurrence of CDI can be treated with the initial regimen if it induced appropriate clinical response; (9) the second recurrence of CDI should be treated with pulsed vancomycin; (10) the third recurrence or unresponsive severe CDI, fecal microbiota transplant should be considered; (11) current data suggests limited if any value, of probiotics for CDI treatment or prevention of relapse. The use of these agents in patients with central venous catheters should be avoided given possible infectious complications; and (12) high level disinfection of environmental surfaces for bathroom and if inpatient, contact surfaces is recommended. We routinely have patients discard toothbrush and change any device or implement that may allow oral contact ingestion of aerosolized spores in patients with CDI.

REFERENCES

1 **McDonald LC**, Lessa F, Sievert D, Wise M, Herrera R, Gould C, Malpiedi P, Dudeck M, Srinivasan A, Fridkin S, Cardo D. Vital signs: preventing *Clostridium* difficile infections. *MMWR* 2012; **61**: 157-162

2 **Miller BA**, Chen LF, Sexton DJ, Anderson DJ. Comparison of the burdens of hospital-onset, healthcare facility-associated *Clostridium* difficile Infection and of healthcare-associated infection due to methicillin-resistant Staphylococcus aureus in community hospitals. *Infect Control Hosp Epidemiol* 2011; **32**: 387-390 [PMID: 21460491 DOI: 10.1086/659156]

3 **Klein E**, Smith DL, Laxminarayan R. Hospitalizations and deaths caused by methicillin-resistant Staphylococcus aureus, United States, 1999-2005. *Emerg Infect Dis* 2007; **13**: 1840-1846 [PMID: 18258033 DOI: 10.3201/eid1312.070629]

4 **Hall AC**, Curns AT, McDonald LC, Parashar UD, Lopman BA. The roles of norovirus and *Clostridium* difficile among gastroenteritis deaths in the United States, 1997-2007. Presentation and the 49th annual Meeting of the Infectious Disease Society of America; 2011 Oct 22; Boston, MA

5 **McDonald LC**, Kilgore GE, Thompson A, Owens, Jr. RC, Kazakova SV, Sambol SP, Johnson S, Gerding DN. An epidemic, toxin gene-variant strain of *Clostridium* difficile. *N Engl J Med* 2005; **353**: 2433-2441 [DOI: 10.1056/NEJMoa051590]

6 **Pépin J**, Valiquette L, Alary ME, Villemure P, Pelletier A, Forget K, Pépin K, Chouinard D. *Clostridium* difficile-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. *CMAJ* 2004; **171**: 466-472 [PMID: 15337727 DOI: 10.1503/cmaj.1041104]

7 **Hensgens MP**, Goorhuis A, Dekkers OM, Kuijper EJ. Time interval of increased risk for *Clostridium* difficile infection after exposure to antibiotics. *J Antimicrob Chemother* 2012; **67**: 742-748 [PMID: 22146873 DOI: 10.1093/jac/dkr508]

8 **Riley TV**. Antibiotic-associated diarrhoea. A costly problem. *Pharmacoeconomics* 1996; **10**: 1-3 [PMID: 10160466 DOI: 10.2165/00019053-199610010-00001]

9 **Thomas C**, Stevenson M, Williamson DJ, Riley TV. *Clostridium* difficile-associated diarrhea: epidemiological data from Western Australia associated with a modified antibiotic policy. *Clin Infect Dis* 2002; **35**: 1457-1462 [PMID: 12471563 DOI: 10.1086/342691]

10 **Gerding DN**, Olson MM, Peterson LR, Teasley LR, Gebhard RL, Schwartz ML, Lee JT Jr. Clostridium difficile -associated diarrhea and colitis in adults. *Arch Intern Med* 1986; **146**: 95-100 [DOI: 10.1001/archinte.1986.00360130117016]

11 **Brown E**, Talbot GH, Axelrod P, Provencher M, Hoegg C. Risk factors for Clostridium difficile toxin-associated diarrhea. *Infect Control Hosp Epidemiol* 1990; **11**: 283-290 [DOI: 10.1086/646173]

12 **Centers for Disease Control and Prevention (CDC)**. Severe Clostridium difficile-associated disease in populations previously at low risk--four states, 2005. *MMWR Morb Mortal Wkly Rep* 2005; **54**: 1201-1205 [PMID: 16319813]

13 **Anand A**, Glatt AE. Clostridium difficile infection associated with antineoplastic chemotherapy: a review. *Clin Infect Dis* 1993; **17**: 109-113 [PMID: 8353229 DOI: 10.1093/clinids/17.1.109]

14 **Raza S**, Baig MA, Russell H, Gourdet Y, Berger BJ. Clostridium difficile infection following chemotherapy. *Recent Pat Antiinfect Drug Discov* 2010; **5**: 1-9 [PMID: 19929843 DOI: 10.2174/157489110790112608]

15 **Baskerville A**, Batter-Hatton D. Intestinal lesions induced experimentally by methotrexate. *Br J Exp Pathol* 1977; **58**: 663-669 [PMID: 607986]

16 **Riddle DJ**, Dubberke ER. Clostridium difficile infection in solid organ transplant recipients. *Curr Opin Organ Transplant* 2008; **13**: 592-600 [PMID: 19060548 DOI: 10.1097/MOT.0b013e3283186b51]

17 **Dallal RM**, Harbrecht BG, Boujoukas AJ, Sirio CA, Farkas LM, Lee KK, Simmons RL. Fulminant Clostridium difficile: an underappreciated and increasing cause of death and complications. *Ann Surg* 2002; **235**: 363-372 [PMID: 11882758 DOI: 10.1097/00000658-200203000-00008]

18 **Albright JB**, Bonatti H, Mendez J, Kramer D, Stauffer J, Hinder R, Michel JA, Dickson RC, Hughes C, Nguyen J, Chua H, Hellinger W. Early and late onset Clostridium difficile-associated colitis following liver transplantation. *Transpl Int* 2007; **20**: 856-866 [PMID: 17854444 DOI: 10.1111/j.1432-2277.2007.00530.x]

19 **Dubberke ER**, Riddle DJ. Clostridium difficile in solid organ transplant recipients. *Am J Transplant* 2009; **9** Suppl 4: S35-S40 [PMID: 20070693 DOI: 10.1111/j.1600-6143.2009.02891.x]

20 **Kelly CP**, LaMont JT. Clostridium difficile infection. *Annu Rev Med* 1998; **49**: 375-390 [PMID: 9509270 DOI: 10.1146/annurev.med.49.1.375]

21 **McFarland LV**, Surawicz CM, Stamm WE. Risk factors for Clostridium difficile carriage and *C. difficile*-associated diarrhea in a cohort of hospitalized patients. *J Infect Dis* 1990; **162**: 678-684 [PMID: 2387993 DOI: 10.1093/infdis/162.3.678]

22 **Bliss DZ**, Johnson S, Savik K, Clabots CR, Willard K, Gerding DN. Acquisition of Clostridium difficile and Clostridium difficile-associated diarrhea in hospitalized patients receiving tube feeding. *Ann Intern Med* 1998; **129**: 1012-1019 [PMID: 9867755 DOI: 10.7326/0003-4819-129-12-199812150-00004]

23 **Kelly TW**, Patrick MR, Hillman KM. Study of diarrhea in critically ill patients. *Crit Care Med* 1983; **11**: 7-9 [PMID: 6848311 DOI: 10.1097/00003246-198301000-00003]

24 **Wilson KH**, Sheagren JN, Freter R. Population dynamics of ingested Clostridium difficile in the gastrointestinal tract of the Syrian hamster. *J Infect Dis* 1985; **151**: 355-361 [PMID: 3968453 DOI: 10.1093/infdis/151.2.355]

25 **Janarthanan S**, Ditah I, Adler DG, Ehrinpreis MN. Clostridium difficile-associated diarrhea and proton pump inhibitor therapy: a meta-analysis. *Am J Gastroenterol* 2012; **107**: 1001-1010 [PMID: 22710578 DOI: 10.1038/ajg.2012.179]

26 **Leonard J**, Marshall JK, Moayyedi P. Systematic review of the risk of enteric infection in patients taking acid suppression. *Am J Gastroenterol* 2007; **102**: 2047-2056; quiz 2057 [PMID: 17509031 DOI: 10.1111/j.1572-0241.2007.01275.x]

27 **Khanna S**, Aronson SL, Kammer PP, Baddour LM, Pardi DS. Gastric acid suppression and outcomes in Clostridium difficile infection: a population-based study. *Mayo Clin Proc* 2012; **87**: 636-642 [PMID: 22766083 DOI: 10.1016/j.mayocp.2011.12.021]

28 **Leonard AD**, Ho KM, Flexman J. Proton pump inhibitors and diarrhoea related to Clostridium difficile infection in hospitalised patients: a case-control study. *Intern Med J* 2012; **42**: 591-594 [PMID: 22616966 DOI: 10.1111/j.1445-5994.2012.02770.x]

29 **Leekha S**, Aronhalt KC, Sloan LM, Patel R, Orenstein R. Asymptomatic Clostridium difficile colonization in a tertiary care hospital: admission prevalence and risk factors. *Am J Infect Control* 2013; **41**: 390-393 [PMID: 23622704 DOI: 10.1016/j.ajic.2012.09.023]

30 **McCollum DL**, Rodriguez JM. Detection, treatment, and prevention of Clostridium difficile infection. *Clin Gastroenterol Hepatol* 2012; **10**: 581-592 [PMID: 22433924 DOI: 10.1016/j.cgh.2012.03.008]

31 **Cohen SH**, Gerding DN, Johnson S, Kelly CP, Loo VG, McDonald LC, Pepin J, Wilcox MH. Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). *Infect Control Hosp Epidemiol* 2010; **31**: 431-455 [PMID: 20307191 DOI: 10.1086/651706]

32 **Drapkin MS**, Worthington MG, Chang TW, Razvi SA. Clostridium difficile colitis mimicking acute peritonitis. *Arch Surg* 1985; **120**: 1321-1322 [PMID: 3931611 DOI: 10.1001/archsurg.1985.01390350097021]

33 **Tedesco FJ**, Barton RW, Alpers DH. Clindamycin-associated colitis. A prospective study. *Ann Intern Med* 1974; **81**: 429-433 [PMID: 4412460 DOI: 10.7326/0003-4819-81-4-429]

34 **Wanahita A**, Goldsmith EA, Musher DM. Conditions associated with leukocytosis in a tertiary care hospital, with particular attention to the role of infection caused by clostridium difficile. *Clin Infect Dis* 2002; **34**: 1585-1592 [PMID: 12032893 DOI: 10.1086/340536]

35 **Bulusu M**, Narayan S, Shetler K, Triadafilopoulos G. Leukocytosis as a harbinger and surrogate marker of Clostridium difficile infection in hospitalized patients with diarrhea. *Am J Gastroenterol* 2000; **95**: 3137-3141 [PMID: 11095331 DOI: 10.1111/j.1572-0241.2000.03284.x]

36 **Wanahita A**, Goldsmith EA, Marino BJ, Musher DM. Clostridium difficile infections in patients with unexplained leukocytosis. *Am J Med* 2003; **115**: 543-546 [DOI: 10.1016/S0002-9343(03)00420-0]

37 **Dansinger ML**, Johnson S, Jansen PC, Opstad NL, Bettin KM, Gerding DN. Protein-losing enteropathy is associated with Clostridium difficile diarrhea but not with asymptomatic colonization: a prospective, case-control study. *Clin Infect Dis* 1996; **22**: 932-937 [PMID: 8783689 DOI: 10.1093/clinids/22.6.932]

38 **Ramaswamy R**, Grover H, Corpuz M, Daniels P, Pitchumoni CS. Prognostic criteria in Clostridium difficile colitis. *Am J Gastroenterol* 1996; **91**: 460-464 [PMID: 8633491]

39 **Bartlett JG**. Leukocytosis and Clostridium difficile-associated diarrhea. *Am J Gastroenterol* 2000; **95**: 3023-3024 [PMID: 11095311 DOI: 10.1016/S0002-9270(00)02068-2]

40 **Marx CE**, Morris A, Wilson ML, Reller LB. Fecal leukocytes in stool specimens submitted for Clostridium difficile toxin assay. *Diagn Microbiol Infect Dis* 1993; **16**: 313-315 [PMID: 8495586 DOI: 10.1016/0732-8893(93)90081-H]

41 **Yong WH**, Mattia AR, Ferraro MJ. Comparison of fecal lactoferrin latex agglutination assay and methylene blue microscopy for detection of fecal leukocytes in Clostridium difficile-associated disease. *J Clin Microbiol* 1994; **32**: 1360-1361 [PMID: 8051268]

42 **Boland GW**, Lee MJ, Cats AM, Gaa JA, Saini S, Mueller PR. Antibiotic-induced diarrhea: specificity of abdominal CT for the diagnosis of Clostridium difficile disease. *Radiology* 1994; **191**: 103-106 [PMID: 8134552]

43 **Fekety R**. Guidelines for the diagnosis and management of Clostridium difficile-associated diarrhea and colitis. American College of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol* 1997; **92**: 739-750 [PMID: 9149180]

44 **Bartlett JG**. Clinical practice. Antibiotic-associated diarrhea. *N Engl J Med* 2002; **346**: 334-339 [PMID: 11821511 DOI: 10.1056/NEJMcp011603]

45 **Olson MM**, Shanholtzer CJ, Lee JT, Gerding DN. Ten years of prospective Clostridium difficile-associated disease surveillance and treatment at the Minneapolis VA Medical Center, 1982-1991. *Infect Control Hosp Epidemiol* 1994; **15**: 371-381 [PMID: 7632199]

46 **Tedesco FJ**, Corless JK, Brownstein RE. Rectal sparing in antibiotic-associated pseudomembranous colitis: a prospective study. *Gastroenterology* 1982; **83**: 1259-1260 [PMID: 7129030]

47 **Price AB**, Davies DR. Pseudomembranous colitis. *J Clin Pathol* 1977; **30**: 1-12 [PMID: 838865 DOI: 10.1136/jcp.30.1.1]

48 **Sumner HW**, Tedesco FJ. Rectal biopsy in clindamycin-associated colitis. An analysis of 23 cases. *Arch Pathol* 1975; **99**: 237-241 [PMID: 1131112]

49 **Curry S**. Clostridium difficile. *Clin Lab Med* 2010; **30**: 329-342 [PMID: 20513554 DOI: 10.1016/j.cll.2010.04.001]

50 **Kyne L**, Warny M, Qamar A, Kelly CP. Asymptomatic carriage of Clostridium difficile and serum levels of IgG antibody against toxin A. *N Engl J Med* 2000; **342**: 390-397 [PMID: 10666429 DOI: 10.1056/NEJM200002103420604]

51 **Simor AE**, Bradley SF, Strausbaugh LJ, Crossley K, Nicolle LE. Clostridium difficile in long-term-care facilities for the elderly. *Infect Control Hosp Epidemiol* 2002; **23**: 696-703 [PMID: 12452300 DOI: 10.1086/501997]

52 **Wilcox MH**. Overcoming barriers to effective recognition and diagnosis of Clostridium difficile infection. *Clin Microbiol Infect* 2012; **18** Suppl 6: 13-20 [PMID: 23121550 DOI: 10.1111/1469-0691.12057]

53 **Bartlett JG**, Gerding DN. Clinical recognition and diagnosis of Clostridium difficile infection. *Clin Infect Dis* 2008; **46** Suppl 1: S12-S18 [PMID: 18177217 DOI: 10.1086/521863]

54 **American Society for Microbiology**. A practical guidance document for the laboratory detection of toxigenic Clostridium difficile, September 21, 2010. Available from: URL: http//www.alere.com/content/dam/alere/docs/guidelines/2010_ASM_guidelines_dated_9.21.10.pdf

55 **Ticehurst JR**, Aird DZ, Dam LM, Borek AP, Hargrove JT, Carroll KC. Effective detection of toxigenic Clostridium difficile by a two-step algorithm including tests for antigen and cytotoxin. *J Clin Microbiol* 2006; **44**: 1145-1149 [PMID: 16517916 DOI: 10.1128/JCM.44.3.1145-1149.2006]

56 **Planche T**, Aghaizu A, Holliman R, Riley P, Poloniecki J, Breathnach A, Krishna S. Diagnosis of Clostridium difficile infection by toxin detection kits: a systematic review. *Lancet Infect Dis* 2008; **8**: 777-784 [PMID: 18977696 DOI: 10.1016/S1473-3099(08)70233-0]

57 **Stelfox HT**, Bates DW, Redelmeier DA. Safety of patients isolated for infection control. *JAMA* 2003; **290**: 1899-1905 [PMID: 14532319 DOI: 10.1001/jama.290.14.1899]

58 **Wilkins TD**, Lyerly DM. Clostridium difficile testing: after 20 years, still challenging. *J Clin Microbiol* 2003; **41**: 531-534 [PMID: 12574241 DOI: 10.1128/JCM.41.2.531-534.2003]

59 **Peterson LR**, Robicsek A. Does my patient have Clostridium difficile infection? *Ann Intern Med* 2009; **151**: 176-179 [PMID: 19652187 DOI: 10.7326/0003-4819-151-3-200908040-00005]

60 **Tenover FC**, Novak-Weekley S, Woods CW, Peterson LR, Davis T, Schreckenberger P, Fang FC, Dascal A, Gerding DN, Nomura JH, Goering RV, Akerlund T, Weissfeld AS, Baron EJ, Wong E, Marlowe EM, Whitmore J, Persing DH. Impact of strain type on detection of toxigenic Clostridium difficile: comparison of molecular diagnostic and enzyme immunoassay approaches. *J Clin Microbiol* 2010; **48**: 3719-3724 [PMID: 20702676 DOI: 10.1128/JCM.00427-10]

61 **Goldenberg SD**, Cliff PR, Smith S, Milner M, French GL. Two-step glutamate dehydrogenase antigen real-time polymerase chain reaction assay for detection of toxigenic Clostridium difficile. *J Hosp Infect* 2010; **74**: 48-54 [PMID: 19900734 DOI: 10.1016/j.jhin.2009.08.014]

62 **Kvach EJ**, Ferguson D, Riska PF, Landry ML. Comparison of BD GeneOhm Cdiff real-time PCR assay with a two-step algorithm and a toxin A/B enzyme-linked immunosorbent assay for diagnosis of toxigenic Clostridium difficile infection. *J Clin Microbiol* 2010; **48**: 109-114 [PMID: 19864479 DOI: 10.1128/JCM.01630-09]

63 **O'Horo JC**, Jones A, Sternke M, Harper C, Safdar N. Molecular techniques for diagnosis of Clostridium difficile infection: systematic review and meta-analysis. *Mayo Clin Proc* 2012; **87**: 643-651 [PMID: 22766084 DOI: 10.1016/j.mayocp.2012.02.024]

64 **Longtin Y**, Trottier S, Brochu G, Paquet-Bolduc B, Garenc C, Loungnarath V, Beaulieu C, Goulet D, Longtin J. Impact of the type of diagnostic assay on Clostridium difficile infection and complication rates in a mandatory reporting program. *Clin Infect Dis* 2013; **56**: 67-73 [PMID: 23011147 DOI: 10.1093/cid/cis840]

65 **Fong KS**, Fatica C, Hall G, Procop G, Schindler S, Gordon SM, Fraser TG. Impact of PCR testing for Clostridium difficile on incident rates and potential on public reporting: is the playing field level? *Infect Control Hosp Epidemiol* 2011; **32**: 932-933 [PMID: 21828981 DOI: 10.1086/661789]

66 **Sethi AK**, Al-Nassir WN, Nerandzic MM, Bobulsky GS, Donskey CJ. Persistence of skin contamination and environmental shedding of Clostridium difficile during and after treatment of *C. difficile* infection. *Infect Control Hosp Epidemiol* 2010; **31**: 21-27 [PMID: 19929371 DOI: 10.1086/649016]

67 **Musher DM**, Stager C. Diagnosis of Clostridium difficile infection. *Clin Infect Dis* 2012; **54**: 1675-1676 [PMID: 22503848 DOI: 10.1093/cid/cis259]

68 **Bamber AI**, Fitzsimmons K, Cunniffe JG, Beasor CC, Mackintosh CA, Hobbs G. Diagnosis of Clostridium difficile-associated disease: examination of multiple algorithms using toxin EIA, glutamate dehydrogenase EIA and loop-mediated isothermal amplification. *Br J Biomed Sci* 2012; **69**: 112-118 [PMID: 23057158]

69 **Larson AM**, Fung AM, Fang FC. Evaluation of tcdB real-time PCR in a three-step diagnostic algorithm for detection of toxigenic Clostridium difficile. *J Clin Microbiol* 2010; **48**: 124-130 [PMID: 19923482 DOI: 10.1128/JCM.00734-09]

70 **Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infection (ARHAI).** Updated guidance on the diagnosis and reporting of Clostridium difficile (March 2012). Available from: URL: http://www.gov.uk/government/publications/updated-guidance-on-the-diagnosis-and-reporting-of-clostridium-difficile

71 **Surawicz CM**, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, McFarland LV, Mellow M, Zuckerbraun BS. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. *Am J Gastroenterol* 2013; **108**: 478-498; quiz 499 [PMID: 23439232 DOI: 10.1038/ajg.2013.4]

72 **Yassin SF**, Young-Fadok TM, Zein NN, Pardi DS. Clostridium difficile-associated diarrhea and colitis. *Mayo Clin Proc* 2001; **76**: 725-730 [PMID: 11444405 DOI: 10.4065/76.7.725]

73 **Renshaw AA**, Stelling JM, Doolittle MH. The lack of value of repeated Clostridium difficile cytotoxicity assays. *Arch Pathol Lab Med* 1996; **120**: 49-52 [PMID: 8554444]

74 **Aichinger E**, Schleck CD, Harmsen WS, Nyre LM, Patel R. Nonutility of repeat laboratory testing for detection of Clostridium difficile by use of PCR or enzyme immunoassay. *J Clin Microbiol* 2008; **46**: 3795-3797 [PMID: 18784320 DOI: 10.1128/JCM.00684-08]

75 **Mohan SS**, McDermott BP, Parchuri S, Cunha BA. Lack of value of repeat stool testing for Clostridium difficile toxin. *Am J Med* 2006; **119**: 356.e7-356.e8 [PMID: 16564786]

76 **Issack MI**, Elliott TS. Clostridium difficile carriage after infection. *Lancet* 1990; **335**: 610-611 [PMID: 1968608 DOI: 10.1016/0140-6736(90)90400-Y]

77 **Wilcox MH**, Planche T, Fang FC, Gilligan P. What is the current role of algorithmic approaches for diagnosis of Clostridium difficile infection? *J Clin Microbiol* 2010; **48**: 4347-4353 [PMID: 20980568 DOI: 10.1128/JCM.02028-10]

78 **Pepin J**. Improving the treatment of Clostridium difficile-associated disease: where should we start? *Clin Infect Dis* 2006; **43**: 553-555 [PMID: 16886145 DOI: 10.1086/506357]

79 **Nelson R**. Antibiotic treatment for Clostridium difficile – associated diarrhea in adults (Review). *Cochrane Database Systematic Review* 2011; **(9)**: CD004610 [DOI: 10.1002/14651858.CD004610.pub3]

80 **Nelson RL**, Kelsey P, Leeman H, Leeman H, Meardon N, Patel H, Paul K, Rees R, Taylor B, Wood E, Malakun R. Antibiotic treatment for Clostridium difficile – associated diarrhea in adults (Review). *Cochrane Database Systematic Review* 2007; **(3)**: CD004610 [DOI: 10.1002/14651858.CD004610.pub4]

81 **Aspevall O**, Lundberg A, Burman LG, Akerlund T, Svenungsson B. Antimicrobial susceptibility pattern of Clostridium difficile and its relation to PCR ribotypes in a Swedish university hospital. *Antimicrob Agents Chemother* 2006; **50**: 1890-1892 [PMID: 16641471 DOI: 10.1128/AAC.50.5.1890-1892.2006]

82 **Hecht DW**, Galang MA, Sambol SP, Osmolski JR, John-

son S, Gerding DN. *In vitro* activities of 15 antimicrobial agents against 110 toxigenic clostridium difficile clinical isolates collected from 1983 to 2004. *Antimicrob Agents Chemother* 2007; **51**: 2716-2719 [PMID: 17517836 DOI: 10.1128/AAC.01623-06]

83 **Bourgault AM**, Lamothe F, Loo VG, Poirier L. *In vitro* susceptibility of Clostridium difficile clinical isolates from a multi-institutional outbreak in Southern Québec, Canada. *Antimicrob Agents Chemother* 2006; **50**: 3473-3475 [PMID: 17005836 DOI: 10.1128/AAC.00479-06]

84 **Sebaihia M**, Wren BW, Mullany P, Fairweather NF, Minton N, Stabler R, Thomson NR, Roberts AP, Cerdeño-Tárraga AM, Wang H, Holden MT, Wright A, Churcher C, Quail MA, Baker S, Bason N, Brooks K, Chillingworth T, Cronin A, Davis P, Dowd L, Fraser A, Feltwell T, Hance Z, Holroyd S, Jagels K, Moule S, Mungall K, Price C, Rabbinowitsch E, Sharp S, Simmonds M, Stevens K, Unwin L, Whithead S, Dupuy B, Dougan G, Barrell B, Parkhill J. The multidrug-resistant human pathogen Clostridium difficile has a highly mobile, mosaic genome. *Nat Genet* 2006; **38**: 779-786 [PMID: 16804543 DOI: 10.1038/ng1830]

85 **Salgado CD**, Giannetta ET, Farr BM. Failure to develop vancomycin-resistant Enterococcus with oral vancomycin treatment of Clostridium difficile. *Infect Control Hosp Epidemiol* 2004; **25**: 413-417 [PMID: 15188848 DOI: 10.1086/502415]

86 Anon (edition. Red Book Online.). via Drugdex system [internet database]. Thompson Healthcare; 2011. Available from: URL: http://pharmacyservices.utah.edu/bulletins/NDB_239.pdf

87 **Ensom MH**, Decarie D, Lakhani A. Stability of Vancomycin 25 mg/mL in Ora-Sweet and Water in Unit-Dose Cups and Plastic Bottles at 4°C and 25°C. *Can J Hosp Pharm* 2010; **63**: 366-372 [PMID: 22479004]

88 **Teasley DG**, Gerding DN, Olson MM, Peterson LR, Gebhard RL, Schwartz MJ, Lee JT. Prospective randomised trial of metronidazole versus vancomycin for Clostridium-difficile-associated diarrhoea and colitis. *Lancet* 1983; **2**: 1043-1046 [PMID: 6138597 DOI: 10.1016/S0140-6736(83)91036-X]

89 **Wenisch C**, Parschalk B, Hasenhündl M, Hirschl AM, Graninger W. Comparison of vancomycin, teicoplanin, metronidazole, and fusidic acid for the treatment of Clostridium difficile-associated diarrhea. *Clin Infect Dis* 1996; **22**: 813-818 [PMID: 8722937 DOI: 10.1093/clinids/22.5.813]

90 **Johnson S**, Homann SR, Bettin KM, Quick JN, Clabots CR, Peterson LR, Gerding DN. Treatment of asymptomatic Clostridium difficile carriers (fecal excretors) with vancomycin or metronidazole. A randomized, placebo-controlled trial. *Ann Intern Med* 1992; **117**: 297-302 [PMID: 1322075 DOI: 10.7326/0003-4819-117-4-297]

91 **Peláez T**, Alcalá L, Alonso R, Rodríguez-Créixems M, García-Lechuz JM, Bouza E. Reassessment of Clostridium difficile susceptibility to metronidazole and vancomycin. *Antimicrob Agents Chemother* 2002; **46**: 1647-1650 [PMID: 12019070 DOI: 10.1128/AAC.46.6.1647-1650.2002]

92 **Musher DM**, Aslam S, Logan N, Nallacheru S, Bhaila I, Borchert F, Hamill RJ. Relatively poor outcome after treatment of Clostridium difficile colitis with metronidazole. *Clin Infect Dis* 2005; **40**: 1586-1590 [PMID: 15889354 DOI: 10.1086/430311]

93 **Odenholt I**, Walder M, Wullt M. Pharmacodynamic studies of vancomycin, metronidazole and fusidic acid against Clostridium difficile. *Chemotherapy* 2007; **53**: 267-274 [PMID: 17595541 DOI: 10.1159/000104471]

94 **Al-Nassir WN**, Sethi A, Bobulsky G, Riggs M, Donskey C. Metronidazole treatment failure in Clostridium difficile – associated disease is associated with suboptimal microbiologic response. 45th Annual Infectious Diseases Society of America, October 2007. LB-7: 64. Available from: URL: http://idsa.confex.com/idsa/2007/webprogram/Paper24530.html

95 **Freeman J**, Baines SD, Saxton K, Wilcox MH. Effect of metronidazole on growth and toxin production by epidemic Clostridium difficile PCR ribotypes 001 and 027 in a human gut model. *J Antimicrob Chemother* 2007; **60**: 83-91 [PMID: 17483547 DOI: 10.1093/jac/dkm113]

96 **Freeman J**, Baines SD, Jabes D, Wilcox MH. Comparison of the efficacy of ramoplanin and vancomycin in both *in vitro* and in vivo models of clindamycin-induced Clostridium difficile infection. *J Antimicrob Chemother* 2005; **56**: 717-725 [PMID: 16143709 DOI: 10.1093/jac/dki321]

97 **Nagy E**, Földes J. Inactivation of metronidazole by Enterococcus faecalis. *J Antimicrob Chemother* 1991; **27**: 63-70 [PMID: 1904851 DOI: 10.1093/jac/27.1.63]

98 **Modena S**, Gollamudi S, Friedenberg F. Continuation of antibiotics is associated with failure of metronidazole for Clostridium difficile-associated diarrhea. *J Clin Gastroenterol* 2006; **40**: 49-54 [PMID: 16340634 DOI: 10.1097/01.mcg.0000190761.80615.0f]

99 **Ensminger PW**, Counter FT, Thomas LJ, Lubbehusen PP. Susceptibility, resistance development, and synergy of antimicrobial combinations against Clostridium difficile . *Current Microbiology* 1982; **7**: 59-62 [DOI: 10.1007/BF01570982]

100 **Lagrotteria D**, Holmes S, Smieja M, Smaill F, Lee C. Prospective, randomized inpatient study of oral metronidazole versus oral metronidazole and rifampin for treatment of primary episode of Clostridium difficile-associated diarrhea. *Clin Infect Dis* 2006; **43**: 547-552 [PMID: 16886144 DOI: 10.1086/506354]

101 **Pépin J**, Valiquette L, Gagnon S, Routhier S, Brazeau I. Outcomes of Clostridium difficile-associated disease treated with metronidazole or vancomycin before and after the emergence of NAP1/027. *Am J Gastroenterol* 2007; **102**: 2781-2788 [PMID: 17900327 DOI: 10.1111/j.1572-0241.2007.01539.x]

102 **Zar FA**, Bakkanagari SR, Moorthi KM, Davis MB. A comparison of vancomycin and metronidazole for the treatment of Clostridium difficile-associated diarrhea, stratified by disease severity. *Clin Infect Dis* 2007; **45**: 302-307 [PMID: 17599306 DOI: 10.1086/519265]

103 **Bishara J**, Wattad M, Paul M. Vancomycin and metronidazole for the treatment of Clostridium difficile-associated diarrhea. *Clin Infect Dis* 2007; **45**: 1646-1667; author reply 1649-1651 [PMID: 18190329 DOI: 10.1086/523720]

104 **Fujitani S**, George WL, Murthy AR. Comparison of clinical severity score indices for Clostridium difficile infection. *Infect Control Hosp Epidemiol* 2011; **32**: 220-228 [PMID: 21460506 DOI: 10.1086/658336]

105 **Adachi JA**, DuPont HL. Rifaximin: a novel nonabsorbed rifamycin for gastrointestinal disorders. *Clin Infect Dis* 2006; **42**: 541-547 [PMID: 16421799 DOI: 10.1086/499950]

106 **Rubin DT**, Sohi S, Glathar M, Thomas T, Yadron N, Surma BL. Rifaximin Is Effective for the Treatment of Clostridium difficile-Associated Diarrhea: Results of an Open-Label Pilot Study. *Gastroenterol Res Pract* 2011; **2011**: 106978 [PMID: 22114587 DOI: 10.1155/2011/106978]

107 **Boero M**, Berti E, Morgando A, Verme G. Treatment of colitis caused by Clostridium difficile: results of a randomized open study of rifaximin vs. vancomycin. *Microbiologia Medica* 1990; **5**: 74-77

108 **O'Connor JR**, Galang MA, Sambol SP, Hecht DW, Vedantam G, Gerding DN, Johnson S. Rifampin and rifaximin resistance in clinical isolates of Clostridium difficile. *Antimicrob Agents Chemother* 2008; **52**: 2813-2817 [PMID: 18559647 DOI: 10.1128/AAC.00342-08]

109 **Fox LM**, Saravolatz LD. Nitazoxanide: a new thiazolide antiparasitic agent. *Clin Infect Dis* 2005; **40**: 1173-1180 [PMID: 15791519 DOI: 10.1086/428839]

110 **Dubreuil L**, Houcke I, Mouton Y, Rossignol JF. *In vitro* evaluation of activities of nitazoxanide and tizoxanide against anaerobes and aerobic organisms. *Antimicrob Agents Chemother* 1996; **40**: 2266-2270 [PMID: 8891127]

111 **McVay CS**, Rolfe RD. *In vitro* and in vivo activities of ni-

tazoxanide against Clostridium difficile. *Antimicrob Agents Chemother* 2000; **44**: 2254-2258 [PMID: 10952564 DOI: 10.1128/AAC.44.9.2254-2258.2000]
112 **Freeman J**, Baines SD, Todhunter SL, Huscroft GS, Wilcox MH. Nitazoxanide is active against Clostridium difficile strains with reduced susceptibility to metronidazole. *J Antimicrob Chemother* 2011; **66**: 1407-1408 [DOI: 10.1093/jac/dkr077]
113 **Musher DM**, Logan N, Hamill RJ, Dupont HL, Lentnek A, Gupta A, Rossignol JF. Nitazoxanide for the treatment of Clostridium difficile colitis. *Clin Infect Dis* 2006; **43**: 421-427 [PMID: 16838229 DOI: 10.1086/506351]
114 **Musher DM**, Logan N, Mehendiratta V, Melgarejo NA, Garud S, Hamill RJ. Clostridium difficile colitis that fails conventional metronidazole therapy: response to nitazoxanide. *J Antimicrob Chemother* 2007; **59**: 705-710 [PMID: 17337513 DOI: 10.1093/jac/dkl553]
115 **Musher DM**, Logan N, Bressler AM, Johnson DP, Rossignol JF. Nitazoxanide versus vancomycin in Clostridium difficile infection: a randomized, double-blind study. *Clin Infect Dis* 2009; **48**: e41-e46 [PMID: 19133801 DOI: 10.1086/596552]
116 **Johnson AP**. Drug evaluation: OPT-80, a narrow-spectrum macrocyclic antibiotic. *Curr Opin Investig Drugs* 2007; **8**: 168-173 [PMID: 17328233]
117 **Weiss K**, Allgren RL, Sellers S. Safety analysis of fidaxomicin in comparison with oral vancomycin for Clostridium difficile infections. *Clin Infect Dis* 2012; **55** Suppl 2: S110-S115 [PMID: 22752858 DOI: 10.1093/cid/cis390]
118 **Sears P**, Crook DW, Louie TJ, Miller MA, Weiss K. Fidaxomicin attains high fecal concentrations with minimal plasma concentrations following oral administration in patients with Clostridium difficile infection. *Clin Infect Dis* 2012; **55** Suppl 2: S116-S120 [PMID: 22752859 DOI: 10.1093/cid/cis337]
119 **Venugopal AA**, Johnson S. Fidaxomicin: a novel macrocyclic antibiotic approved for treatment of Clostridium difficile infection. *Clin Infect Dis* 2012; **54**: 568-574 [PMID: 22156854 DOI: 10.1093/cid/cir830]
120 **Louie TJ**, Cannon K, Byrne B, Emery J, Ward L, Eyben M, Krulicki W. Fidaxomicin preserves the intestinal microbiome during and after treatment of Clostridium difficile infection (CDI) and reduces both toxin reexpression and recurrence of CDI. *Clin Infect Dis* 2012; **55** Suppl 2: S132-S142 [PMID: 22752862 DOI: 10.1093/cid/cis338]
121 **Tannock GW**, Munro K, Taylor C, Lawley B, Young W, Byrne B, Emery J, Louie T. A new macrocyclic antibiotic, fidaxomicin (OPT-80), causes less alteration to the bowel microbiota of Clostridium difficile-infected patients than does vancomycin. *Microbiology* 2010; **156**: 3354-3359 [PMID: 20724385 DOI: 10.1099/mic.0.042010-0]
122 **Nerandzic MM**, Mullane K, Miller MA, Babakhani F, Donskey CJ. Reduced acquisition and overgrowth of vancomycin-resistant enterococci and Candida species in patients treated with fidaxomicin versus vancomycin for Clostridium difficile infection. *Clin Infect Dis* 2012; **55** Suppl 2: S121-S126 [PMID: 22752860 DOI: 10.1093/cid/cis440]
123 **Louie TJ**, Miller MA, Mullane KM, Weiss K, Lentnek A, Golan Y, Gorbach S, Sears P, Shue YK. Fidaxomicin versus vancomycin for Clostridium difficile infection. *N Engl J Med* 2011; **364**: 422-431 [PMID: 21288078 DOI: 10.1056/NEJMoa0910812]
124 **Cornley OA**, Crook DW, Esposio R, Poirier A, Somero MS, Weiss K, Sears P, Gorbach S. Fidaxomycin versus vancomycin for infection with Clostridium difficile in Europe, Canada, and the USA: a double-blind, non-inferiority, randomized control trial. *Lancet Infect Dis* 2012; **12**: 281-289 [DOI: 10.1016/S1473-3099(11)70374-7]
125 **Crook DW**, Walker AS, Kean Y, Weiss K, Cornely OA, Miller MA, Esposito R, Louie TJ, Stoesser NE, Young BC, Angus BJ, Gorbach SL, Peto TE. Fidaxomicin versus vancomycin for Clostridium difficile infection: meta-analysis of pivotal randomized controlled trials. *Clin Infect Dis* 2012; **55** Suppl 2: S93-103 [PMID: 22752871 DOI: 10.1093/cid/cis499]
126 **Baird DR**. Comparison of two oral formulations of vancomycin for treatment of diarrhoea associated with Clostridium difficile. *J Antimicrob Chemother* 1989; **23**: 167-169 [PMID: 2745253 DOI: 10.1093/jac/23.1.167]
127 **Owens RC**. Clostridium difficile-associated disease: changing epidemiology and implications for management. *Drugs* 2007; **67**: 487-502 [PMID: 17352510 DOI: 10.2165/00003495-200767040-00001]
128 **Apisarnthanarak A**, Razavi B, Mundy LM. Adjunctive intracolonic vancomycin for severe Clostridium difficile colitis: case series and review of the literature. *Clin Infect Dis* 2002; **35**: 690-696 [PMID: 12203166 DOI: 10.1086/342334]
129 **Baines SD**, Saxton K, Freeman J, Wilcox MH. Tigecycline does not induce proliferation or cytotoxin production by epidemic Clostridium difficile strains in a human gut model. *J Antimicrob Chemother* 2006; **58**: 1062-1065 [PMID: 17030519 DOI: 10.1093/jac/dkl364]
130 **Jump RL**, Li Y, Pultz MJ, Kypriotakis G, Donskey CJ. Tigecycline exhibits inhibitory activity against Clostridium difficile in the colon of mice and does not promote growth or toxin production. *Antimicrob Agents Chemother* 2011; **55**: 546-549 [PMID: 21135181 DOI: 10.1128/AAC.00839-10]
131 **Larson KC**, Belliveau PP, Spooner LM. Tigecycline for the treatment of severe Clostridium difficile infection. *Ann Pharmacother* 2011; **45**: 1005-1010 [PMID: 21730279 DOI: 10.1345/aph.1Q080]
132 **El-Herte RI**, Baban TA, Kanj SS. Recurrent refractory Clostridium difficile colitis treated successfully with rifaximin and tigecycline: a case report and review of the literature. *Scand J Infect Dis* 2012; **44**: 228-230 [PMID: 22077098 DOI: 10.3109/00365548.2011.616224]
133 **Lipsett PA**, Samantaray DK, Tam ML, Bartlett JG, Lillemoe KD. Pseudomembranous colitis: a surgical disease? *Surgery* 1994; **116**: 491-496 [PMID: 8079179]
134 **Synnott K**, Mealy K, Merry C, Kyne L, Keane C, Quill R. Timing of surgery for fulminating pseudomembranous colitis. *Br J Surg* 1998; **85**: 229-231 [PMID: 9501823 DOI: 10.1046/j.1365-2168.1998.00519.x]
135 **Medich DS**, Lee KK, Simmons RL, Grubbs PE, Yang HC, Showalter DP. Laparotomy for fulminant pseudomembranous colitis. *Arch Surg* 1992; **127**: 847-852; discussion 852-853 [PMID: 1524485 DOI: 10.1001/archsurg.1992.01420070111020]
136 **Koss K**, Clark MA, Sanders DS, Morton D, Keighley MR, Goh J. The outcome of surgery in fulminant Clostridium difficile colitis. *Colorectal Dis* 2006; **8**: 149-154 [PMID: 16412077 DOI: 10.1111/j.1463-1318.2005.00876.x]
137 **Longo WE**, Mazuski JE, Virgo KS, Lee P, Bahadursingh AN, Johnson FE. Outcome after colectomy for Clostridium difficile colitis. *Dis Colon Rectum* 2004; **47**: 1620-1626 [PMID: 15540290 DOI: 10.1007/s10350-004-0672-2]
138 **Lamontagne F**, Labbé AC, Haeck O, Lesur O, Lalancette M, Patino C, Leblanc M, Laverdière M, Pépin J. Impact of emergency colectomy on survival of patients with fulminant Clostridium difficile colitis during an epidemic caused by a hypervirulent strain. *Ann Surg* 2007; **245**: 267-272 [PMID: 17245181 DOI: 10.1097/01.sla.0000236628.79550.e5]
139 **Neal MD**, Alverdy JC, Hall DE, Simmons RL, Zuckerbraun BS. Diverting loop ileostomy and colonic lavage: an alternative to total abdominal colectomy for the treatment of severe, complicated Clostridium difficile associated disease. *Ann Surg* 2011; **254**: 423-427; discussion 427-429; [PMID: 21865943 DOI: 10.1097/SLA.0b013e31822ade48]
140 **Butala P**, Divino CM. Surgical aspects of fulminant Clostridium difficile colitis. *Am J Surg* 2010; **200**: 131-135 [PMID: 20409527 DOI: 10.1016/j.amjsurg.2009.07.040]
141 **Fekety R**, McFarland LV, Surawicz CM, Greenberg RN, Elmer GW, Mulligan ME. Recurrent Clostridium difficile diar-

rhea: characteristics of and risk factors for patients enrolled in a prospective, randomized, double-blind trial. *Clin Infect Dis* 1997; **24**: 324-333 [DOI: 10.1093/clinids/24.3.324]
142 **Norén T**, Akerlund T, Bäck E, Sjöberg L, Persson I, Alriksson I, Burman LG. Molecular epidemiology of hospital-associated and community-acquired Clostridium difficile infection in a Swedish county. *J Clin Microbiol* 2004; **42**: 3635-3643 [PMID: 15297509 DOI: 10.1128/JCM.42.8.3635-3643.2004]
143 **Pepin J**, Alary ME, Valiquette L, Raiche E, Ruel J, Fulop K, Godin D, Bourassa C. Increasing risk of relapse after treatment of Clostridium difficile colitis in Quebec, Canada. *Clin Infect Dis* 2005; **40**: 1591-1597 [PMID: 15889355 DOI: 10.1086/430315]
144 **Pépin J**, Routhier S, Gagnon S, Brazeau I. Management and outcomes of a first recurrence of Clostridium difficile-associated disease in Quebec, Canada. *Clin Infect Dis* 2006; **42**: 758-764 [PMID: 16477549 DOI: 10.1086/501126]
145 **Musher DM**, Nuila F, Logan N. The long-term outcome of treatment of Clostridium difficile colitis. *Clin Infect Dis* 2007; **45**: 523-524 [PMID: 17638211 DOI: 10.1086/520008]
146 **Tedesco F**, Markham R, Gurwith M, Christie D, Bartlett JG. Oral vancomycin for antibiotic-associated pseudomembranous colitis. *Lancet* 1978; **2**: 226-228 [PMID: 79026 DOI: 10.1016/S0140-6736(78)91741-5]
147 **Tedesco FJ**, Gordon D, Fortson WC. Approach to patients with multiple relapses of antibiotic-associated pseudomembranous colitis. *Am J Gastroenterol* 1985; **80**: 867-868 [PMID: 4050760]
148 **McFarland LV**, Elmer GW, Surawicz CM. Breaking the cycle: treatment strategies for 163 cases of recurrent Clostridium difficile disease. *Am J Gastroenterol* 2002; **97**: 1769-1775 [PMID: 12135033 DOI: 10.1111/j.1572-0241.2002.05839.x]
149 **Bartlett JG**, Gupta A. Clostridium difficile infection: state of the field with John G. Bartlett and Amita Gupta. Available from: URL: http://www.medscape.com/viewarticle/568043
150 **Surawicz CM**. Treatment of recurrent Clostridium difficile-associated disease. *Nat Clin Pract Gastroenterol Hepatol* 2004; **1**: 32-38 [PMID: 16265042 DOI: 10.1038/ncpgasthep0018]
151 **Johnson S**, Schriever C, Galang M, Kelly CP, Gerding DN. Interruption of recurrent Clostridium difficile-associated diarrhea episodes by serial therapy with vancomycin and rifaximin. *Clin Infect Dis* 2007; **44**: 846-848 [PMID: 17304459 DOI: 10.1086/511870]
152 **Garey KW**, Ghantoji SS, Shah DN, Habib M, Arora V, Jiang ZD, DuPont HL. A randomized, double-blind, placebo-controlled pilot study to assess the ability of rifaximin to prevent recurrent diarrhoea in patients with Clostridium difficile infection. *J Antimicrob Chemother* 2011; **66**: 2850-2855 [PMID: 21948965 DOI: 10.1093/jac/dkr377]
153 **Mattila E**, Arkkila P, Mattila PS, Tarkka E, Tissari P, Anttila VJ. Rifaximin in the treatment of recurrent Clostridium difficile infection. *Aliment Pharmacol Ther* 2013; **37**: 122-128 [PMID: 23095030 DOI: 10.1111/apt.12111]
154 **Brown EL**, Xue Q, Jiang ZD, Xu Y, Dupont HL. Pretreatment of epithelial cells with rifaximin alters bacterial attachment and internalization profiles. *Antimicrob Agents Chemother* 2010; **54**: 388-396 [PMID: 19858255 DOI: 10.1128/AAC.00691-09]
155 **Borody TJ**, Warren EF, Leis SM, Surace R, Ashman O, Siarakas S. Bacteriotherapy using fecal flora: toying with human motions. *J Clin Gastroenterol* 2004; **38**: 475-483 [PMID: 15220681 DOI: 10.1097/01.mcg.0000128988.13808.dc]
156 **Guo B**, Harstall C, Louie T, Veldhuyzen van Zanten S, Dieleman LA. Systematic review: faecal transplantation for the treatment of Clostridium difficile-associated disease. *Aliment Pharmacol Ther* 2012; **35**: 865-875 [PMID: 22360412 DOI: 10.1111/j.1365-2036.2012.05033.x]
157 **Brandt LJ**, Aroniadis OC, Mellow M, Kanatzar A, Kelly C, Park T, Stollman N, Rohlke F, Surawicz C. Long-term follow-up of colonoscopic fecal microbiota transplant for recurrent Clostridium difficile infection. *Am J Gastroenterol* 2012; **107**: 1079-1087 [PMID: 22450732 DOI: 10.1038/ajg.2012.60]
158 **van Nood E**, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JF, Tijssen JG, Speelman P, Dijkgraaf MG, Keller JJ. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med* 2013; **368**: 407-415 [PMID: 23323867 DOI: 10.1056/NEJMoa1205037]
159 **Jorup-Rönström C**, Håkanson A, Sandell S, Edvinsson O, Midtvedt T, Persson AK, Norin E. Fecal transplant against relapsing Clostridium difficile-associated diarrhea in 32 patients. *Scand J Gastroenterol* 2012; **47**: 548-552 [PMID: 22468996 DOI: 10.3109/00365521.2012.672587]
160 **Petrof EO**, Gloor GB, Vanner SJ, Weese SJ, Carter D, Daigneault MC, Brown EM, Schroeter K, Allen-Vercoe E. Stool substitute transplant therapy for the eradication of Clostridium difficile infection: 'RePOOPulating' the gut. *Microbiome* 2013; **1**: 1-12
161 **Kassam Z**, Lee CH, Yuan Y, Hunt RH. Fecal microbiota transplantation for Clostridium difficile infection: systematic review and meta-analysis. *Am J Gastroenterol* 2013; **108**: 500-508 [PMID: 23511459 DOI: 10.1038/ajg.2013.59]
162 Available from: URL: http://www.fda.gov/BiologicsBloodVaccines/NewsEvents/WorkshopsMeetingsConferences/ucm357258.htm. Accessed June 2013
163 **Kyne L**, Warny M, Qamar A, Kelly CP. Association between antibody response to toxin A and protection against recurrent Clostridium difficile diarrhoea. *Lancet* 2001; **357**: 189-193 [PMID: 11213096 DOI: 10.1016/S0140-6736(00)03592-3]
164 **Wilcox MH**. Descriptive study of intravenous immunoglobulin for the treatment of recurrent Clostridium difficile diarrhoea. *J Antimicrob Chemother* 2004; **53**: 882-884 [PMID: 15073160 DOI: 10.1093/jac/dkh176]
165 **Salcedo J**, Keates S, Pothoulakis C, Warny M, Castagliuolo I, LaMont JT, Kelly CP. Intravenous immunoglobulin therapy for severe Clostridium difficile colitis. *Gut* 1997; **41**: 366-370 [PMID: 9378393 DOI: 10.1136/gut.41.3.366]
166 **Johnson S**. Antibody responses to clostridial infection in humans. *Clin Infect Dis* 1997; **25** Suppl 2: S173-S177 [PMID: 9310668]
167 **McPherson S**, Rees CJ, Ellis R, Soo S, Panter SJ. Intravenous immunoglobulin for the treatment of severe, refractory, and recurrent Clostridium difficile diarrhea. *Dis Colon Rectum* 2006; **49**: 640-645 [PMID: 16525744 DOI: 10.1007/s10350-006-0511-8]
168 **Juang P**, Skledar SJ, Zgheib NK, Paterson DL, Vergis EN, Shannon WD, Ansani NT, Branch RA. Clinical outcomes of intravenous immune globulin in severe clostridium difficile-associated diarrhea. *Am J Infect Control* 2007; **35**: 131-137 [PMID: 17327194 DOI: 10.1016/j.ajic.2006.06.007]
169 **Kreutzer EW**, Milligan FD. Treatment of antibiotic-associated pseudomembranous colitis with cholestyramine resin. *Johns Hopkins Med J* 1978; **143**: 67-72 [PMID: 691920]
170 **Taylor NS**, Bartlett JG. Binding of Clostridium difficile cytotoxin and vancomycin by anion-exchange resins. *J Infect Dis* 1980; **141**: 92-97 [PMID: 7365273 DOI: 10.1093/infdis/141.1.92]
171 Drugs Facts and Comparisons. 46th ed. St. Louis: Walters Kluwer Co, 1992
172 **Miller MA**. Clinical management of Clostridium difficile-associated disease. *Clin Infect Dis* 2007; **45** Suppl 2: S122-S128 [PMID: 17683016]
173 **Hoffman FA**, Heimbach JT, Sanders ME, Hibberd PL. Executive summary: scientific and regulatory challenges of development of probiotics as foods and drugs. *Clin Infect Dis* 2008; **46** Suppl 2: S53-S57 [PMID: 18181723 DOI: 10.1086/523342]
174 **Reddy S**. Probiotics' benefits may be more than a gut feeling (Wall Street J 26 Nov 2012). Available from: URL: http://online.wsj.com/article/SB10001424127887324784404

578143402702878118.html accessed 22 March 2013.
175 **Watson K**. Digesting the category performance of probiotics foods and supplements. SPINS, LLC. Available from: URL: http://www.spins.com/sites/default/files/Probiotics_Growth.pdf
176 Watson E. US per capita spending on probiotic supplements expected to nearly double by 2016. Available from: URL: http://www.nutraingredients-usa.com/Consumer-Trends/US-per-capita-spending-on-probiotic-supplements-expected-to-nearly-double-by-2016
177 **Hempel S**, Newberry SJ, Maher AR, Wang Z, Miles JN, Shanman R, Johnsen B, Shekelle PG. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. *JAMA* 2012; **307**: 1959-1969 [PMID: 22570464 DOI: 10.1001/jama.2012.3507]
178 **Videlock EJ**, Cremonini F. Meta-analysis: probiotics in antibiotic-associated diarrhoea. *Aliment Pharmacol Ther* 2012; **35**: 1355-1369 [PMID: 22531096 DOI: 10.1111/j.1365-2036.2012.05104.x]
179 **Pothoulakis C**, Kelly CP, Joshi MA, Gao N, O'Keane, Castagliuolo I, LaMont JT. Saccharomyces boulardii inhibits Clostridium difficile toxin A binding and enterotoxicity in rat ileum. *Gastroenterol* 1993; **104**: 1108-1115
180 **Castagliuolo I**, Riegler MF, Valenick L, LaMont JT, Pothoulakis C. Saccharomyces boulardii protease inhibits the effects of Clostridium difficile toxins A and B in human colonic mucosa. *Infect Immun* 1999; **67**: 302-307
181 **Toothaker RD**, Elmer GW. Prevention of clindamycin-induced mortality in hamsters by Saccharomyces boulardii. *Antimicrob Agents Chemother* 1984; **26**: 552-556 [PMID: 6517545 DOI: 10.1128/AAC.26.4.552]
182 **Czerucka D**, Piche T, Rampal P. Review article: yeast as probiotics -- Saccharomyces boulardii. *Aliment Pharmacol Ther* 2007; **26**: 767-778 [PMID: 17767461 DOI: 10.1111/j.1365-2036.2007.03442.x]
183 **Dalmasso G**, Cottrez F, Imbert V, Lagadec P, Peyron JF, Rampal P, Czerucka D, Groux H, Foussat A, Brun V. Saccharomyces boulardii inhibits inflammatory bowel disease by trapping T cells in mesenteric lymph nodes. *Gastroenterology* 2006; **131**: 1812-1825 [PMID: 17087945 DOI: 10.1053/j.gastro.2006.10.001]
184 **Tasteyre A**, Barc MC, Karjalainen T, Bourlioux P, Collignon A. Inhibition of *in vitro* cell adherence of Clostridium difficile by Saccharomyces boulardii. *Microb Pathog* 2002; **32**: 219-225 [PMID: 12071678 DOI: 10.1006/mpat.2002.0495]
185 **Dendukuri N**, Costa V, McGregor M, Brophy JM. Probiotic therapy for the prevention and treatment of Clostridium difficile-associated diarrhea: a systematic review. *CMAJ* 2005; **173**: 167-170 [PMID: 16027434 DOI: 10.1503/cmaj.050350]
186 **Szajewska H**, Mrukowicz J. Meta-analysis: non-pathogenic yeast Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea. *Aliment Pharmacol Ther* 2005; **22**: 365-372 [PMID: 16128673 DOI: 10.1111/j.1365-2036.2005.02624.x]
187 **Katz JA**. Probiotics for the prevention of antibiotic-associated diarrhea and Clostridium difficile diarrhea. *J Clin Gastroenterol* 2006; **40**: 249-255 [PMID: 16633131 DOI: 10.1097/00004836-200603000-00017]
188 **McFarland LV**, Surawicz CM, Greenberg RN, Fekety R, Elmer GW, Moyer KA, Melcher SA, Bowen KE, Cox JL, Noorani Z. A randomized placebo-controlled trial of Saccharomyces boulardii in combination with standard antibiotics for Clostridium difficile disease. *JAMA* 1994; **271**: 1913-1918 [PMID: 8201735 DOI: 10.1001/jama.1994.03510480037031]
189 **Surawicz CM**, McFarland LV, Greenberg RN, Rubin M, Fekety R, Mulligan ME, Garcia RJ, Brandmarker S, Bowen K, Borjal D, Elmer GW. The search for a better treatment for recurrent Clostridium difficile disease: use of high-dose vancomycin combined with Saccharomyces boulardii. *Clin Infect Dis* 2000; **31**: 1012-1017 [PMID: 11049785 DOI: 10.1086/318130]
190 **McFarland LV**. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease. *Am J Gastroenterol* 2006; **101**: 812-822 [PMID: 16635227 DOI: 10.1111/j.1572-0241.2006.00465.x]
191 **Gerding EN**, Muto CA, Owens, Jr. RC. Treatment of Clostridium difficile infection. *Clin Infect Dis* 2008; **46**: S32-S42
192 **Enache-Angoulvant A**, Hennequin C. Invasive Saccharomyces infection: a comprehensive review. *Clin Infect Dis* 2005; **41**: 1559-1568 [PMID: 16267727 DOI: 10.1086/497832]
193 **Segarra-Newnham M**. Probiotics for Clostridium difficile-associated diarrhea: focus on *Lactobacillus* rhamnosus GG and Saccharomyces boulardii. *Ann Pharmacother* 2007; **41**: 1212-1221 [PMID: 17595306 DOI: 10.1345/aph.1K110]
194 **Besselink MG**, van Santvoort HC, Buskens E, Boermeester MA, van Goor H, Timmerman HM, Nieuwenhuijs VB, Bollen TL, van Ramshorst B, Witteman BJ,Rosman C, Ploeg RJ, Brink MA, Schaapherder AF, Dejong CH, Wahab PJ, van Laarhoven CJ, van der Harst E, van Eijck CH, Cuesta MA, Akkermans LM,Gooszen HG. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomized, double-blind, placebo-controlled trial. *Lancet* 2008; **371**: 651-659 [DOI: 10.1016/S0140-6736(08)60207-X]
195 **Hickson M**, D'Souza AL, Muthu N, Rogers TR, Want S, Rajkumar C, Bulpitt CJ. Use of probiotic Lactobacillus preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. *BMJ* 2007; **335**: 80 [PMID: 17604300 DOI: 10.1136/bmj.39231.599815.55]
196 **Billyard T**. Probiotics and diarrhea: No high risk antibiotics? *BMJ* 2007; **335**: 171 [PMID: 17656513]
197 **Pillai A**, Nelson R. Probiotics for treatment of Clostridium difficile-associated colitis in adults. *Cochrane Database Syst Rev* 2008;**(1)**: CD004611 [PMID: 18254055 DOI: 10.1002/14651858.CD004611]
198 **Johnston BC**, Ma SS, Goldenberg JZ, Thorlund K, Vandvik PO, Loeb M, Guyatt GH. Probiotics for the prevention of Clostridium difficile-associated diarrhea: a systematic review and meta-analysis. *Ann Intern Med* 2012; **157**: 878-888 [PMID: 23362517 DOI: 10.7326/0003-4819-157-12-201212180-00563]
199 **Allen SJ**, Wareham K, Wang D, Bradley C, Hutchings H, Harris W, Dhar A, Brown H, Foden A, Gravenor MB, Mack D. Lactobacilli and bifidobactria in the prevention of antibiotic-associated diarrhoea and Clostridium difficile diarrhoea in older inpatients (PLACIDE): a randomized, double-blind, placebo controlled, multicenter trial. *The Lancet* 2013; **382**: 1249-1257 [DOI: 10.1016/S0140-6736(13)61218-0]
200 **Lawrence SJ**, Korzenik JR, Mundy LM. Probiotics for recurrent Clostridium difficile disease. *J Med Microbiol* 2005; **54**: 905-906 [PMID: 16091446 DOI: 10.1099/jmm.0.46096-0]
201 **Coeuret V**, Gueguen M, Vernoux JP. Numbers and strains of lactobacilli in some probiotic products. *Int J Food Microbiol* 2004; **97**: 147-156 [PMID: 15541801 DOI: 10.1016/j.ijfoodmicro.2004.04.015]
202 **Howerton A**, Ramierz N, Abel-Santos E. Mapping interactions between germinants and Clostridium difficile spores. *J Bacteriol* 2010; **190**: 2505-2512
203 **Howerton A**, Patra M, Abel-Santos E. A new strategy for the prevention of Clostridium difficile infection. *J Infect Dis* 2013; **207**: 1498-1504 [PMID: 23420906]
204 **Anton PM**, O'Brien M, Kokkotou E, Eisenstein B, Michaelis A, Rothstein D, Paraschos S, Kelly CP, Pothoulakis C. Rifalazil treats and prevents relapse of clostridium difficile-associated diarrhea in hamsters. *Antimicrob Agents Chemother* 2004; **48**: 3975-3979 [PMID: 15388461 DOI: 10.1128/AAC.48.10.3975-3979.2004]
205 **Peláez T**, Alcalá L, Alonso R, Martín-López A, García-Arias V, Marín M, Bouza E. *In vitro* activity of ramoplanin against Clostridium difficile, including strains with reduced susceptibility to vancomycin or with resistance to metronidazole.

Antimicrob Agents Chemother 2005; **49**: 1157-1159 [PMID: 15728918 DOI: 10.1128/AAC.49.3.1157-1159.2005]

206 **Rayman MK**, Aris B, Hurst A. Nisin: a possible alternative or adjunct to nitrite in the preservation of meats. *Appl Environ Microbiol* 1981; **41**: 375-380 [PMID: 7195188]

207 **Critchley IA**, Green LS, Young CL, Bullard JM, Evans RJ, Price M, Jarvis TC, Guiles JW, Janjic N, Ochsner UA. Spectrum of activity and mode of action of REP3123, a new antibiotic to treat Clostridium difficile infections. *J Antimicrob Chemother* 2009; **63**: 954-963 [PMID: 19258353 DOI: 10.1093/jac/dkp041]

208 **Citron DM**, Warren YA, Tyrrell KL, Merriam V, Goldstein EJ. Comparative *in vitro* activity of REP3123 against Clostridium difficile and other anaerobic intestinal bacteria. *J Antimicrob Chemother* 2009; **63**: 972-976 [PMID: 19240076 DOI: 10.1093/jac/dkp037]

209 **Barker RH**, Dagher R, Davidson DM, Marquis JK. Review article: tolevamer, a novel toxin-binding polymer: overview of preclinical pharmacology and physicochemical properties. *Aliment Pharmacol Ther* 2006; **24**: 1525-1534 [PMID: 17206941 DOI: 10.1111/j.1365-2036.2006.03157.x]

210 **Louie TJ**, Peppe J, Watt CK, Johnson D, Mohammed R, Dow G, Weiss K, Simon S, John JF, Garber G, Chasan-Taber S, Davidson DM. Tolevamer, a novel nonantibiotic polymer, compared with vancomycin in the treatment of mild to moderately severe Clostridium difficile-associated diarrhea. *Clin Infect Dis* 2006; **43**: 411-420 [PMID: 16838228 DOI: 10.1086/506349]

211 **Peppe J**, Porzio A, Davidson DM. A new formulation of tolevamer, a novel nonantibiotic polymer, is safe and well-tolerated in healthy volunteers: a randomized phase I trial. *Br J Clin Pharmacol* 2008; **66**: 102-109 [PMID: 18341677 DOI: 10.1111/j.1365-2125.2008.03151.x]

212 **Bouza E**, Dryden M, Mohammed R, Peppe J, Chasan-Taber S, Donovan J, Davidson S, Short G. Results of a phase III trial comparing tolevamer, vancomycin and metronidazole in patients with Clostridium difficile -associated diarrhea. In: Program and abstracts of the 18th European Congress of Clinical Microbiology and Infectious Diseases (Barcelona). 2008; O464

213 **Baines SD**, Freeman J, Wilcox MH. Tolevamer is not efficacious in the neutralization of cytotoxin in a human gut model of Clostridium difficile infection. *Antimicrob Agents Chemother* 2009; **53**: 2202-2204 [PMID: 19223641 DOI: 10.1128/AAC.01085-08]

214 **Davies NL**, Compson JE, Mackenzie B, O'Dowd VL, Oxbrow AK, Heads JT, Turner A, Sarkar K, Dugdale SL, Jairaj M, Christodoulou L, Knight DE, Cross AS, Hervé KJ, Tyson KL, Hailu H, Doyle CB, Ellis M, Kriek M, Cox M, Page MJ, Moore AR, Lightwood DJ, Humphreys DP. A mixture of functionally oligoclonal humanized monoclonal antibodies that neutralize Clostridium difficile TcdA and TcdB with high levels of *in vitro* potency shows in vivo protection in a hamster infection model. *Clin Vaccine Immunol* 2013; **20**: 377-390 [PMID: 23324518 DOI: 10.1128/CVI.00625-12]

215 **Corthier G**, Muller MC, Wilkins TD, L'Haridon R. Protection against experimental pseudomembranous colitis in gnotobiotic mice by use of monoclonal antibodies against Clostridium difficile toxin A. *Infect Immun* 1991; **59**: 2123-2127

216 **Steele J**, Mukherjee J, Parry N, Tzipori S. Antibody against TcdB, but not TcdA, prevents development of gastrointestinal and systemic Clostridium difficile disease. *J Infect Dis* 2013; **207**: 323-330 [PMID: 23125448 DOI: 10.1093/infdis/jis669]

217 **Lowy I**, Molrine DC, Leav BA, Blair BM, Baxter R, Gerding DN, Nichol G, Thomas WD, Leney M, Sloan S, Hay CA, Ambrosino DM. Treatment with monoclonal antibodies against Clostridium difficile toxins. *N Engl J Med* 2010; **362**: 197-205 [PMID: 20089970 DOI: 10.1056/NEJMoa0907635]

218 **Safdar A**. Treatment with monoclonal antibodies against Clostridium difficile toxins. *N Engl J Med* 2010; **362**: 1444-145; author reply 1444-1445; [PMID: 20397289]

219 **Parks T**, Gkrania-Klotsas E, Nicholl C. Treatment with monoclonal antibodies against Clostridium difficile toxins. *N Engl J Med* 2010; **362**: 1444; author reply 1445-1446 [PMID: 20393181]

220 NCT01241552. Available from: URL: http://www.clinicaltrials.gov. Accessed 21 March 2013.

221 NCT01513239. Available from: URL: http://www.clinicaltrials.gov. Accessed 21 March 2013

222 **Marozsan AJ**, Ma D, Nagashima KA, Kennedy BJ, Kang YK, Arrigale RR, Donovan GP, Magargal WW, Maddon PJ, Olson WC. Protection against Clostridium difficile infection with broadly neutralizing antitoxin monoclonal antibodies. *J Infect Dis* 2012; **206**: 706-713 [PMID: 22732923 DOI: 10.1093/infdis/jis416]

223 **Sougioultzis S**, Kyne L, Drudy D, Keates S, Maroo S, Pothoulakis C, Giannasca PJ, Lee CK, Warny M, Monath TP, Kelly CP. Clostridium difficile toxoid vaccine in recurrent *C. difficile*-associated diarrhea. *Gastroenterology* 2005; **128**: 764-770 [PMID: 15765411 DOI: 10.1053/j.gastro.2004.11.004]

224 **Foglia G**, Shah S, Luxemburger C, Pietrobon PJ. Clostridium difficile: development of a novel candidate vaccine. *Vaccine* 2012; **30**: 4307-4309 [PMID: 22682287 DOI: 10.1016/j.vaccine.2012.01.056]

225 Available from: URL: http://www.sanofipasteur.com/EN/our-commitment/r-d/vaccines-for-tomorrow/vaccines-for-tomorrow.html. Accessed 21 March 2013

226 **Wang H**, Sun X, Zhang Y, Li S, Chen K, Shi L, Nie W, Kumar R, Tzipori S, Wang J, Savidge T, Feng H. A chimeric toxin vaccine protects against primary and recurrent Clostridium difficile infection. *Infect Immun* 2012; **80**: 2678-2688 [PMID: 22615245 DOI: 10.1128/IAI.00215-12]

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REVIEW

Role of chemoprophylaxis with either NSAIDs or statins in patients with Barrett's esophagus

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Abstract

The incidence of esophageal adenocarcinoma, a poor prognosis neoplasia, has risen dramatically in recent decades. Barrett's esophagus represents the best-known risk factor for esophageal adenocarcinoma development. Non-steroidal anti-inflammatory drugs through cyclooxygenase-2 inhibition and prostaglandin metabolism regulation could control cell proliferation, increase cell apoptosis and regulate the expression of growth and angiogenic factors. Statins can achieve equivalent effects through prenylation and subsequently control of cellular signaling cascades. At present, epidemiological studies are small and underpowered. Their data could not justify either medication as a chemo-preventive agent. Population based studies have shown a 43% reduction of the odds of developing an esophageal adenocarcinoma, leaving out or stating a 25% reduction in patients consuming non-aspirin nonsteroidal anti-inflammatory drugs and a 50% reduction in those patients consuming aspirin. They have also stated a 19% reduction of esophageal cancer incidence when statins have been used. Observational studies have shown that non-steroidal anti-inflammatory drugs could reduce the adenocarcinoma incidence in patients with Barrett's esophagus by 41%, while statins could reduce the risk by 43%. The cancer preventive effect has been enhanced in those patients taking a combination of non-steroidal anti-inflammatory drugs and statins (a 74% decrease). Observational data are equivocal concerning the efficacy of non-steroidal anti-inflammatory drug subclasses. Non-steroidal anti-inflammatory drugs clearly have substantial potential for toxicity, while statins are rather safe drugs. In conclusion, both non-steroidal anti-inflammatory drugs and statins are promising chemopreventive agents and deserve further exploration with interventional studies. In the meanwhile, their use is justified only in patients with cardiovascular disease.

Key words: Esophageal adenocarcinoma; Barrett's esophagus; Non-steroidal anti-inflammatory drugs; Aspirin; Statins; Cancer chemoprevention

Core tip: Esophageal adenocarcinoma remains a major burden upon health. Experimental studies have suggested that non-steroidal anti-inflammatory drugs and statins may have useful actions against esophageal cancer cells. This review of observational studies shows that non-steroidal anti-inflammatory drugs reduced adenocarcinoma incidence in patients with Barrett's esophagus by 41%, while statins reduced the risk by 43%. The cancer preventive effect is enhanced in those patients taking a combination of non-steroidal anti-inflammatory drugs and statins (a 74% decrease). Non-steroidal anti-inflammatory drugs clearly have substantial potential for toxicity, while statins are rather safe drugs. Their combination offers promise for chemoprevention and further interventional studies are warranted.

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INTRODUCTION

A rapid increase in incidence and mortality from esophageal adenocarcinoma (EAC) has been observed over the past four decades in the Western world[1,2]. Although the absolute incidence of EAC varies dramatically by gender and race, few demographic groups have been spared from the increases[3]. Moreover, survival of persons with EAC remains abysmal, with most succumbing to the disease within a year[4], while the 5-year survival rate is less than 15%[5].

Barrett's esophagus (BE), replacement of the squamous esophageal mucosa by metaplastic columnar epithelium due to prolonged reflux[6] of the gastric content into the esophagus, represents the best-known risk factor for EAC development[7]. The annual incidence of EAC development in patients with BE is 0.2%-0.5%[8,9].

Clinical and demographic factors that have shown some promise in being predictive of malignant transformation in BE are male gender[10,11], increasing age[11], length of Barrett's segment[12-14], duration of BE[13] and size of hiatal hernia[14]. There is little evidence to suggest that total alcohol consumption or specific alcoholic beverages modifies the risk of EAC in the general population[15,16], while smoking[16-19] and obesity[16,20] raise the risk for neoplastic progression.

According to the most popular theory, carcinogenesis in BE patients is completed in three stages. During the first, a distinct stem cell population develops in the bone marrow of genetically predisposed patients with gastroesophageal reflux disease. Those cells migrate during the second stage to the gastroesophageal junction and lower esophagus, producing a macroscopically visible BE. The inflammatory milieu in the lower esophagus produces the driving force for stem cell migration. Repeat call for repair in the hostile environment of lower esophagus in BE patients leads to increase cell proliferation and frequent mutations. As noxious mutations sum up by a multistep process, metaplastic epithelium evolves into low-grade dysplasia, high-grade dysplasia, early EAC and ultimately invasive cancer[21].

Although cancer surveillance is performed in most institutions, once diagnosis of BE is rendered, the true cost-benefit ratio of this endeavour is still essentially unknown[22]. Surveillance does not interfere with the neoplastic process and could not affect the pre-neoplastic stem cell population generated in the bone marrow. Thus, there is a quest for global and more interventional strategies. Chemoprevention is attractive, especially for the high-risk group of BE individuals, since it can affect the neoplastic process from its early beginning. Moreover, because it could be effective even under insufficient gastric acid suppression[23], it may be superior to BE ablative techniques that presuppose adequate acid suppression to prevent BE recurrence[24] and may prove too expensive[25]. Finally, since BE surveillance cost-effectiveness has been undermined by recent data suggesting a low risk of malignant transformation[26,27], chemoprevention seems to represent an attractive alternative[28]. At present, there are no proven chemo-preventive agents, although non-steroidal anti-inflammatory drugs (NSAIDs) and statins appear to offer the most attractive combination of risks and benefits.

This review is to assess current experimental and epidemiological data that NSAIDs and statins could reduce the risk of developing EAC in BE. Moreover, we aim to clarify how existing findings could be included in the EAC etiological models, as well as any side effects, that would follow clinical application of NSAIDs and statins for cancer prevention.

NSAIDS AND EAC CHEMOPREVENTION

Numerous *in vitro* and animal studies support the possible chemo-preventive effect of cyclooxygenase-2 (COX-2) inhibition in BE. COX-2 inhibitors, either drugs or naturally occurring in plant foods, could produce significant suppression of cell proliferation and induce cell cycle arrest in cultured EAC cells[29]. Selective COX-2 inhibitors have a similar effect in cell cultures from endoscopic biopsies taken from BE patients[30]. Adding COX-2 inhibitors in rat diet after esophagojejunostomy had reduced progression to EAC[31,32] in some studies, while indomethacin, but not selective COX-2 inhibitors, produced a similar effect in others[33].

Several case control studies comparing EAC patients to healthy controls have shown that NSAID use can effectively prevent EAC. A meta-analysis of all human studies published prior to 2003, showed an overall 43% reduction of the odds of developing an EAC in NSAID takers, comprising a 25% reduction in patients consuming non-aspirin NSAIDs and 50% reduction in aspirin users[34], but the analysis included only one small case-control study comparing BE and EAC patients[35] and no prospective study. Thus, it cannot differentiate whether any beneficial effect of NSAID use is produced before or after BE appearance. In 2009, a questionnaire based study that included approximately 300000 members of the American Association of Retired Persons found no significant association between EAC and the use of aspirin or non-aspirin NSAIDs[36].

Since 2003, several observational studies comparing BE and EAC patients have been published (Table 1). These were either case-control retrospective[23,37,38] or cohort studies[39-43]. Prospective chemoprevention trials are underway to evaluate the efficacy of aspirin and NSAIDs. In the United Kingdom, the AspECT trial is currently evaluating the combination of high-dose proton pump inhibitors and aspirin in minimizing the risk of progression to cancer in 9000 BE sufferers[44]. A similar

Table 1 Available epidemiological evidence of benefit of non-steroidal anti-inflammatory drugs and aspirin in prevention of esophageal adenocarcinoma in patients with Barrett's esophagus

Ref.	Type of study	Size-follow-up	Effect on EAC rate	Beneficial effect
Abnet *et al*[36]	Population based	311115 AARP members	Aspirin OR = 1.1 (0.78-1.57) Non-aspirin NSAIDs OR = 0.90 (0.55-1.43)	None
Tsibouris *et al*[23]	Case-control	BE: 382 EAC: 114	Daily use of non-aspirin NSAIDs OR = 0.30 (0.10-0.91) Daily use of low-dose aspirin OR = 1.21 (0.52-2.83)	Non-aspirin NSAIDs
Beales *et al*[37]	Case-control	BE: 170 EAC: 85	Statins + aspirin OR = 0.31 (0.04-0.69)	Statins + aspirin
Nguyen *et al*[38]	Case-control	BE: 696 EAC: 116	All NSAIDs OR = 0.64 (0.42-0.97)	All NSAIDs
Vaughan *et al*[39]	Cohort	BE: 350 1731 PY	All NSAIDs OR = 0.20 (0.10-0.41)	All NSAIDs
Kastelein *et al*[40]	Cohort	BE: 570 4.5years	Non-aspirin NSAIDs OR = 0.50 (0.26-0.97) Aspirin OR = 0.67 (0.31-1.46)	Non-aspirin NSAIDs
Nguyen *et al*[41]	Cohort	BE: 344 2620 PY	All NSAIDs OR = 0.51 (0.25-1.04)	None
Gatenby *et al*[42]	Cohort	BE: 650 3683 PY	Non-aspirin NSAIDs OR = 0.90 (0.34-2.37) Aspirin OR = 0.72 (0.41-1.31)	None
Kantor *et al*[43]	Cohort	BE: 411 2805 PY	All NSAIDs OR = 0.46 (0.34-1.10)	None

PY: Patient years; EAC: Esophageal adenocarcinoma; BE: Barrett's esophagus; NSAIDs: Non-steroidal anti-inflammatory drugs.

prospective study is running in the United States[45]. In the only prospective interventional study published today, Heath *et al*[46] randomized 100 patients who had either low or high-grade dysplasia and BE to receive either a COX-2 selective NSAID (celecoxib) or placebo. After 48 wk of treatment, there was no significant difference between the 2 groups in the proportion of esophageal biopsy specimens showing dysplasia or cancer[46]. This study has limitations (*e.g.*, the use of dysplasia as the primary outcome, the use of a low dose of celecoxib) that prevent definite conclusions on the utility of NSAID chemoprevention.

Although all case control studies have shown that NSAID use is beneficial, there is considerable diversity concerning NSAID subclasses that could reduce EAC risk. Our case control study has shown that daily use of non-aspirin NSAIDs was beneficial; while a daily low dose, as well as infrequent use of either aspirin or non-aspirin NSAIDs, was not[23]. Beales *et al*[37] found that statin and aspirin combination reduced incidence of EAC and Nguyen *et al*[38] that all NSAIDs are beneficial, without a separate report of NSAID subclasses.

Cohort study results are more diverse. Vaughan *et al*[39] found that, comparing current NSAID users to those who never used, NSAIDs had a significantly decreased risk of EAC. Kantor *et al*[43] reported that non-aspirin NSAID use reduced the risk of neoplastic progression but not aspirin use. The other 3 cohort studies were negative[41-43], although Kantor found that NSAID use was beneficial only for patients with high-grade dysplasia. A pooled analysis of 6 population-based studies within the Barrett's and Esophageal Adenocarcinoma Consortium have shown that daily NSAID use can reduce the risk of developing EAC by more than 40% (OR = 0.56, 95%CI: 0.43-0.73, $P < 0.001$)[47]. A meta-analysis of all published observational studies calculated the pooled effect size for COX-inhibitors to 0.59 (95%CI: 0.45-0.77) with minimal heterogeneity[48].

Many of the observational studies have inherent limitations because not all confounding variables (such as socioeconomic status, tobacco and alcohol use, *H. pylori* status, dietary intake) have been taken into account, especially in case-control studies. Use of aspirin and/or NSAIDs may have been associated with certain patient-led behaviors that have an influence on risk. Such behaviors may include vitamin supplementation[49] and dietary habits. Furthermore, patients on aspirin may indeed have been more health conscious and might have been more likely to have their cancers detected than others. Finally, it is likely that those with upper gastrointestinal symptoms such as heartburn and regurgitation, risk factors for EAC, are less likely to have been prescribed NSAIDs or aspirin. The use of acid-reducing agents with the sole aim of reducing BE has not been proven in a long-term controlled trial[45]. Although most studies suggest a synergy between sufficient acid suppression and NSAIDs chemopreventive effect[38,40], we have shown that NSAIDs could be effective despite financially driven reduction of proton pump inhibitor treatment[23].

Typically, diagnosis of BE is made in men older than 50 years of age, a group with elevated frequency of cardiovascular disease. Low-dose aspirin is beneficial for primary cardiovascular events in men older than 50 years of age who are at risk of developing coronary artery disease[50-52]. Today, data concerning BE patients with ischemic heart disease are scarce. We have reported that low-dose aspirin could reduce the risk of EAC in BE patients with ischemic heart disease, but it had no beneficial effect in patients without cardiovascular co-morbidities[53], possibly due to cofactors common to the etiology of ischemic heart disease and EAC, such as alcohol, tobacco, diet and exercise.

Limited data suggest that biomarkers might have a role in identifying those patients with BE who are most likely to benefit from chemopreventive therapies. In BE patients with DNA content abnormalities, such as 17p loss of heterozygosity (LOH), and/or 9p LOH in their esophageal biopsy specimens, NSAID use was associated with a significant reduction in the risk of EAC after 6-10 years of follow-up. In contrast, no beneficial effect was seen in patients without those abnormalities[54].

MECHANISMS OF NSAID CHEMOPREVENTIVE EFFECT

Esophageal carcinogenesis is mainly related to the inflammatory process in macroscopically visible Barrett epithelium, due to persistent gastroesophageal reflux[47] in addition to angiogenesis up-regulation[55]. The inflamed mucosa produces several inflammatory intermediates. Interleukin-1 and tumor necrosis factor induce nuclear factor (NF)-κB over-expression[56]. After activation, NF-κB translocates to nucleus, where it activates gene transcription[57]. In BE patients, NF-κB binds the promoter region of *COX-2* gene, increasing COX-2 expression[58]. Most observational studies suggest that NSAIDs, in doses adequate to suppress COX-2, can effectively prevent BE progression to EAC[23,37-40].

Reactive oxygen species may damage DNA, RNA, lipids and proteins, leading to increased mutation and altered functions of enzymes and proteins (*e.g.*, activation of oncogene products and/or inhibition of tumor suppressor proteins). They also related to cellular immunity, signal transduction and modification of extracellular matrix. In normal esophagus, low levels of reactive oxygen species are produced in non-phagocytic cells and are thought to be by-products of aerobic metabolism[59]. Pulsed acid treatment and bile significantly increases H_2O_2 production in BE cells *via* NADPH oxidase NOX-5-S overexpression. It also increases calcium ion influx and cyclic adenosine monophosphate (AMP) reactive element binding protein[60,61]. Increased cellular calcium ion influx causes up-regulation of NADPH oxidase NOX5-S[62]. Overproduction of reactive oxygen species derived from up-regulation of NADPH oxidase NOX5-S, as well as H_2O_2 overproduction, can up-regulate NF-κB[63] and as a result leads to COX-2 over-expression[64].

Because acid and bile contents of refluxate represent the main driving forces for COX-2 over-expression in BE patients[65] and because proton pump inhibitors enhance COX-2 anti-proliferated effect *in vitro* and prevent vascular endothelial growth factor overexpression[66], acid suppression should be an essential cofactor of EAC chemoprevention. This suggestion is also supported by epidemiological data[38,40,45].

COXs (or prostaglandin H synthases) are a family of myeloperoxidases located at the luminal side of the endoplasmic reticulum and nuclear membrane, which catalyze the rate-limiting step of prostaglandin biosynthesis from arachidonic acid[67]. COX-2 induction or over-expression is associated with an increased production of prostaglandin E2 (PGE2), which is known to modulate cell proliferation, cell death and tumor invasion in many types of cancer. In addition to COX overexpression, pulsed acid exposure can up-regulate microsomal PGE synthase 1 and through it, PGE2 production and cell proliferation. Acid-induced microsomal PGE synthase 1 over-expression depends on NADPH oxidase 5S activation and NF-κB1 over-expression[68] and it is regulated through the increase of cytosolic calcium[69]. Epidemiological data suggest that the acid related route of PGE2 production is of minor importance since COX-2 inhibitors can be effective even under inadequate acid suppression[23].

PGE2 acts through different membrane receptors called EP receptors (EP1, EP2, EP3 and EP4). These receptors are all located on the cell surface but trigger different signaling pathways. Thus, it is known that EP1 signaling acts through phospholipase C/inositol triphosphate signaling, leading to intracellular mobilization of calcium. EP2 and EP4 receptors are coupled with G proteins and activate adenylate cyclase, leading to an increase of intracellular cyclic AMP[70]. Cyclic AMP is then able to activate various kinases, such as protein kinase A, phosphoinositide-3 kinase and glycogen synthetase kinase-3, leading to an activation of β-catenin, a pathway regulating cell proliferation[71]. Contrary to EP2 and EP4, EP3 is coupled with Gi protein, leading to an inhibition of adenylate cyclase and decreases of cAMP inside the cells[70]. Dietary elements entering arachidonic acid metabolism can interfere with PGE2[49] and therefore they should not be overlooked. Unfortunately, almost all epidemiological studies ignore this parameter[23,37-43].

Cell circle regulatory mechanisms form checkpoints where the cell cycle can be stopped after cellular damage in order to allow repair and to maintain cellular integrity or, alternatively, to eliminate mutated and potentially dangerous cells. Different serine-threonine kinase proteins called cyclin-dependent kinases (Cdk) are important cell circle regulators. They interfere with the cell cycle by phosphorylating many substrates[72]. The inhibitors of cyclin kinase 4 (INK4) family (p16, p15, p18 and p19) and the Cip/Kip family (p21, p27 and p57)[72,73] are key regulators of cell transition from G1 to S phase. INK4 family inhibits Cdk4 and Cdk6, whereas Cip/Kip family inhibits all Cdks. After DNA damage, p53, a tumor suppressor gene, activates transcription of p21, which inhibits cyclin E phosphorylation, leading to hypophosphorylation of retinoblastoma protein[71]. After phosphorylation, retinoblastoma protein releases transcription factor E2F activating genes involved in the S phase-like proliferating cell nuclear antigen[74]. p53 also regulates cell transition from G_2 to M phase through cyclin B-Cdk 2 complex activation. Cyclin B-Cdk 2 complex accumulates during the previous step of the cell cycle. It is inactivated by phosphorylation at tyrosine 15 and threonine 14 by Wee 1 and Myt 1 and can be reactivated when these phosphate groups are removed by the phosphatase CDC25A, a cyclin related phosphatase, when cells enter mitosis[75].

COX-2 up-regulation increases Barrett's epithelium

and esophageal adenocarcinoma cell proliferation by induction of retinoblastoma tumor suppressor protein phosphorylation and up-regulation of cyclins, cyclin-dependent kinases[76] and p53 LOH[77]. NSAIDs could also increase the proportion of Barrett cells in G_0-G_1 phase and reduce those in S and G_2-M phase[78,79].

Two major cascades of intracellular events are commonly involved in mediating apoptosis. (1) The intrinsic pathway, also called the mitochondrial or stress-induced apoptotic pathway, which is activated in response to damaging stresses; and (2) the extrinsic, or physiological, apoptotic pathway. Typical hallmarks of the intrinsic pathway are mitochondrial outer membrane permeabilization, accompanied by a collapse of the mitochondrial membrane potential[80]. These events lead to the release of cytochrome *c* into the cytosol and the death complex formation by apoptotic protease activating factor-1 and procaspase-9. Once recruited, procaspase-9 is cleaved to its activated form (caspase-9) to further activate the executor caspase-3 and to finalize the apoptotic program. The intrinsic pathway can be triggered upon binding of specific ligands to death receptors characterized by the presence of a death effector domain[81]. Ligands include cytokines, such as tumor necrosis factor α, tumor necrosis factor-related apoptosis inducing ligand-induced apoptosis or Fas. After binding, death inducing silencing complex is formed. The adaptor proteins, tumor necrosis factor receptor-associated death domain and Fas associated death domain, form the death inducing silencing complex that is able to recruit and activate pro-caspase-8. The latter activates caspase-3 in order to trigger the final steps of apoptosis.

Cross talks between the two pathways take place. The extrinsic apoptotic pathway can activate the intrinsic pathway *via* truncation of the BH3-only protein Bid by caspase-8. BH3-only protein Bid interacts with mitochondria, by favoring the activation of the pro-apoptotic Bcl-2 family members Bak and Bax, thus leading to mitochondrial outer membrane permeabilization and caspase-9 activation[80]. The intrinsic apoptotic pathway may, in turn, activate caspase-8, downstream to caspase-3[82]. NSAIDs can inhibit programmed cell death in BE cells *via* prevention of Bcl-2 suppression[83], a key checkpoint in COX-2 controlled apoptotic cascade[84].

Anoikis is a form of apoptosis mediated by the loss of cell anchorage. This pathway plays a fundamental role during development and maintenance of tissue homeostasis by killing damaged cells or detached cells in order to maintain tissue architecture. It is dependent on caspase activation and cytochrome *c* release by mitochondria and is regulated by Bcl-2 family members[71]. Cell anchorage is due to cell-cell and cell-matrix interactions. Cell-cell interactions are mainly mediated by integrins, transmembrane receptors located at the cell surface[85]. Many intracellular signals can act downstream to integrins, which, correctly switched on, can ensure cell survival. Some of them are mediated by kinases such as focal-adhesion-kinase or integrin-linked kinase. Focal-adhesion-kinase is phosphorylated upon integrin adhesion, leading to activation of other signaling pathways like phosphoinositide 3 kinase and mitogen-activated protein kinase (MAPK)[71].

NSAIDs can up-regulate MAPK signaling cascade[86] through Cl/HCO3 membrane exchange channel after intracellular acidification[87]. COX-2 inhibitors can regulate mesenchymal-epidermal cross talk[88]. In non-dysplastic Barrett, COX-2 is selectively increased only in stromal cells, while in adenocarcinoma it is also increased in neoplastic epithelium[89,90].

COX-2 can also regulate the expression of angiogenetic factors, especially vascular endothelial growth factor[90], mainly through a MAPK dependent pathway[91]. Because reactive oxygen species are overproduced in the ischemic tissue[92] and various angiogenic factors are abundant in patients with cardiovascular diseases[93], NSAIDs are expected to be more effective in this patient group. Nevertheless, non-aspirin NSAID are not effective in BE patients with ischemic heart disease, while aspirin is especially effective in this patient group[53].

Although low-dose aspirin clearly prevents EAC when prescribed in healthy controls, it suppresses COX insufficiently[34]. Thus, apart from COX-related, there are also other mechanisms implicated in NSAID chemopreventive action. Epidemiological data doubt the significance of COX-independent mechanisms[23,37-43].

Independently to COX, NSAIDs can bind and inhibit protein kinase B/Akt, an important mediator of cell proliferation and in apoptosis. Protein kinase B is able to phosphorylate Cdk inhibitors, such as p21 and p27, leading to proliferating cell nuclear antigen activation[94]. Moreover, it inhibits apoptosis by phosphorylating the pro-apoptotic protein Bad and by inhibiting caspase-9 cleavage[80]. Independently to COX, NSAIDs can also activate the extrinsic apoptotic pathway by modulating the sensitivity of several tumor cells to Fas and tumor necrosis factor-related apoptosis inducing ligand[95]. They can also up-regulate Bax expression and mitochondrial cytochrome c translocation[96]. Finally, NSAIDs are able to decrease intracellular content of glutathione, the most important intracellular non-protein antioxidant defense against free radicals and, in such a way, affect both cell proliferation and apoptosis[97].

Although *in vitro* studies suggest that NO-aspirin is more effective than aspirin to prevent Barrett cell hyperproliferation[98], this did not prove to be the case in a clinical study[23].

COST-EFFECTIVENESS AND SIDE EFFECTS OF NSAIDS CHEMOPREVENTION

Assuming that aspirin use can reduce EAC development risk by 50% in BE patients, the cost of the chemo-preventive intervention was calculated to 40000 Euros for every quality year of life saved[28].

NSAIDs clearly have substantial potential for toxic-

ity, including serious gastrointestinal and cardiovascular side effects that should be balanced with their potential cancer-preventive effects. Generally the risk for low-dose aspirin is low. A meta-analysis of randomized controlled trials comparing low-dose aspirin (75-325 mg) and placebo for cardiovascular prophylaxis found that the absolute annual increase in risk attributable to aspirin was only 0.13% (95%CI: 0.08-0.20) for major bleeding, 0.12% (95%CI: 0.07-0.19) for major gastrointestinal bleeding, and 0.03% (95%CI: 0.01-0.08) for intracranial bleeding[99]. Moreover, concomitant proton pump inhibitor therapy could reduce the risk of gastrointestinal bleeding by a factor of 2 to 9[100,101].

We have shown that complications, including upper gastrointestinal bleeding, esophageal ulcers and benign esophageal strictures, were no more common in NSAID users with BE than NSAID non-users. Moreover, the majority of those complications were acid related and could be prevented by adequate acid suppression, preferentially with high dose proton pump inhibitors. On high dose proton pump inhibitors, only 14% of BE patients consuming NSAIDs presented with any complication[23]. In accordance to our findings, Hillman *et al*[102] have shown that esophageal ulcers and stenosis can be effectively prevented with adequate acid suppression[102].

Because thromboxane biosynthesis depends on sustained inhibition of COX-1, several NSAIDs present serious cardiovascular side effects. In the two meta-analyses published today, major vascular events were increased by about a third for COX-2 selective and non-selective NSAIDs, with the exception of naproxen. Analyses showed that the excess risk was mainly attributable to an increase of about three quarters in the risk of major coronary events. Vascular death increased by about two-thirds, heart failure risk roughly doubled, while risk for stroke was not affected[103,104].

Nitro-NSAIDs represent an NSAID subclass with lower risk for gastrointestinal bleeding[105]. We have shown that combination of NSAID use to nitrates in BE patients neither affected EAC risk nor improved NSAID safety profile[23].

STATINS AND EAC CHEMOPREVENTION

Cellular effects of statins on EAC cell lines have been evaluated in three *in vitro* studies. All reported anti-proliferative and pro-apoptotic effects[106-108]. Qresearch, a prospective study based on 24 general practice research databases from England and Wales, have shown that statins were protective against esophageal cancer development in both men and women. The risk of esophageal carcinoma decreased in both men and women prescribed simvastatin, as well as in men prescribed atorvastatin. There were inadequate data for other statins. There was some evidence of a dose-response associated with simvastatin in men only[109]. A more recent analysis of the same database revealed no protective effect from statin use[110]. An analysis of General Practice United Kingdom Research Database in 2002 that included only 9 esophageal carcinoma cases revealed no protective effected related to statin use[111]. Bhutta *et al*[112] case control study found that statin use was negatively associated with the development of esophageal carcinoma. Both lipophilic and hydrophilic statins were protective. The magnitude of this negative association was similar for time periods extending beyond one year. When statin use and cancer development was accessed through a health care program database from northern Calofornia, esophageal carcinoma was more common among statin users[113]. Population studies published today, although they have been adjusted for many covariates including age, body mass index, smoking, do not differentiate between EAC and squamous carcinomas and do not allow evaluation of statin use in EAC prevention.

Three population studies[110-112] were included in the meta-analysis of risk of esophageal carcinoma among general population cohorts with statin use. The pooled effect size was 0.86 (95%CI: 0.78-0.94, $P = 0.001$) with minimal heterogeneity[114]. A recent meta-analysis of all published studies calculated the pooled effect size for statins to 0.81 (95%CI: 0.75-0.88) with substantial heterogeneity[48].

Several observational studies evaluating NSAIDs chemopreventive effect have also analyzed the utility of statin use (Table 2). In their case control study, Beales *et al*[37] found that regular statin use was associated with a significantly lower incidence of EAC. Longer duration of statin use and higher doses were both associated with a significantly greater reduction in EAC. Kastelein *et al*[40] reported that statin use for greater than one mo was associated with a statistically significant inverse risk for neoplastic progression, although this was only observed in men over 60 years of age. The concomitant use of both statins and NSAIDs was associated with a greater risk reduction. Nguyen *et al*[41] reported that having any filled statin prescription was associated with 45% lower risk of EAC. Patients with a cumulative filled statin prescription for > 12 mo have a reduced risk of EAC compared to those with ⩽ 12 mo or those with no statin prescription. Kantor *et al*[43] found that statin use was not associated with a reduced risk of neoplastic progression in BE patients. Nevertheless, when the analysis was limited to persons with high-grade dysplasia at baseline, a subgroup at particularly high risk of EAC development, statin use was definitively protective. The combination of statins and NSAIDs was also protective. The main draw back of most observational studies was that authors did not adjust for important covariates, namely body mass index and smoking[40,41,43].

Kastelein *et al*[40] and Nguyen *et al*[41] cohort studies were included in the meta-analysis of risk of EAC. The pooled effect size was 0.53 (95%CI: 0.36-0.78, $P = 0.001$) with minimal heterogeneity[115]. A recent meta-analysis, including all 5 studies published today, calculated the pooled effect size for statins to 0.57 (95%CI: 0.43-0.75) with minimal heterogeneity. For the combination of statins and COX inhibitors, pooled effect size was 0.26 (95%CI: 0.10-0.68)[48].

Table 2 Available epidemiological evidence of benefit of statin use in prevention of esophageal adenocarcinoma in patients with Barrett's esophagus

Ref.	Type of study	Size-follow-up	Effect on EAC rate	Beneficial effect
Hippisley-Cox *et al*[109]	Population based	General population 2004692 cases	Statins Men[1] OR = 0.78 (0.66-0.91) Women[1] OR = 0.68 (0.52-0.88) Simvastatin Men[1] OR = 0.69 (0.50-0.94) Women[1] OR = 0.82 (0.68-0.99) Atorvastatin Men[1] OR = 0.73 (0.55-0.96) Women[1] OR = 0.73 (0.47-1.13)	Statins[1]
Vinogradova *et al*[110]	Population based	General population 2004692 cases	Statins[1] OR = 0.88 (077-1.01)	None[1]
Kaye *et al*[111]	Population based	Esophageal cancer: 9	Statins[2] OR = 0.8 (0.3-1.8)	None[2]
Bhutta *et al*[112]	Population based	Esophageal cancer: 4242 Controls: 17233	Statins[1] OR = 0.84 (0.73-0.95) Lipophylic statins[1] OR = 0.86 (0.75-0.98) Hydrophilic statins[1] OR = 0.71 (0.51-0.98)	Statins[1]
Beales *et al*[37]	Case-control	BE: 170 EAC: 85	Statins OR = 0.57 (0.28-0.94)	Statins
Kastelein *et al*[40]	Cohort	BE: 570 4.5 years	Statins OR = 0.46 (0.21-0.99) Statins + NSAIDs OR = 0.22 (0.06-0.85)	Statins Statins + NSAIDs
Nguyen *et al*[41]	Cohort	BE: 344 2620 PY	Statins OR = 0.55 (0.36-0.86)	Statins
Kantor *et al*[43]	Cohort	BE: 411 2805 PY	Statins OR = 0.68 (0.30-1.54) Statins + NSAIDs OR = 0.41 (0.13-1.26)	Statins + NSAIDs

[1]Results pertain to esophageal carcinoma; [2]Results pertaining to all cancers. PY: Patient years; EAC: Esophageal adenocarcinoma; BE: Barrett's esophagus; NSAIDs: Non-steroidal anti-inflammatory drugs.

At present, there are no published observational studies evaluating statin chemopreventive effect in BE patients in the general population. Moreover, there are no interventional studies underway.

MECHANISMS OF STATIN CHEMOPREVENTIVE EFFECT

Statins competitively inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate-limiting enzyme in the biosynthesis of cholesterol. Although this is their most appreciated biological action, statins have several other important roles. They inhibit biosynthesis of L-mevalonate[116], a precursor of cholesterol, and they produce two isoprenoid intermediates: farnesyl pyrophosphate and geranylgeranyl pyrophosphate[117]. Farnesyl pyrophosphate and geranylgeranyl pyrophosphate attach to several cellular proteins including G proteins by a posttranslational modification termed isoprenylation. The isoprenylation of G proteins is crucial for membrane attachment and normal functioning. The low molecular weight G proteins, including Ras, Rho, Rab and Cdk 42, play crucial roles in signal transduction and therefore influence important cellular functions, such as proliferation, apoptosis and differentiation. Ras represents the most important G protein and is predominantly farnesylated, while all other GTPases are predominantly geranylated. Ras mutations in preneoplastic cells determine their susceptibility to statin treatment[118]. Because Ras in EAC cells is very susceptible to statin treatment[106], statins are very effective in all available epidemiological studies[37,40,41,43].

Ras pathway down-regulation could reduce phosphoinositide 3 kinase/Akt and extra-cellular signal regulating kinase activities, enhancing cell proliferation and modulating cell-cell interactions. Moreover, it up-regulates the pro-apoptotic proteins Bad and Bax through phosphoinositide 3 kinase/Akt pathway, preventing cell apoptosis[106,107,118-120]. Through Ras modification, statins attenuate total cellular and cell-surface intracellular adhesion molecule-1 expression and activate NF-κB[121].

Through a G-protein independent mechanism, statins can suppress angiogenesis. Angiogenesis inhibition is a result of the inhibition of the expression or activity of monocyte chemoattractant protein-1, inhibition of metalloproteinase, angiotensin-2, preproendothelin gene, as well as inhibition of actin filament and by focal adhesion molecules formation[122]. Finally, they present with an anti-inflammatory effect by reducing tumor necrosis factor-alpha[107] and intracellular adhesion molecule-1 (a critical adhesion molecule involved in transendothelial tumor cell migration)[123,124].

Because statins do not interfere with various proliferation pathways, such as MAPK pathway and transcription factor AP1/c-jun terminate kinase[106], NSAIDs can enhance statin chemopreventive effect by blocking those metabolic routes[125]. As a result, whenever statins and NSAIDs are combined, lower doses of either chemopreventive agent are necessary, leading to a reduction of side effects[106]. Current epidemiological data unanimously verify NSAID and statin synergy[37,40,41,43].

Because adiponectin and ghrelin can interfere in vitro with EAC cell apoptosis[126], obesity, a parameter overlooked by most observational studies[40,41,43], mandates further attention.

SIDE EFFECTS OF STATIN CHEMOPREVENTION

Statins are generally safe medications. Out of the various adverse effects of statins, only liver and muscle-related toxicity is consistently reported[127]. Between 1987 and 2001, the Food and Drug Administration (FDA) recorded 42 deaths from rhabdomyolysis induced by statins, translated to one death per million prescriptions (30 day supply). Although 5%-10% of patients complain of muscle symptoms, only 1%-3% of them are actually statin related. Muscle symptoms usually occur within the first 6 mo of starting statins but can occur months or years after the initiation of statin therapy and automatically resolve within 2 mo of discontinuing statin therapy[128]. The incidence of statin-associated myopathy is quite low (approximately 0.01%) and rhabdomyolysis even lower (0.002%)[129]. Fatal rhabdomyolysis has been estimated to occur in approximately 1.5 in 10 million prescriptions[130].

Post-marketing surveillance studies of statins revealed that elevation in hepatic aminotransferases are dose related, mild and unrelated to low-density lipoprotein lowering effect. Thus, most hepatologists no longer consider statins to have any significant hepatotoxicity[131]. Although serious hepatotoxicity is rare, 30 cases of liver failure associated with statin use were reported to the FDA between 1987 and 2000, the rate being about one case per million person-years of use. Thus, the occurrence of acute liver failure thought to be caused by statins is well below the background rate of idiopathic acute liver failure in the general population[132].

Evidence from four cohort studies and case reports suggest that statins cause reversible peripheral neuropathy. Nevertheless, the attributable risk is small (12 per 100000 person-years) and no change in cognitive function was found in randomized trials of statins in elderly patients[130].

Because BE patients are usually old with various multi-systemic comorbidities[36], increased toxicity is expected[133] with statin use. No study today has specifically addressed statin toxicity in BE patients.

FUTURE DIRECTIONS

The poor prognosis of patients diagnosed with EAC presents a challenge to the clinician. Consequently there is burgeoning interest in potential chemo-preventive strategies. Considerable evidence of medium quality is available of a protective effect of NSAIDs, yet because of their side-effect profile, widespread use cannot be currently justified. Although statin safety profile is good, epidemiological and animal data are limited to justify their use as chemo-preventive agents. Because mortality due to cardiovascular disease is high in BE patients, "technical review on the management of Barrett's esophagus today" suggests screening for cardiovascular factors in BE patients and aspirin and statin use as warranted[45]. Because we have shown no benefit for non-aspirin NSAID use in BE patients with ischemic heart disease[53] and substantial cardiovascular side effects are expected[103,104], use of non-aspirin NSAIDs should be withheld in patients with BE and cardiovascular co-morbidities, at least until more clinical data might justify their use.

Large randomized control trials in the near future are expected to safely evaluate NSAIDs and statins as chemopreventive agents and possibly introduce their widespread use in patients with BE. Because of their synergistic effect[106], such trials ought to test either and both medications against proton pump inhibitors alone.

REFERENCES

1 **Steevens J**, Botterweck AA, Dirx MJ, van den Brandt PA, Schouten LJ. Trends in incidence of oesophageal and stomach cancer subtypes in Europe. *Eur J Gastroenterol Hepatol* 2010; **22**: 669-678 [PMID: 19474750 DOI: 10.1097/MEG.0b013e32832ca091]

2 **Cook MB**, Chow WH, Devesa SS. Oesophageal cancer incidence in the United States by race, sex, and histologic type, 1977-2005. *Br J Cancer* 2009; **101**: 855-859 [PMID: 19672254 DOI: 10.1038/sj.bjc.6605246]

3 Esophageal cancer: epidemiology, pathogenesis and prevention. *Nat Clin Pract Gastroenterol Hepatol* 2008; **5**: 517-526 [PMID: 18679388 DOI: 10.1038/ncpgasthep1223]

4 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009; **59**: 225-249 [PMID: 19474385 DOI: 10.3322/caac.20006]

5 **Spechler SJ**, Goyal RK. The columnar-lined esophagus, intestinal metaplasia, and Norman Barrett. *Gastroenterology* 1996; **110**: 614-621 [PMID: 8566611 DOI: 10.1053/gast.1996.v110.agast960614]

6 **Lund O**, Kimose HH, Aagaard MT, Hasenkam JM, Erlandsen M. Risk stratification and long-term results after surgical treatment of carcinomas of the thoracic esophagus and cardia. A 25-year retrospective study. *J Thorac Cardiovasc Surg* 1990; **99**: 200-209 [PMID: 2299857]

7 **Naef AP**, Savary M, Ozzello L. Columnar-lined lower esophagus: an acquired lesion with malignant predisposition. Report on 140 cases of Barrett's esophagus with 12 adenocarcinomas. *J Thorac Cardiovasc Surg* 1975; **70**: 826-835 [PMID: 1186274]

8 **Aldulaimi DM**, Cox M, Nwokolo CU, Loft DE. Barrett's surveillance is worthwhile and detects curable cancers. A prospective cohort study addressing cancer incidence, treatment outcome and survival. *Eur J Gastroenterol Hepatol* 2005; **17**: 943-950 [PMID: 16093872 DOI: 10.1097/00042737-200509000-00010]

9 **Drewitz DJ**, Sampliner RE, Garewal HS. The incidence of adenocarcinoma in Barrett's esophagus: a prospective study of 170 patients followed 4.8 years. *Am J Gastroenterol* 1997; **92**: 212-215 [PMID: 9040193]

10 **de Jonge PJ**, van Blankenstein M, Looman CW, Casparie MK, Meijer GA, Kuipers EJ. Risk of malignant progression in patients with Barrett's oesophagus: a Dutch nationwide cohort study. *Gut* 2010; **59**: 1030-1036 [PMID: 20639249 DOI: 10.1136/gut.2009.176701]

11 **Yousef F**, Cardwell C, Cantwell MM, Galway K, Johnston BT, Murray L. The incidence of esophageal cancer and high-grade dysplasia in Barrett's esophagus: a systematic review

and meta-analysis. *Am J Epidemiol* 2008; **168**: 237-249 [PMID: 18550563 DOI: 10.1093/aje/kwn121]
12 **Jung KW**, Talley NJ, Romero Y, Katzka DA, Schleck CD, Zinsmeister AR, Dunagan KT, Lutzke LS, Wu TT, Wang KK, Frederickson M, Geno DM, Locke GR, Prasad GA. Epidemiology and natural history of intestinal metaplasia of the gastroesophageal junction and Barrett's esophagus: a population-based study. *Am J Gastroenterol* 2011; **106**: 1447-155; quiz 1456 [PMID: 21483461 DOI: 10.1038/ajg.2011.130]
13 **Sikkema M**, Looman CW, Steyerberg EW, Kerkhof M, Kastelein F, van Dekken H, van Vuuren AJ, Bode WA, van der Valk H, Ouwendijk RJ, Giard R, Lesterhuis W, Heinhuis R, Klinkenberg EC, Meijer GA, ter Borg F, Arends JW, Kolkman JJ, van Baarlen J, de Vries RA, Mulder AH, van Tilburg AJ, Offerhaus GJ, ten Kate FJ, Kusters JG, Kuipers EJ, Siersema PD. Predictors for neoplastic progression in patients with Barrett's Esophagus: a prospective cohort study. *Am J Gastroenterol* 2011; **106**: 1231-1238 [PMID: 21577245 DOI: 10.1038/ajg.2011.153]
14 **Weston AP**, Sharma P, Mathur S, Banerjee S, Jafri AK, Cherian R, McGregor D, Hassanein RS, Hall M. Risk stratification of Barrett's esophagus: updated prospective multivariate analysis. *Am J Gastroenterol* 2004; **99**: 1657-1666 [PMID: 15330898 DOI: 10.1111/j.1572-0241.2004.30426.x]
15 **Freedman ND**, Murray LJ, Kamangar F, Abnet CC, Cook MB, Nyrén O, Ye W, Wu AH, Bernstein L, Brown LM, Ward MH, Pandeya N, Green AC, Casson AG, Giffen C, Risch HA, Gammon MD, Chow WH, Vaughan TL, Corley DA, Whiteman DC. Alcohol intake and risk of oesophageal adenocarcinoma: a pooled analysis from the BEACON Consortium. *Gut* 2011; **60**: 1029-1037 [PMID: 21406386 DOI: 10.1136/gut.2010.233866]
16 **Hardikar S**, Onstad L, Blount PL, Odze RD, Reid BJ, Vaughan TL. The role of tobacco, alcohol, and obesity in neoplastic progression to esophageal adenocarcinoma: a prospective study of Barrett's esophagus. *PLoS One* 2013; **8**: e52192 [PMID: 23300966 DOI: 10.1371/journal.pone.0052192]
17 **Smith KJ**, O'Brien SM, Green AC, Webb PM, Whiteman DC. Current and past smoking significantly increase risk for Barrett's esophagus. *Clin Gastroenterol Hepatol* 2009; **7**: 840-848 [PMID: 19410015 DOI: 10.1016/j.cgh.2009.04.018]
18 **Kubo A**, Levin TR, Block G, Rumore G, Quesenberry CP, Buffler P, Corley DA. Cigarette smoking and the risk of Barrett's esophagus. *Cancer Causes Control* 2009; **20**: 303-311 [PMID: 18853262 DOI: 10.1007/s10552-008-9244-4]
19 **Coleman HG**, Bhat S, Johnston BT, McManus D, Gavin AT, Murray LJ. Tobacco smoking increases the risk of high-grade dysplasia and cancer among patients with Barrett's esophagus. *Gastroenterology* 2012; **142**: 233-240 [PMID: 22062359]
20 **Cook MB**, Kamangar F, Whiteman DC, Freedman ND, Gammon MD, Bernstein L, Brown LM, Risch HA, Ye W, Sharp L, Pandeya N, Webb PM, Wu AH, Ward MH, Giffen C, Casson AG, Abnet CC, Murray LJ, Corley DA, Nyrén O, Vaughan TL, Chow WH. Cigarette smoking and adenocarcinomas of the esophagus and esophagogastric junction: a pooled analysis from the international BEACON consortium. *J Natl Cancer Inst* 2010; **102**: 1344-1353 [PMID: 20716718 DOI: 10.1093/jnci/djq289]
21 **Fitzgerald RC**, Triadafilopoulos G. Recent developments in the molecular characterization of Barrett's esophagus. *Dig Dis* 1998; **16**: 63-80 [PMID: 9571372 DOI: 10.1159/000016850]
22 **Wang KK**, Sampliner RE. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. *Am J Gastroenterol* 2008; **103**: 788-797 [PMID: 18341497 DOI: 10.1111/j.1572-0241.2008.01835.x]
23 **Tsibouris P**, Hendrickse MT, Isaacs PE. Daily use of non-steroidal anti-inflammatory drugs is less frequent in patients with Barrett's oesophagus who develop an oesophageal adenocarcinoma. *Aliment Pharmacol Ther* 2004; **20**: 645-655 [PMID: 15352913 DOI: 10.1111/j.1365-2036.2004.02150.x]
24 **Orman ES**, Li N, Shaheen NJ. Efficacy and durability of radiofrequency ablation for Barrett's Esophagus: systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2013; **11**: 1245-1255 [PMID: 23644385 DOI: 10.1016/j.cgh.2013.03.039]
25 **Hur C**, Choi SE, Rubenstein JH, Kong CY, Nishioka NS, Provenzale DT, Inadomi JM. The cost effectiveness of radiofrequency ablation for Barrett's esophagus. *Gastroenterology* 2012; **143**: 567-575 [PMID: 22626608 DOI: 10.1053/j.gastro.2012.05.010]
26 **Sikkema M**, de Jonge PJ, Steyerberg EW, Kuipers EJ. Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2010; **8**: 235-244; quiz e32 [PMID: 19850156 DOI: 10.1016/j.cgh.2009.10.010]
27 **Reid BJ**, Li X, Galipeau PC, Vaughan TL. Barrett's oesophagus and oesophageal adenocarcinoma: time for a new synthesis. *Nat Rev Cancer* 2010; **10**: 87-101 [PMID: 20094044 DOI: 10.1038/nrc2773]
28 **Hur C**, Nishioka NS, Gazelle GS. Cost-effectiveness of aspirin chemoprevention for Barrett's esophagus. *J Natl Cancer Inst* 2004; **96**: 316-325 [PMID: 14970280 DOI: 10.1093/jnci/djh039]
29 **Cheong E**, Ivory K, Doleman J, Parker ML, Rhodes M, Johnson IT. Synthetic and naturally occurring COX-2 inhibitors suppress proliferation in a human oesophageal adenocarcinoma cell line (OE33) by inducing apoptosis and cell cycle arrest. *Carcinogenesis* 2004; **25**: 1945-1952 [PMID: 15155531 DOI: 10.1093/carcin/bgh184]
30 **Buttar NS**, Wang KK, Anderson MA, Dierkhising RA, Pacifico RJ, Krishnadath KK, Lutzke LS. The effect of selective cyclooxygenase-2 inhibition in Barrett's esophagus epithelium: an in vitro study. *J Natl Cancer Inst* 2002; **94**: 422-429 [PMID: 11904314 DOI: 10.1093/jnci/94.6.422]
31 **Buttar NS**, Wang KK, Leontovich O, Westcott JY, Pacifico RJ, Anderson MA, Krishnadath KK, Lutzke LS, Burgart LJ. Chemoprevention of esophageal adenocarcinoma by COX-2 inhibitors in an animal model of Barrett's esophagus. *Gastroenterology* 2002; **122**: 1101-1112 [PMID: 11910360 DOI: 10.1053/gast.2002.32371]
32 **Oyama K**, Fujimura T, Ninomiya I, Miyashita T, Kinami S, Fushida S, Ohta T, Koichi M. A COX-2 inhibitor prevents the esophageal inflammation-metaplasia-adenocarcinoma sequence in rats. *Carcinogenesis* 2005; **26**: 565-570 [PMID: 15564290 DOI: 10.1093/carcin/bgh340]
33 **Esquivias P**, Morandeira A, Escartín A, Cebrián C, Santander S, Esteva F, García-González MA, Ortego J, Lanas A, Piazuelo E. Indomethacin but not a selective cyclooxygenase-2 inhibitor inhibits esophageal adenocarcinogenesis in rats. *World J Gastroenterol* 2012; **18**: 4866-4874 [PMID: 23002358 DOI: 10.3748/wjg.v18.i35.4866]
34 **Corley DA**, Kerlikowske K, Verma R, Buffler P. Protective association of aspirin/NSAIDs and esophageal cancer: a systematic review and meta-analysis. *Gastroenterology* 2003; **124**: 47-56 [PMID: 12512029 DOI: 10.1053/gast.2003.50008]
35 **Tsibouris P**, Hendrickse M, Zissis M, Isaacs P. NSAIDs do not protect patients with Barrett's oesophagus from adenocarcinoma development. *Gastoenterology* 2001; **120**: A595
36 **Abnet CC**, Freedman ND, Kamangar F, Leitzmann MF, Hollenbeck AR, Schatzkin A. Non-steroidal anti-inflammatory drugs and risk of gastric and oesophageal adenocarcinomas: results from a cohort study and a meta-analysis. *Br J Cancer* 2009; **100**: 551-557 [PMID: 19156150 DOI: 10.1038/sj.bjc.6604880]
37 **Beales IL**, Vardi I, Dearman L. Regular statin and aspirin use in patients with Barrett's oesophagus is associated with a reduced incidence of oesophageal adenocarcinoma. *Eur J Gastroenterol Hepatol* 2012; **24**: 917-923 [PMID: 22569083 DOI: 10.1097/MEG.0b013e3283543f01]
38 **Nguyen DM**, El-Serag HB, Henderson L, Stein D, Bhattacharyya A, Sampliner RE. Medication usage and the risk of neo-

plasia in patients with Barrett's esophagus. *Clin Gastroenterol Hepatol* 2009; **7**: 1299-1304 [PMID: 19523538 DOI: 10.1016/j.cgh.2009.06.001]

39 **Vaughan TL**, Dong LM, Blount PL, Ayub K, Odze RD, Sanchez CA, Rabinovitch PS, Reid BJ. Non-steroidal anti-inflammatory drugs and risk of neoplastic progression in Barrett's oesophagus: a prospective study. *Lancet Oncol* 2005; **6**: 945-952 [PMID: 16321762 DOI: 10.1016/S1470-2045(05)70431-9]

40 **Kastelein F**, Spaander MC, Biermann K, Steyerberg EW, Kuipers EJ, Bruno MJ. Nonsteroidal anti-inflammatory drugs and statins have chemopreventative effects in patients with Barrett's esophagus. *Gastroenterology* 2011; **141**: 2000-2008; quiz e13- e14; [PMID: 21878200 DOI: 10.1053/j.gastro.2011.08.036]

41 **Nguyen DM**, Richardson P, El-Serag HB. Medications (NSAIDs, statins, proton pump inhibitors) and the risk of esophageal adenocarcinoma in patients with Barrett's esophagus. *Gastroenterology* 2010; **138**: 2260-2266 [PMID: 20188100 DOI: 10.1053/j.gastro.2010.02.045]

42 **Gatenby PA**, Ramus JR, Caygill CP, Winslet MC, Watson A. Aspirin is not chemoprotective for Barrett's adenocarcinoma of the oesophagus in multicentre cohort. *Eur J Cancer Prev* 2009; **18**: 381-384 [PMID: 19620873 DOI: 10.1097/CEJ.0b013e32832e0955]

43 **Kantor ED**, Onstad L, Blount PL, Reid BJ, Vaughan TL. Use of statin medications and risk of esophageal adenocarcinoma in persons with Barrett's esophagus. *Cancer Epidemiol Biomarkers Prev* 2012; **21**: 456-461 [PMID: 22241250 DOI: 10.1158/1055-9965.EPI-11-1014]

44 **Jankowski J**, Sharma P. Review article: approaches to Barrett's oesophagus treatment-the role of proton pump inhibitors and other interventions. *Aliment Pharmacol Ther* 2004; **19** Suppl 1: 54-59 [PMID: 14725580 DOI: 10.1111/j.0953-0673.2004.01839.x]

45 **Spechler SJ**, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American Gastroenterological Association technical review on the management of Barrett's esophagus. *Gastroenterology* 2011; **140**: e18-52; quiz e13 [PMID: 21376939 DOI: 10.1053/j.gastro.2011.01.031]

46 **Heath EI**, Canto MI, Piantadosi S, Montgomery E, Weinstein WM, Herman JG, Dannenberg AJ, Yang VW, Shar AO, Hawk E, Forastiere AA. Secondary chemoprevention of Barrett's esophagus with celecoxib: results of a randomized trial. *J Natl Cancer Inst* 2007; **99**: 545-557 [PMID: 17405999 DOI: 10.1093/jnci/djk112]

47 **Liao LM**, Vaughan TL, Corley DA, Cook MB, Casson AG, Kamangar F, Abnet CC, Risch HA, Giffen C, Freedman ND, Chow WH, Sadeghi S, Pandeya N, Whiteman DC, Murray LJ, Bernstein L, Gammon MD, Wu AH. Nonsteroidal anti-inflammatory drug use reduces risk of adenocarcinomas of the esophagus and esophagogastric junction in a pooled analysis. *Gastroenterology* 2012; **142**: 442-452.e5; quiz e22-23 [PMID: 22108196 DOI: 10.1053/j.gastro.2011.11.019]

48 **Beales IL**, Hensley A, Loke Y. Reduced esophageal cancer incidence in statin users, particularly with cyclo-oxygenase inhibition. *World J Gastrointest Pharmacol Ther* 2013; **4**: 69-79 [PMID: 23919219 DOI: 10.4292/wjgpt.v4.i3.69]

49 **Patterson RE**, Neuhouser ML, White E, Hunt JR, Kristal AR. Cancer-related behavior of vitamin supplement users. *Cancer Epidemiol Biomarkers Prev* 1998; **7**: 79-81 [PMID: 9456246]

50 **Hur C**, Broughton DE, Ozanne E, Yachimski P, Nishioka NS, Gazelle GS. Patient preferences for the chemoprevention of esophageal adenocarcinoma in Barrett's esophagus. *Am J Gastroenterol* 2008; **103**: 2432-2442 [PMID: 18775019 DOI: 10.1111/j.1572-0241.2008.02117.x]

51 **Antithrombotic Trialists' Collaboration**. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ* 2002; **324**: 71-86 [PMID: 11786451 DOI: 10.1136/bmj.324.7329.71]

52 **Hayden M**, Pignone M, Phillips C, Mulrow C. Aspirin for the primary prevention of cardiovascular events: a summary of the evidence for the U.S. Preventive Services Task Force. *Ann Intern Med* 2002; **136**: 161-172 [PMID: 11790072 DOI: 10.7326/0003-4819-136-2-200201150-00061]

53 **Tsibouris P**. Ischemic heart disease, factor predisposing to Barrett's adenocarcinoma [Abstract]. *Gut* 2000; **47** Suppl III: A69

54 **Galipeau PC**, Li X, Blount PL, Maley CC, Sanchez CA, Odze RD, Ayub K, Rabinovitch PS, Vaughan TL, Reid BJ. NSAIDs modulate CDKN2A, TP53, and DNA content risk for progression to esophageal adenocarcinoma. *PLoS Med* 2007; **4**: e67 [PMID: 17326708 DOI: 10.1371/journal.pmed.0040067]

55 **Auvinen MI**, Sihvo EI, Ruohtula T, Salminen JT, Koivistoinen A, Siivola P, Rönnholm R, Rämö JO, Bergman M, Salo JA. Incipient angiogenesis in Barrett's epithelium and lymphangiogenesis in Barrett's adenocarcinoma. *J Clin Oncol* 2002; **20**: 2971-2979 [PMID: 12089227 DOI: 10.1200/JCO.2002.09.011]

56 **Yan X**, Wu Xiao C, Sun M, Tsang BK, Gibb W. Nuclear factor kappa B activation and regulation of cyclooxygenase type-2 expression in human amnion mesenchymal cells by interleukin-1beta. *Biol Reprod* 2002; **66**: 1667-1671 [PMID: 12021045 DOI: 10.1095/biolreprod66.6.1667]

57 **Karin M**, Cao Y, Greten FR, Li ZW. NF-kappaB in cancer: from innocent bystander to major culprit. *Nat Rev Cancer* 2002; **2**: 301-310 [PMID: 12001991 DOI: 10.1038/nrc780]

58 **Tazawa R**, Xu XM, Wu KK, Wang LH. Characterization of the genomic structure, chromosomal location and promoter of human prostaglandin *H synthase-2* gene. *Biochem Biophys Res Commun* 1994; **203**: 190-199 [PMID: 8074655 DOI: 10.1006/bbrc.1994.2167]

59 **Ohshima H**, Tatemichi M, Sawa T. Chemical basis of inflammation-induced carcinogenesis. *Arch Biochem Biophys* 2003; **417**: 3-11 [PMID: 12921773 DOI: 10.1016/S0003-9861(03)00283-2]

60 **Zhou X**, Li D, Resnick MB, Behar J, Wands J, Cao W. Signaling in H2O2-induced increase in cell proliferation in Barrett's esophageal adenocarcinoma cells. *J Pharmacol Exp Ther* 2011; **339**: 218-227 [PMID: 21750116]

61 **Aiyer HS**, Li Y, Harper N, Myers SR, Martin RC. Molecular changes in the esophageal epithelium after a subchronic exposure to cigarette smoke in the presence of bile-acid reflux. *Inhal Toxicol* 2011; **23**: 304-311 [PMID: 21506880 DOI: 10.3109/08958378.2011.568977]

62 **Demaurex N**, Lew DP, Krause KH. Cyclopiazonic acid depletes intracellular Ca2+ stores and activates an influx pathway for divalent cations in HL-60 cells. *J Biol Chem* 1992; **267**: 2318-2324 [PMID: 1531138]

63 **Kim H**, Rhee SH, Kokkotou E, Na X, Savidge T, Moyer MP, Pothoulakis C, LaMont JT. Clostridium difficile toxin A regulates inducible cyclooxygenase-2 and prostaglandin E2 synthesis in colonocytes via reactive oxygen species and activation of p38 MAPK. *J Biol Chem* 2005; **280**: 21237-21245 [PMID: 15767259 DOI: 10.1074/jbc.M413842200]

64 **Flohé L**, Brigelius-Flohé R, Saliou C, Traber MG, Packer L. Redox regulation of NF-kappa B activation. *Free Radic Biol Med* 1997; **22**: 1115-1126 [PMID: 9034250 DOI: 10.1016/S0891-5849(96)00501-1]

65 **Shirvani VN**, Ouatu-Lascar R, Kaur BS, Omary MB, Triadafilopoulos G. Cyclooxygenase 2 expression in Barrett's esophagus and adenocarcinoma: Ex vivo induction by bile salts and acid exposure. *Gastroenterology* 2000; **118**: 487-496 [PMID: 10702199 DOI: 10.1016/S0016-5085(00)70254-X]

66 **Lanas A**, Ortego J, Sopeña F, Alcedo J, Barrio E, Bujanda L, Cosme A, Bajador E, Parra-Blanco A, Ferrandez A, Piazuelo E, Quintero E, Pique JM. Effects of long-term cyclo-oxygenase 2 selective and acid inhibition on Barrett's oesophagus. *Aliment Pharmacol Ther* 2007; **26**: 913-923 [PMID: 17767476 DOI: 10.1111/j.1365-2036.2007.03429.x]

67 **Chandrasekharan NV**, Simmons DL. The cyclooxygenases.

Genome Biol 2004; **5**: 241 [PMID: 15345041 DOI: 10.1186/gb-2004-5-9-241]
68 **Zhou X**, Li D, Resnick MB, Wands J, Cao W. NADPH oxidase NOX5-S and nuclear factor κB1 mediate acid-induced microsomal prostaglandin E synthase-1 expression in Barrett's esophageal adenocarcinoma cells. *Mol Pharmacol* 2013; **83**: 978-990 [PMID: 23439561 DOI: 10.1124/mol.112.083287]
69 **Si J**, Fu X, Behar J, Wands J, Beer DG, Souza RF, Spechler SJ, Lambeth D, Cao W. NADPH oxidase NOX5-S mediates acid-induced cyclooxygenase-2 expression via activation of NF-kappaB in Barrett's esophageal adenocarcinoma cells. *J Biol Chem* 2007; **282**: 16244-16255 [PMID: 17403674 DOI: 10.1074/jbc.M700297200]
70 **Breyer RM**, Bagdassarian CK, Myers SA, Breyer MD. Prostanoid receptors: subtypes and signaling. *Annu Rev Pharmacol Toxicol* 2001; **41**: 661-690 [PMID: 11264472 DOI: 10.1146/annurev.pharmtox.41.1.661]
71 **Grossmann J**. Molecular mechanisms of "detachment-induced apoptosis--Anoikis". *Apoptosis* 2002; **7**: 247-260 [PMID: 11997669 DOI: 10.1023/A:1015312119693]
72 **Stewart ZA**, Westfall MD, Pietenpol JA. Cell-cycle dysregulation and anticancer therapy. *Trends Pharmacol Sci* 2003; **24**: 139-145 [PMID: 12628359 DOI: 10.1016/S0165-6147(03)00026-9]
73 **Cobrinik D**. Pocket proteins and cell cycle control. *Oncogene* 2005; **24**: 2796-2809 [PMID: 15838516 DOI: 10.1038/sj.onc.1208619]
74 **Kelman Z**. PCNA: structure, functions and interactions. *Oncogene* 1997; **14**: 629-640 [PMID: 9038370 DOI: 10.1038/sj.onc.1200886]
75 **Taylor WR**, Stark GR. Regulation of the G2/M transition by p53. *Oncogene* 2001; **20**: 1803-1815 [PMID: 11313928 DOI: 10.1038/sj.onc.1204252]
76 **Lagarde SM**, ten Kate FJ, Richel DJ, Offerhaus GJ, van Lanschot JJ. Molecular prognostic factors in adenocarcinoma of the esophagus and gastroesophageal junction. *Ann Surg Oncol* 2007; **14**: 977-991 [PMID: 17122988 DOI: 10.1245/s10434-006-9262-y]
77 **Kaur BS**, Triadafilopoulos G. Acid- and bile-induced PGE(2) release and hyperproliferation in Barrett's esophagus are COX-2 and PKC-epsilon dependent. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G327-G334 [PMID: 12121879]
78 **Shiff SJ**, Koutsos MI, Qiao L, Rigas B. Nonsteroidal antiinflammatory drugs inhibit the proliferation of colon adenocarcinoma cells: effects on cell cycle and apoptosis. *Exp Cell Res* 1996; **222**: 179-188 [PMID: 8549662 DOI: 10.1006/excr.1996.0023]
79 **Elder DJ**, Hague A, Hicks DJ, Paraskeva C. Differential growth inhibition by the aspirin metabolite salicylate in human colorectal tumor cell lines: enhanced apoptosis in carcinoma and in vitro-transformed adenoma relative to adenoma relative to adenoma cell lines. *Cancer Res* 1996; **56**: 2273-2276 [PMID: 8625297]
80 **Burlacu A**. Regulation of apoptosis by Bcl-2 family proteins. *J Cell Mol Med* 1996; **7**: 249-257 [PMID: 14594549 DOI: 10.1111/j.1582-4934.2003.tb00225.x]
81 **Coppola S**, Ghibelli L. GSH extrusion and and the mitochondrial pathway of apoptotic signalling. *Biochem Soc Trans* 2000; **28**: 56-61 [PMID: 10816099]
82 **Cha YJ**, Kim HS, Rhim H, Kim BE, Jeong SW, Kim IK. Activation of caspase-8 in 3-deazaadenosine-induced apoptosis of U-937 cells occurs downstream of caspase-3 and caspase-9 without Fas receptor-ligand interaction. *Exp Mol Med* 2001; **33**: 284-292 [PMID: 11795493 DOI: 10.1038/emm.2001.46]
83 **Tsujii M**, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell* 1995; **83**: 493-501 [PMID: 8521479 DOI: 10.1016/0092-8674(95)90127-2]
84 **Shimizu D**, Vallböhmer D, Kuramochi H, Uchida K, Schneider S, Chandrasoma PT, Shimada H, DeMeester TR, Danenberg KD, Peters JH, DeMeester SR, Danenberg PV. Increasing cyclooxygenase-2 (cox-2) gene expression in the progression of Barrett's esophagus to adenocarcinoma correlates with that of Bcl-2. *Int J Cancer* 2006; **119**: 765-770 [PMID: 16550596 DOI: 10.1002/ijc.21922]
85 **Giancotti FG**, Ruoslahti E. Integrin signaling. *Science* 1999; **285**: 1028-1032 [PMID: 10446041 DOI: 10.1126/science.285.5430.1028]
86 **Sarosi GA**, Jaiswal K, Herndon E, Lopez-Guzman C, Spechler SJ, Souza RF. Acid increases MAPK-mediated proliferation in Barrett's esophageal adenocarcinoma cells via intracellular acidification through a Cl-/HCO3- exchanger. *Am J Physiol Gastrointest Liver Physiol* 2005; **289**: G991-G997 [PMID: 16081761 DOI: 10.1152/ajpgi.00215.2005]
87 **Souza RF**, Shewmake K, Beer DG, Cryer B, Spechler SJ. Selective inhibition of cyclooxygenase-2 suppresses growth and induces apoptosis in human esophageal adenocarcinoma cells. *Cancer Res* 2000; **60**: 5767-5772 [PMID: 11059772]
88 **Wilson KT**, Fu S, Ramanujam KS, Meltzer SJ. Increased expression of inducible nitric oxide synthase and cyclooxygenase-2 in Barrett's esophagus and associated adenocarcinomas. *Cancer Res* 1998; **58**: 2929-2934 [PMID: 9679948]
89 **Zimmermann KC**, Sarbia M, Weber AA, Borchard F, Gabbert HE, Schrör K. Cyclooxygenase-2 expression in human esophageal carcinoma. *Cancer Res* 1999; **59**: 198-204 [PMID: 9892207]
90 **Buskens CJ**, Ristimäki A, Offerhaus GJ, Richel DJ, van Lanschot JJ. Role of cyclooxygenase-2 in the development and treatment of oesophageal adenocarcinoma. *Scand J Gastroenterol Suppl* 2003; **(239)**: 87-93 [PMID: 14743889 DOI: 10.1080/00855920310002753]
91 **Jones MK**, Wang H, Peskar BM, Levin E, Itani RM, Sarfeh IJ, Tarnawski AS. Inhibition of angiogenesis by nonsteroidal anti-inflammatory drugs: insight into mechanisms and implications for cancer growth and ulcer healing. *Nat Med* 1999; **5**: 1418-1423 [PMID: 10581086]
92 **Zhou XL**, Wan L, Xu QR, Zhao Y, Liu JC. Notch signaling activation contributes to cardioprotection provided by ischemic preconditioning and postconditioning. *J Transl Med* 2013; **11**: 251 [PMID: 24098939 DOI: 10.1186/1479-5876-11-251]
93 **Iribarren C**, Phelps BH, Darbinian JA, McCluskey ER, Quesenberry CP, Hytopoulos E, Vogelman JH, Orentreich N. Circulating angiopoietins-1 and -2, angiopoietin receptor Tie-2 and vascular endothelial growth factor-A as biomarkers of acute myocardial infarction: a prospective nested case-control study. *BMC Cardiovasc Disord* 2011; **11**: 31 [PMID: 21672190 DOI: 10.1186/1471-2261-11-31]
94 **Liang J**, Slingerland JM. Multiple roles of the PI3K/PKB (Akt) pathway in cell cycle progression. *Cell Cycle* 2003; **2**: 339-345 [PMID: 12851486 DOI: 10.4161/cc.2.4.433]
95 **Totzke G**, Schulze-Osthoff K, Jänicke RU. Cyclooxygenase-2 (COX-2) inhibitors sensitize tumor cells specifically to death receptor-induced apoptosis independently of COX-2 inhibition. *Oncogene* 2003; **22**: 8021-8030 [PMID: 12970750]
96 **Aggarwal S**, Taneja N, Lin L, Orringer MB, Rehemtulla A, Beer DG. Indomethacin-induced apoptosis in esophageal adenocarcinoma cells involves upregulation of Bax and translocation of mitochondrial cytochrome C independent of COX-2 expression. *Neoplasia* 2000; **2**: 346-356 [PMID: 11005569 DOI: 10.1038/sj.neo.7900097]
97 **Ryan EP**, Bushnell TP, Friedman AE, Rahman I, Phipps RP. Cyclooxygenase-2 independent effects of cyclooxygenase-2 inhibitors on oxidative stress and intracellular glutathione content in normal and malignant human B-cells. *Cancer Immunol Immunother* 2008; **57**: 347-358 [PMID: 17668203 DOI: 10.1007/s00262-007-0374-4]
98 **Konturek PC**, Kania J, Burnat G, Hahn EG. NO-releasing aspirin exerts stronger growth inhibitory effect on Barrett's adenocarcinoma cells than traditional aspirin. *J Physiol Pharmacol* 2006; **57** Suppl 12: 15-24 [PMID: 17244951]
99 **McQuaid KR**, Laine L. Systematic review and meta-analysis

of adverse events of low-dose aspirin and clopidogrel in randomized controlled trials. *Am J Med* 2006; **119**: 624-638 [PMID: 16887404 DOI: 10.1016/j.amjmed.2005.10.039]

100 **Chan FK**, Chung SC, Suen BY, Lee YT, Leung WK, Leung VK, Wu JC, Lau JY, Hui Y, Lai MS, Chan HL, Sung JJ. Preventing recurrent upper gastrointestinal bleeding in patients with Helicobacter pylori infection who are taking low-dose aspirin or naproxen. *N Engl J Med* 2001; **344**: 967-973 [PMID: 11274623 DOI: 10.1056/NEJM200103293441304]

101 **Lai KC**, Lam SK, Chu KM, Wong BC, Hui WM, Hu WH, Lau GK, Wong WM, Yuen MF, Chan AO, Lai CL, Wong J. Lansoprazole for the prevention of recurrences of ulcer complications from long-term low-dose aspirin use. *N Engl J Med* 2002; **346**: 2033-2038 [PMID: 12087138 DOI: 10.1056/NEJMoa012877]

102 **Hillman LC**, Chiragakis L, Clarke AC, Kaushik SP, Kaye GL. Barrett's esophagus: Macroscopic markers and the prediction of dysplasia and adenocarcinoma. *J Gastroenterol Hepatol* 2003; **18**: 526-533 [PMID: 12702044 DOI: 10.1046/j.1440-1746.2003.02965.x]

103 **Kearney PM**, Baigent C, Godwin J, Halls H, Emberson JR, Patrono C. Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials. *BMJ* 2006; **332**: 1302-1308 [PMID: 16740558 DOI: 10.1136/bmj.332.7553.1302]

104 **Bhala N**, Emberson J, Merhi A, Abramson S, Arber N, Baron JA, Bombardier C, Cannon C, Farkouh ME, FitzGerald GA, Goss P, Halls H, Hawk E, Hawkey C, Hennekens C, Hochberg M, Holland LE, Kearney PM, Laine L, Lanas A, Lance P, Laupacis A, Oates J, Patrono C, Schnitzer TJ, Solomon S, Tugwell P, Wilson K, Wittes J, Baigent C. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. *Lancet* 2013; **382**: 769-779 [PMID: 23726390 DOI: 10.1016/S0140-6736(13)60900-9]

105 **Abraham P**, K I, K D. Nitro-arginine methyl ester, a non-selective inhibitor of nitric oxide synthase reduces ibuprofen-induced gastric mucosal injury in the rat. *Dig Dis Sci* 2005; **50**: 1632-1640 [PMID: 16133962 DOI: 10.1007/s10620-005-2908-y]

106 **Ogunwobi OO**, Beales IL. Statins inhibit proliferation and induce apoptosis in Barrett's esophageal adenocarcinoma cells. *Am J Gastroenterol* 2008; **103**: 825-837 [PMID: 18371146 DOI: 10.1111/j.1572-0241.2007.01773.x]

107 **Konturek PC**, Burnat G, Hahn EG. Inhibition of Barret's adenocarcinoma cell growth by simvastatin: involvement of COX-2 and apoptosis-related proteins. *J Physiol Pharmacol* 2007; **58** Suppl 3: 141-148 [PMID: 17901590]

108 **Sadaria MR**, Reppert AE, Yu JA, Meng X, Fullerton DA, Reece TB, Weyant MJ. Statin therapy attenuates growth and malignant potential of human esophageal adenocarcinoma cells. *J Thorac Cardiovasc Surg* 2011; **142**: 1152-1160 [PMID: 22014341 DOI: 10.1016/j.jtcvs.2011.08.004]

109 **Hippisley-Cox J**, Coupland C. Unintended effects of statins in men and women in England and Wales: population based cohort study using the QResearch database. *BMJ* 2010; **340**: c2197 [PMID: 20488911 DOI: 10.1136/bmj.c2197]

110 **Vinogradova Y**, Coupland C, Hippisley-Cox J. Exposure to statins and risk of common cancers: a series of nested case-control studies. *BMC Cancer* 2011; **11**: 409 [PMID: 21943022 DOI: 10.1186/1471-2407-11-409]

111 **Kaye JA**, Jick H. Statin use and cancer risk in the General Practice Research Database. *Br J Cancer* 2004; **90**: 635-637 [PMID: 14760377 DOI: 10.1038/sj.bjc.6601566]

112 **Bhutta H**, Clark A, Holt S, Lewis M, Hart A. Oesophageal cancer – an aetiological investigation into the potential protective effect of statins in the UK general practice research database (GPRD). *Gut* 2011; **60**: A36–A37 [DOI: 10.1136/gut.2011.239301.72]

113 **Friedman GD**, Flick ED, Udaltsova N, Chan J, Quesenberry CP, Habel LA. Screening statins for possible carcinogenic risk: up to 9 years of follow-up of 361,859 recipients. *Pharmacoepidemiol Drug Saf* 2008; **17**: 27-36 [PMID: 17944002 DOI: 10.1002/pds.1507]

114 **Alexandre L**, Clark AB, Cheong E, Lewis MP, Hart AR. Systematic review: potential preventive effects of statins against oesophageal adenocarcinoma. *Aliment Pharmacol Ther* 2012; **36**: 301-311 [PMID: 22716127 DOI: 10.1111/j.1365-2036.2012.05194.x]

115 **Lindblad M**, Rodríguez LA, Lagergren J. Body mass, tobacco and alcohol and risk of esophageal, gastric cardia, and gastric non-cardia adenocarcinoma among men and women in a nested case-control study. *Cancer Causes Control* 2005; **16**: 285-294 [PMID: 15947880 DOI: 10.1007/s10552-004-3485-7]

116 **Maron DJ**, Fazio S, Linton MF. Current perspectives on statins. *Circulation* 2000; **101**: 207-213 [PMID: 10637210 DOI: 10.1161/01.CIR.101.2.207]

117 **Edwards PA**, Ericsson J. Sterols and isoprenoids: signaling molecules derived from the cholesterol biosynthetic pathway. *Annu Rev Biochem* 1999; **68**: 157-185 [PMID: 10872447 DOI: 10.1146/annurev.biochem.68.1.157]

118 **Hawk ET**, Viner JL. Statins in esophageal cancer cell lines: promising lead? *Am J Gastroenterol* 2008; **103**: 838-841 [PMID: 18371147 DOI: 10.1111/j.1572-0241.2007.01768.x]

119 **Hillman LC**, Chiragakis L, Shadbolt B, Kaye GL, Clarke AC. Effect of proton pump inhibitors on markers of risk for high-grade dysplasia and oesophageal cancer in Barrett's oesophagus. *Aliment Pharmacol Ther* 2008; **27**: 321-326 [PMID: 18047565 DOI: 10.1111/j.1365-2036.2007.03579.x]

120 **Fang D**, Das KM, Cao W, Malhotra U, Triadafilopoulos G, Najarian RM, Hardie LJ, Lightdale CJ, Beales IL, Felix VN, Schneider PM, Bellizzi AM. Barrett's esophagus: progression to adenocarcinoma and markers. *Ann N Y Acad Sci* 2011; **1232**: 210-229 [PMID: 21950815 DOI: 10.1111/j.1749-6632.2011.06053.x]

121 **Hayes SH**, Seigel GM. Immunoreactivity of ICAM-1 in human tumors, metastases and normal tissues. *Int J Clin Exp Pathol* 2009; **2**: 553-560 [PMID: 19636402]

122 **Weis M**, Heeschen C, Glassford AJ, Cooke JP. Statins have biphasic effects on angiogenesis. *Circulation* 2002; **105**: 739-745 [PMID: 11839631 DOI: 10.1161/hc0602.103393]

123 **Roland CL**, Harken AH, Sarr MG, Barnett CC. ICAM-1 expression determines malignant potential of cancer. *Surgery* 2007; **141**: 705-707 [PMID: 17560245 DOI: 10.1016/j.surg.2007.01.016]

124 **Lin YC**, Shun CT, Wu MS, Chen CC. A novel anticancer effect of thalidomide: inhibition of intercellular adhesion molecule-1-mediated cell invasion and metastasis through suppression of nuclear factor-kappaB. *Clin Cancer Res* 2006; **12**: 7165-7173 [PMID: 17145842 DOI: 10.1158/1078-0432.CCR-06-1393]

125 **Looby E**, Abdel-Latif MM, Athié-Morales V, Duggan S, Long A, Kelleher D. Deoxycholate induces COX-2 expression via Erk1/2-, p38-MAPK and AP-1-dependent mechanisms in esophageal cancer cells. *BMC Cancer* 2009; **9**: 190 [PMID: 19534809 DOI: 10.1186/1471-2407-9-190]

126 **Konturek PC**, Burnat G, Rau T, Hahn EG, Konturek S. Effect of adiponectin and ghrelin on apoptosis of Barrett adenocarcinoma cell line. *Dig Dis Sci* 2008; **53**: 597-605 [PMID: 17763959 DOI: 10.1007/s10620-007-9922-1]

127 **Armitage J**. The safety of statins in clinical practice. *Lancet* 2007; **370**: 1781-1790 [PMID: 17559928 DOI: 10.1016/S0140-6736(07)60716-8]

128 **Jacobson TA**. Toward "pain-free" statin prescribing: clinical algorithm for diagnosis and management of myalgia. *Mayo Clin Proc* 2008; **83**: 687-700 [PMID: 18533086 DOI: 10.4065/83.6.687]

129 **Omar MA**, Wilson JP, Cox TS. Rhabdomyolysis and HMG-CoA reductase inhibitors. *Ann Pharmacother* 2001; **35**: 1096-1107 [PMID: 11573861 DOI: 10.1345/aph.10228]

130 **Law M**, Rudnicka AR. Statin safety: a systematic review. *Am J Cardiol* 2006; **97**: 52C-60C [PMID: 16581329 DOI: 10.1016/j.amjcard.2005.12.010]
131 **Davidson MH**, Robinson JG. Safety of aggressive lipid management. *J Am Coll Cardiol* 2007; **49**: 1753-1762 [PMID: 17466224 DOI: 10.1016/j.jacc.2007.01.067]
132 **Bader T**. The myth of statin-induced hepatotoxicity. *Am J Gastroenterol* 2010; **105**: 978-980 [PMID: 20445507 DOI: 10.1038/ajg.2010.102]
133 **Pasternak RC**, Smith SC, Bairey-Merz CN, Grundy SM, Cleeman JI, Lenfant C. ACC/AHA/NHLBI clinical advisory on the use and safety of statins. *J Am Coll Cardiol* 2002; **40**: 567-572 [PMID: 12142128 DOI: 10.1016/S0735-1097(02)02030-2]

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MINIREVIEWS

Aspirin, cyclooxygenase inhibition and colorectal cancer

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Abstract

Colorectal cancer (CRC) is the third most common type of cancer worldwide. Screening measures are far from adequate and not widely available in resource-poor settings. Primary prevention strategies therefore remain necessary to reduce the risk of developing CRC. Increasing evidence from epidemiological studies, randomized clinical trials and basic science supports the effectiveness of aspirin, as well as other non-steroidal anti-inflammatory drugs, for chemoprevention of several types of cancer, including CRC. This includes the prevention of adenoma recurrence and reduction of CRC incidence and mortality. The detectable benefit of daily low-dose aspirin (at least 75 mg), as used to prevent cardiovascular disease events, strongly suggests that its antiplatelet action is central to explaining its antitumor efficacy. Daily low-dose aspirin achieves complete and persistent inhibition of cyclooxygenase (COX)-1 in platelets (in pre-systemic circulation) while causing a limited and rapidly reversible inhibitory effect on COX-2 and/or COX-1 expressed in nucleated cells. Aspirin has a short half-life in human circulation (about 20 minutes); nucleated cells have the ability to resynthesize acetylated COX isozymes within a few hours, while platelets do not. COX-independent mechanisms of aspirin have been suggested to explain its chemopreventive effects but this concept remains to be demonstrated *in vivo* at clinical doses.

Key words: Aspirin; Colorectal cancer; Cyclooxygenase inhibition; Mechanisms; Risk; Benefits

Core tip: Colorectal cancer (CRC) is a major cause of morbidity and mortality worldwide. Currently, CRC screening programs are not widely available and need to be improved. New prevention strategies are therefore necessary. Daily low-dose aspirin, as given for the prevention of cardiovascular disease events, has demonstrated benefits in clinical and basic studies in terms of preventing adenoma recurrence and decreasing the incidence of CRC and attributable mortality. These findings indicate that the antiplatelet action of aspirin plays a central role in its antitumor effect. Cyclooxygenase-dependent and independent mechanisms have been suggested to explain this effect. Extensive translational medical research is mandatory for future progress in CRC prevention.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common can-

cer worldwide, accounting for an estimated 9.8% of all new cancers (1.2 million cases annually) and 8.1% of all cancer mortality[1]. It arises through the cumulative effects of inherited genetic predisposition and environmental factors. Genomic instability is an integral part of the transformation of normal colonic or rectal mucosa into carcinoma. Three molecular pathways have been identified: chromosomal instability, microsatellite instability and CpG island methylator phenotype pathways. These pathways are not mutually exclusive, with some tumors exhibiting features of multiple pathways. Germline mutations are responsible for hereditary CRC syndromes (accounting for less than 5% of all CRC), while a stepwise accumulation of genetic and epigenetic alterations results in sporadic CRC.

Today it is well known that screening reduces CRC mortality and is recommended, beginning at age 50, for average risk individuals, although compliance is far from adequate and screening is not widely available in resource-poor settings[2,3]. Primary prevention strategies are therefore still necessary to reduce the risk of CRC, especially because of the limitations of population-based secondary prevention programs that rely on detection and removal of adenomas.

Aspirin has demonstrated its efficacy in the prevention of adverse events related to cardiovascular disease (CVD). It is one of the most widely used drugs in the world. One survey suggested that over one-third of the United States adult population use low-dose aspirin (LDA) regularly[4]. In England in 2007, over 30 million primary care prescriptions were issued for aspirin[5]. Hence, both physicians and patients are largely familiar with the long-term use of aspirin for chronic disease management. In addition, CRC and CVD share the same risk factors, such as older age, being overweight/obesity and physical inactivity.

Today, a large body of clinical and experimental evidence indicates that aspirin can protect against different types of cancer, in particular CRC[6]. A role of the anti-platelet effect of aspirin in its anti-cancer effect is also supported by several studies.

In this review we will discuss clinical results related to the impact of aspirin on the risk of CRC. Then, we will explain the pharmacology of aspirin at low doses in order to provide a mechanistic interpretation of aspirin action as a chemopreventive agent for CRC, in particular the selective inhibition of platelet cyclooxygenase (COX)-1 activity.

CLINICAL EFFECTS OF ASPIRIN ON SPORADIC CRC

Evidence from epidemiological studies

Most case-control and cohort studies have found that regular aspirin use was associated with reduced risk of CRC[7]. A systematic review of case-control studies published in 2012 showed a statistically significant reduction of long-term risk of developing CRC (OR = 0.62, 95%CI: 0.58-0.67) in regular aspirin users compared with non-users, as well as a significant reduction in the proportion of cancers with distant metastasis at diagnosis (OR = 0.69, 95%CI: 0.57-0.83)[8] (Table 1). An analysis of 662424 men and women enrolled in the Cancer Prevention Study Ⅱ cohort showed that daily use of aspirin for at least 5 years was associated with a 32% reduction in risk of CRC[9]. Two cohort studies of United States health professionals (47363 men and 82911 women) showed that regular aspirin users (≥ 2 times/wk) had 21% and 23% lower risk of CRC, respectively, during follow-up periods of 18 and 20 years respectively[10,11]. Moreover, in a separate analysis of a Nurses Health Study cohort, regular aspirin use also reduced the risk of death from CRC by 28% and risk of death from any type of cancer by 12%[12].

Table 1 Summary of the associations between regular use of aspirin and risk of colorectal cancer in case-control and cohort studies

Study type	*n*	Aspirin	Controls	OR (95%CI)	*P* value
Case-control					
Any ASA	26	10464/25618	28300/47834	0.67 (0.60-0.74)	< 0.0001
Maximum reported ASA	17	1551/12659	2664/18153	0.62 (0.58-0.67)	< 0.0001
ASA ≥ 5 yr	10	971/7682	1534/10029	0.68 (0.63-0.75)	< 0.0001
Daily ASA	4	165/1254	349/1523	0.49 (0.40-0.60)	< 0.0001
Daily ASA ≥ 5 yr	1	66/1668	121/1973	0.63 (0.46-0.86)	0.004
Standard cohort					
Any Aspirin	11	3791/2764414	3623/2514652	0.85 (0.82-0.89)	< 0.0001
Maximum reported ASA	8	661/664475	1858/1374905	0.78 (0.71-0.84)	< 0.0001
ASA ≥ 5 yr	4	889/1 022192	1311/1304760	0.76 (0.70-0.82)	< 0.0001
Daily ASA	5	741/658536	1115/819288	0.80 (0.73-0.88)	< 0.0001
Daily ASA ≥ 5 yr	1	60/38302	420/232116	0.68 (0.52-0.90)	0.0060
Nested case-control					
Any ASA	6	2215/8926	65 099/109526	0.87 (0.75-1.00)	0.0700
Maximum reported ASA	5	206/4457	8302/40948	0.67 (0.58-0.77)	< 0.0001
ASA ≥ 5 yr	1	116/228	23704/37935	0.62 (0.48-0.81)	< 0.0001
Daily ASA	1	53/165	8744/22975	0.77 (0.55-1.07)	0.1400
Daily ASA ≥ 5 yr	1	29/141	7274/21505	0.51 (0.34-0.76)	0.0120

Modified from Algra *et al*[8]. Estimates from standard cohort studies are based on results adjusted for age and other baseline clinical characteristics. ASA: Aspirin.

Evidence from clinical trials

In 2010, Rothwell *et al*[13] obtained long-term follow-up data on cancer outcomes from four randomised trials that were originally designed to evaluate the effect of aspirin on the prevention of CVD events (Table 2). These trials studied diverse populations with CVD, including men at low risk (n = 10,224) and men and women at high risk (n = 3809). Dosage ranged from 75-1200 mg/d, median treatment duration was 6 years and median follow-up was 18.3 years. Treatment with aspirin (75-500 mg/d) reduced the 20-year risk of CRC by 24% and CRC-associated

Table 2 Characteristics of trials included in Rothwell *et al* study and details of post-trial follow-up

	Thromobosis prevention trial	Swedish aspirin low dose trial	UK-TIA aspirin trial	British doctors aspirin trial
ASA comparison	75 mg/d *vs* placebo	75 mg/d *vs* placebo	300 mg *vs* 1200 mg/d *vs* placebo	500 mg/d *vs* placebo
Recruitment period	1989-1992	1984-1989	1979-1985	1978-7199
Median duration of scheduled treatment in original trial (yr)	6.9	2.7	4.4	6
Year post-trial follow up extended to	2009	2007	2006	2002

Modified from Algra *et al*[8]. ASA: Aspirin.

Table 3 Clinical effects of aspirin on sporadic colorectal cancer (clinical trials)

	Rothwell *et al* meta analysis	Physician's health study	Women's health study
ASA dosage	75-1200 mg/d	325 mg per every other day	100 mg per every other day
Duration of follow up (yr)	⩾ 20	10	10
Relative risk of CRC over follow up (HR)	0.76 (95%CI: 0.60-0.96)	1.03 (95%CI: 0.83-1.28)	0.97 (95%CI: 0.77-0.24)

Comparison of findings from meta-analysis performed by Rothwell *et al*[13] and incidence data of Physician's Health Study and Women's Health Study studies[13,15,16]. CRC: Colorectal cancer; ASA: Aspirin.

mortality by 35%. The benefit increased with longer duration of treatment and seemed to be higher for proximal CRC compared to distal CRC. An absolute reduction of 1.76% (P = 0.001) in 20-year risk of any fatal CRC after 5 years of daily treatment with aspirin (75-300 mg) was observed. Subsequently, the same authors published a study that examined the effects of daily aspirin on long-term risk of death due to all cancers. They included data from eight randomised trials (25570 patients, 674 cancer deaths) and concluded that aspirin use reduced the risk of death due to cancer (pooled OR = 0.79, P = 0.003), but the benefit was only apparent after 5 years of treatment. Absolute reduction reached 7% in 20-year risk of death due to cancer for patients aged ⩾ 65 years. In the 3 trials reporting data on the specific site of cancer occurrence with treatment duration of 5 years or longer and long-term follow-up, patients randomized to aspirin showed a statistically significant 20 year risk reduction of death due to CRC of 40% (HR = 0.60; 95%CI: 0.45-0.81, P = 0.0007)[14].

Although these data are compelling, it should be taken into account that these studies were secondary analyses of CVD prevention trials and therefore they were not originally designed to examine CRC incidence or mortality. In addition, there are two large randomized trials of alternate-day aspirin treatment in healthy subjects: the Physician's Health Study (PHS)[15] and Women's Health Study (WHS), which showed no effect of aspirin on the incidence of CRC over a 10-year follow-up period[16]. The PHS determined the effect of aspirin 325 mg every other day on CVD in 22,071 healthy male physicians. In this study, the relative risk of CRC over a 10-year follow up was 1.03 (95%CI: 0.83-1.28). The WHS examined the effect of 100 mg every other day in 39876 healthy women. The relative risk of CRC was 0.97 (95%CI: 0.77-1.24). There are several plausible explanations for the discrepancy in results between the meta-analyses performed by Rothwell and incidence data of the PHS and WHS trials. Firstly, both trials used alternate-day dosing regimens in contrast to daily dosing used in the studies included in both meta-analyses. Secondly, in the PHS and WHS trials, the duration of follow-up was shorter and may have been insufficient to detect the aspirin effect. Finally, in the WHS trial, the equivalent daily dose of aspirin was 50 mg, lower than the 75 mg/d shown to be the minimum effective dose in the Rothwell meta-analyses[13] (Table 3).

The precursors of CRC are colorectal adenomas in most cases. It would be expected that the chemopreventive effects of aspirin should begin before the development of CRC. The long duration of aspirin treatment required to show a preventive effect against invasive CRC probably reflects the time required for the development of cancer from precursor lesions (5-10 years). To date, four randomized double-blind placebo-controlled trials with 2967 participants have evaluated aspirin versus placebo for the secondary prevention of colorectal adenomas (in patients who had had colorectal adenomas or CRC)[17-20]. Doses ranged from 81 to 325 mg/d and median follow-up was 33 mo. The meta-analysis of these randomized trials[21] showed a statistically significant 17% reduction of the risk of developing adenoma with any dose of aspirin vs placebo (RR = 0.83; 95%CI: 0.72-0.96). This corresponded to a significant absolute risk reduction of 6.7%. For any advanced lesion, a significant relative risk reduction of 28% for aspirin at any dose was observed. This preventive effect emerged rather quickly (1 year) after the initiation of aspirin use (Table 4).

CLINICAL EFFECTS OF ASPIRIN IN HIGH-RISK POPULATIONS: FAMILIAL ADENOMATOUS POLYPOSIS AND LYNCH SYNDROME

To date, there are two controlled randomized trials that primarily evaluated the efficacy of aspirin in high-risk CRC patients: the Colorectal Adenoma/Carcinoma Programme (CAPP)1[22], which included 206 young individuals with a diagnosis of familial adenomatous polyposis (FAP), and CAPP2[23], which studied 1009 patients with Lynch syndrome. Both studies compared aspirin (600 mg/d), with or without resistant starch or resistant starch placebo. Data from CAPP1 patients were only analyzed

Table 4 Clinical effects of aspirin in incidence of sporadic colorectal adenomas (clinical trials)

Study	Patients	Treatment	RR (95%CI)	Ref.
AFPPS trial	Patients with a recent history of histologically documented (removed) adenomas	ASA (81 or 325 mg/d) or folic acid (1 mg/d) or placebo for 2.7 years	Any adenoma 0.81 (0.69-0.96), ASA 81mg *vs* non ASA 0.96 (0.81-1.13), ASA 325 mg *vs* non ASA Advanced lesion 0.59 (0.38-0.92), ASA 81 mg *vs* non ASA 0.83 (0.55–1.23), ASA 325 mg vs non ASA	[17]
CAPS trial	Patients with a histologically documented colon or rectal cancer with a low risk of recurrent disease	ASA 325 mg/d or placebo for 2.6 years	0.65 (0.46-0.91)	[18]
APACC trial	Patients with a history of colorectal adenomas	ASA 160 or 300 mg/d or placebo for 1 and 4 years	0.73 (0.52-1.04) for both doses, after 1 year 0.96 (0.75-1.22), for both doses, after 4 years	[20]
ukCAP trial	Patients with an adenoma removed in the 6 mo before recruitment	ASA (300 mg/d) plus placebo or ASA plus folic acid (0.5 mg/d) or folic acid plus placebo or double placebo for about 2.6 years	Any adenoma 0.79 (0.63-0.99), ASA *vs* non ASA, Advanced adenoma 0.63 (0.43-0.91), ASA *vs* non ASA	[19]
J-CAPP trial	Patients with previous sporadic colorectal tumors	ASA 100 mg/d or placebo for 2 years	Ongoing	

ASA: Aspirin.

Table 5 Clinical effects of aspirin in high risk population (clinical trials)

Study	Patients	Treatment	RR or HR (95%CI)	Ref.
CAPP1 trial	FAP young patients (10 to 21 years of age)	ASA (600 mg/d) plus placebo or resistant starch (30 g daily) plus placebo or double placebo for 17 years	RR = 0.77 (0.54-1.10), ASA *vs* non ASA	[22]
CAPP2 trial	Hereditary non-polyposis colon cancer or HNPCC	ASA (600 mg/d) or ASA placebo or resistant starch (30 g daily) or starch placebo for up to 4 years	HR = 0.63 (0.35-1.13), for the entire post-randomization period (ASA *vs* placebo) HR = 0.41 (0.19-0.86), for ≥ 2 years of treatment (ASA *vs* placebo)	[23]
J-FAPP Ⅱ trial	FAP patients (≥ 16 years of age)	Placebo *vs* enteric coated ASA (100 mg/d) for 6-10 mo	Ongoing	[25]

ASA: Aspirin; FAP: Familial adenomatous polyposis.

if they had received treatment for at least 1 year. CAPP2 patients received aspirin for a mean of 29 mo.

The CAPP1 trial showed that the mean size of the largest polyps was significantly reduced in aspirin users. Despite a trend to fewer polyps in the rectum and sigmoid colon in aspirin versus non-aspirin users at the end of intervention (from 1 to 12 years), the difference was not significant.

CAPP2 was the first clinical trial that had cancer prevention as a primary endpoint. At the end of the intervention phase, analysis showed that aspirin treatment did not reduce the risk of developing new adenomas (RR = 1.03; 95%CI: 0.7-1.4) or CRC. The study design involved post-intervention follow-up[24]. Over a mean follow-up of 55.7 mo, 48 aspirin users had developed 53 primary CRC, whereas in intention-to-treat analysis of time to first CRC there were no differences (HR = 0.63; $P = 0.12$) and the per-protocol analysis of patients completing 2 years of intervention yielded a HR of 0.41 (0.19-0.86, $P = 0.02$) (Table 5).

In Japan, two chemoprevention studies are currently being performed: one in patients with previous sporadic colorectal tumors [Japan Colorectal Aspirin Polyps Prevention (J-CAPP study)] and the second in patients with familial adenomatous polyposis (J-FAPP study Ⅱ). Both are double-blind randomized controlled trials with low-dose aspirin (100 mg/d) and study the effect of aspirin in colorectal carcinogenesis[25].

CLINICAL EFFECTS OF ASPIRIN IN PATIENTS WITH PREVIOUS CRC

It has been suggested that aspirin may prevent recurrence or death in CRC patients. In a placebo-controlled randomized trial of patients with a history of non-metastatic CRC after resection, daily treatment with LDA was associated with a 35% reduction in risk of recurrent adenoma or carcinoma at 36 mo[18]. In a cohort study of health professionals diagnosed with stage Ⅰ-Ⅲ CRC, regular use of aspirin after diagnosis was associated with higher CRC specific survival compared with non-users[26].

DOSING FOR CHEMOPREVENTION

Because most aspirin-related adverse effects are dose-dependent, to find the minimum effective dose required for CRC prevention remains a critically important issue.

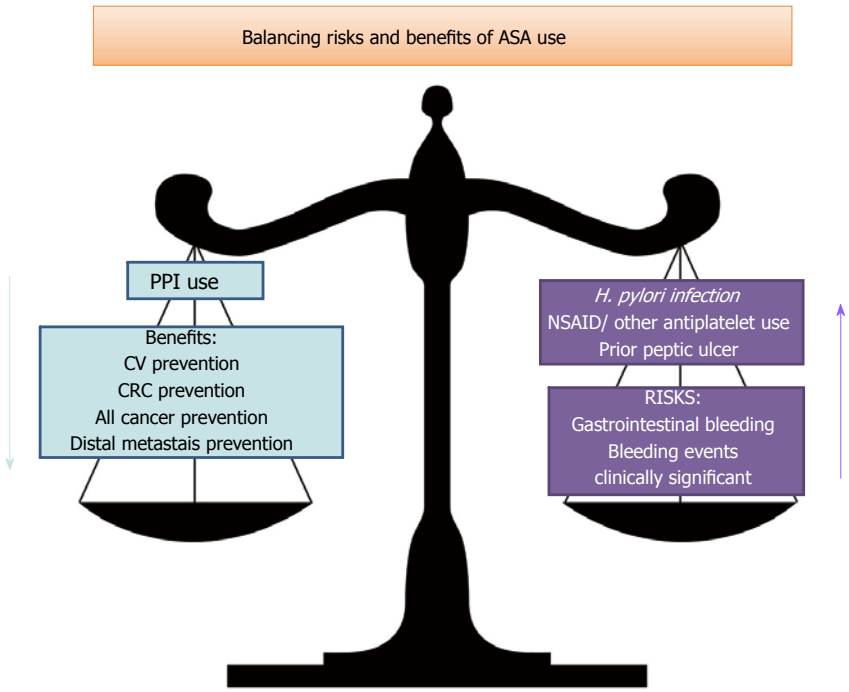

Figure 1 Balancing risk-benefits for the use of low dose aspirin. CRC: Colorectal cancer; NSAID: Nonsteroidal anti-inflammatory drug; CV: Cardiovascular; PPI: Proton pump inhibitor; ASA: Aspirin.

The Rothwell meta-analysis found that daily LDA regimens for the prevention of CVD-related events (75-325 mg) were as effective as daily high-dose aspirin[13]. However, the short-term follow up data from the PHS[15] (aspirin 325 mg every other day) and the WHS[16] (aspirin 100 mg every other day) did not show a reduction in risk of CRC. These negative findings could be attributed to alternate day dosage and/or short follow up and/or the lower dose, especially in the WHS trial. The adenoma trials (REFS) also indicate that LDA (81-325 mg/d) reduces the risk of developing adenomas and advanced adenomas.

Although follow-up of the randomized trial of daily aspirin in CVD prevention and adenoma prevention trials demonstrated that daily LDA of 75-81 mg may be sufficient for CRC prevention, the results of observational studies are controversial. Some suggested that 300-325 mg may be necessary for CRC prevention but most provided incomplete information regarding the dose and duration of aspirin treatment[7,8,10,11,27].

Therefore, taking the clinical trial and observational information together, there is very strong evidence that long-term LDA (75-325 mg/d) reduces the risk of CRC. Importantly, for the prevention of CVD-related events, LDA (75-81 mg/d) seems to be as effective as high-dose aspirin (300-325 mg/d) and, moreover, LDA has a better safety profile. However, daily aspirin at any dose may show greater benefit in patients with CVD than in those at risk of CRC.

BALANCING RISKS AND BENEFITS

Based on current evidence, treatment with LDA for 5 years in patients at risk of CVD-related events will probably prevent between 12 and 40 myocardial infarctions per 1000 patients treated, assuming an overall 10% risk of CVD-related events in this population[28]. Unfortunately, LDA use is also associated with 2-4 upper gastrointestinal bleeding events per 1000 patients[28]. However, the risk of adverse events differs according to patient characteristics (gender, age, history of ulcer, *etc.*). It is of course possible to reduce gastrointestinal risk with proton pump inhibitors, but we cannot reduce the risk of intracranial bleeding. Given the risk of bleeding, clinical guidelines (2007) recommended against the routine use of aspirin for CRC prevention in average-risk individuals[29]. However, the accumulating evidence from randomized clinical trials provides an exciting opportunity to reconsider the potential role of aspirin in cancer prevention; therefore, future practice guidelines recommendation for primary prevention in average-risk individuals for aspirin prophylaxis may also consider the prevention of cancer and not only the benefits of aspirin for the prevention of CVD-related events (Figure 1).

MECHANISM OF ACTION OF ASPIRIN

Aspirin, like other nonsteroidal anti-inflammatory drugs (NSAIDs), has the capacity to reduce prostanoid generation by inhibiting the activity of COX isozymes. Prostanoids are biologically active derivatives of arachidonic acid (AA) released from membrane phospholipids through the activity of different phospholipases[30,31]. There are two isoforms of COX, named COX-1 and COX-2[32]. Both COX isozymes are differently regulated catalytically, transcriptionally and post-transcriptionally, but they share the same catalytic activities.

COX-1 gene is considered a "housekeeping gene" and the protein is highly expressed in platelets where it is responsible for the generation of thromboxane A2 (*TXA2*), which promotes platelet activation and aggregation, vasoconstriction and proliferation of vascular smooth muscle

cells[31,33]. In addition, COX-1 is highly expressed in gastric epithelial cells where it plays an important role in cytoprotection through the generation of prostanoids, such as prostaglandin E2 (PGE2)[31,33]. In contrast, *COX-2* gene, a primary response one with many regulatory sites[34], is constitutively expressed in some tissues in physiological conditions, such as the endothelium, kidney and brain, and in pathological conditions, such as in cancer[35]. In cancer cells, the major prostanoid produced through COX-2 is PGE2, which plays important roles in modulating motility, proliferation and resistance to apoptosis[36,37].

Unlike other NSAIDs, aspirin is able to produce an irreversible inactivation of COX isozymes through the acetylation of a specific serine moiety (Ser529 of COX-1 and Ser516 of COX-2)[38]. Acetylation of the allosteric subunit of COX-1 by aspirin causes an irreversible inhibition of COX activity and, in turn, of the generation of PGG2 from AA. Acetylated COX-2 is not able to form PGG_2 but it generates 15R-hydroxyeicosapentaenoic acid (15R-HETE) from AA[39]. However, there is no convincing evidence that these lipid mediators triggered by aspirin are generated *in vivo* in humans.

ASPIRIN PHARMACOLOGY

Aspirin has a short half-life when administered *in vivo* and it is rapidly inactivated by plasma and tissue esterases into salicylic acid, which is a weak inhibitor of COXs (in the millimolar range)[40-42]. The inhibitory effects of aspirin have been found to be > 100-fold more potent in inhibiting platelet COX-1 than monocyte COX-2[17-20]. Aspirin at low doses (75-100 mg daily) is able to cause nearly complete inhibition of the capacity of platelet COX-1 to generate TXA_2[43,44]. Due to irreversible inhibition of COX-1 and the limited capacity of platelets for *de novo* protein synthesis[45], the profound inhibitory effect of platelet function by aspirin persists throughout the dose interval (*i.e.*, 24 h).

The major part of the inhibitory effect of platelet COX-1 by the oral administration of low-dose aspirin occurs in the presystemic circulation where the drug reaches higher concentrations[46,47]. The impact of low-dose aspirin, administered once daily, on COX-2 activity *in vivo* is marginal. In summary, the pharmacokinetics and pharmacodynamics of low-dose aspirin support the fact that the drug acts mainly by modifying platelet function as a consequence of COX-1 inhibition. At higher doses, aspirin may affect COX-2 in a dose-dependent fashion.

COX-DEPENDENT MECHANISMS FOR ANTITUMOR EFFECTS

Randomised clinical trials have shown that once daily LDA provides a chemopreventive effect against atherothrombosis[24] and CRC[13,14]. This finding suggests that enhanced platelet activation is involved in the development of these two pathological conditions. In fact, these aspirin doses and dosing intervals are consistent with a selective inhibitory effect of aspirin on platelet COX-1 activity and on TXA2-dependent platelet function.

Transcriptional upregulation of the *COX-2* gene has been observed in nearly half of human colorectal adenomas and 80%-90% of CRC, probably related to the disturbed function of the *APC* gene. However, *COX-1* gene and protein expression are not affected[48-52] and the role of this enzyme in CRC carcinogenesis remains unclear. In colonic mucosa, COX-2 is localized predominantly in tumor tissue, including epithelial cells, mononuclear cells, endothelial and stromal cells, but not in nearby normal tissue. Upregulation of COX-2 is associated with increased cell adhesion, phenotypic changes, resistance to apoptosis and tumor angiogenesis[53-57]. COX-2 expression does not always correlate with survival and/or with Duke's stage of the disease[58-60]. This suggests a role of upregulated COX-2 for the initial stages of colon carcinogenesis but not for clinical outcome at advanced stages. The best studied consequence of upregulated COX-2 in CRC is enhanced prostaglandin production[48]. Prostaglandin levels in CRC tissue are 3-4 fold higher than in healthy tissue in the vicinity, with PGE2 being the predominant product[61]. PGE2 inhibits apoptosis and stimulates tumor growth and angiogenesis via stimulation of b-catenin/T-cell factor dependent transcription[62]. In addition, PGE2 acts as an immunosuppressant in patients with CRC[53,63]. The clearest clinical evidence for COX-2 as a pharmacological target for the chemopreventive action of aspirin was the finding that aspirin reduced the risk of CRC exclusively in individuals with elevated COX-2 expression but not in those without[64]. This was associated with a reduction in mortality[65]. Although these findings were from observational studies, they confirmed experimental data that prostaglandins and non-prostaglandin COX-2 products are central to the pathogenesis of CRC. The vast majority of published experimental studies have reported beneficial antitumor effects for aspirin, celecoxib and non-aspirin NSAIDs in a variety of experimental models[65-67]. These data strongly suggest a central role of COX-2 in CRC and its inhibition is an effective chemopreventive measure (Figure 2).

The generation of TXA2, a major product of platelet COX-1 which promotes platelet aggregation and vasoconstriction[68], represents another important mechanism by which platelets can affect tumorigenesis. One study has shown that enhanced TXA2 generation into murine colon-26 adenocarcinoma cell line (C26) stimulated tumor angiogenesis, tumor growth *in vivo*[69] and promoted the interaction between metastasizing tumor cells and the host hemostatic system[69], thus suggesting a role of TXA2 in promoting angiogenesis and the development of tumor metastasis[70].

In one study, the authors demonstrated PGE2 inhibition in rectal biopsies performed 1 mo after treatment with three different doses of aspirin (81, 325 and 650 mg) versus placebo[71]. Unexpectedly, the 81 mg daily aspirin dose suppressed PGE2 levels to the same extent as the 650 mg dose. In another study, treatment with 81 mg of aspirin per day for 3 mo reduced mucosal PGE2

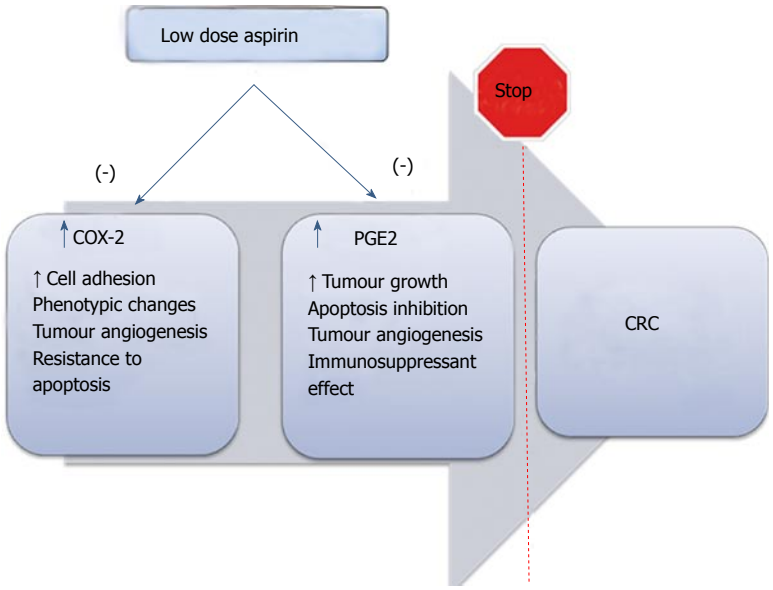

Figure 2 Cyclooxygenase-dependent mechanisms for antitumoral effects of low dose aspirin. CRC: Colorectal cancer; COX: Cyclooxygenase; PGE2: Prostaglandin E2.

and transforming growth factor-α expression in apparently normal rectal mucosa of individuals with a history of adenomatous polyps[72]. Further studies using more appropriate methodologies are required to definitively clarify whether LDA affects COX-1 activity in the gastrointestinal tract.

Some investigators have proposed that both COX-1 and COX-2 pathways are involved in intestinal tumorigenesis and that they operate sequentially. This is strongly supported by the findings of experimental animal studies in which the loss of either *COX-1* or *COX-2* genes blocks intestinal polyposis in mouse models of FAP by about 90%[66,67].

COX-INDEPENDENT MECHANISMS OF ANTITUMOR EFFECTS

Evidence from different lines of research indicates that COX-2 independent mechanisms may also affect apoptosis and cell proliferation in CRC and are sensitive to both aspirin and non-aspirin NSAIDs. Nearly but not all human colon cancer cells express COX-2 and produce prostaglandins[53,73]. Today, several COX-independent mechanisms of aspirin have been reported that might contribute to its chemopreventive effects in tumorigenesis[73]. Most of these effects have been found *in vitro* using supra-therapeutic concentrations of aspirin which cannot be obtained in systemic circulation with low doses of the drug. However, no convincing evidence has been obtained to demonstrate that these mechanisms are operative *in vivo*, particularly with low doses of aspirin which have been associated with chemopreventive benefits in randomised clinical trials. In any case, currently available evidence clearly points to the existence of further cellular targets of NSAIDs, in addition to COX-2 inhibition, which may contribute to their antitumor effects. Further studies are needed to completely understand the mechanisms involved.

CONCLUSION

A large body of clinical evidence supports the protective action of aspirin as a chemopreventive agent for different types of cancer, in particular CRC[6]. Also, increasing indirect evidence has led to the hypothesis that the antiplatelet effect of aspirin is a central mechanism for its antitumor effect[34,41]. The finding of an apparent maximum chemopreventive efficacy against cancer and atherothrombosis by low-dose aspirin lends support to this hypothesis[6]. At low doses every 24 h, aspirin acts as a complete and persistent inhibitor of COX-1 in platelets (in pre-systemic circulation)[47], while causing a limited and rapidly reversible inhibitory effect on COX-2 and/or COX-1 expressed in nucleated cells[39]. Despite uncertainty about the precise mechanisms that underlie aspirin's anticancer benefit, the evidence supporting its effectiveness for the prevention of CRC is substantial; daily aspirin for at least 5 years has been shown to reduce the 20-year risk of CRC by 32% and 20-year mortality by 43%[13]. Therefore, the potential benefit of aspirin in both CVD and prevention of cancer at multiple sites may favor its use for broader chronic disease prevention. It is likely that the benefits in terms of morbidity and mortality will outweigh concerns about gastrointestinal bleeding, which is rarely life threatening, and cerebral bleeding, which is extremely uncommon. Health authorities should consider the possibility of extending recommendations on the routine use of aspirin, taking into account its beneficial effects in cardiovascular disease and cancer prevention.

Extensive translational medical research is required to confirm the hypothesis of platelet-mediated colon tumorigenesis. Importantly, these studies will need to address the current uncertainty concerning the optimal aspirin dose, the dosing regimen for cancer prevention, the possible contribution of individual genetic cancer susceptibility to aspirin response[74] and also the target

population most likely to benefit from daily aspirin use.

REFERENCES

1 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. GLOBOCAN 2008 v1. 2, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet]. Lyon, France: International Agency for Research on Cancer, 2011

2 **Shapiro JA**, Seeff LC, Thompson TD, Nadel MR, Klabunde CN, Vernon SW. Colorectal cancer test use from the 2005 National Health Interview Survey. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 1623-1630 [PMID: 18628413 DOI: 10.1158/1055-9965.EPI-07-2838]

3 **United States Preventive Services Task Force.** Screening for colorectal cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2008; **149**: 627-637 [PMID: 18838716 DOI: 10.7326/0003-4819-149-9-200811040-00243]

4 **Ajani UA**, Ford ES, Greenland KJ, Giles WH, Mokdad AH. Aspirin use among U.S. adults: Behavioral Risk Factor Surveillance System. *Am J Prev Med* 2006; **30**: 74-77 [PMID: 16414427 DOI: 10.1016/j.amepre.2005.08.042]

5 **National Health Service Information Centre**. Prescription cost analysis for England 2007. Available from: URL: http://www.ic.nhs. uk/webfiles/publications/PCA publication/PCA 2007 complete V2.pdf

6 **Thun MJ**, Jacobs EJ, Patrono C. The role of aspirin in cancer prevention. *Nat Rev Clin Oncol* 2012; **9**: 259-267 [PMID: 22473097]

7 **Flossmann E**, Rothwell PM. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. *Lancet* 2007; **369**: 1603-1613 [PMID: 17499602 DOI: 10.1016/S0140-6736(07)60747-8]

8 **Algra AM**, Rothwell PM. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. *Lancet Oncol* 2012; **13**: 518-527 [PMID: 22440112 DOI: 10.1016/S1470-2045(12)70112-2]

9 **Jacobs EJ**, Thun MJ, Bain EB, Rodriguez C, Henley SJ, Calle EE. A large cohort study of long-term daily use of adult-strength aspirin and cancer incidence. *J Natl Cancer Inst* 2007; **99**: 608-615 [PMID: 17440162 DOI: 10.1093/jnci/djk132]

10 **Chan AT**, Giovannucci EL, Meyerhardt JA, Schernhammer ES, Wu K, Fuchs CS. Aspirin dose and duration of use and risk of colorectal cancer in men. *Gastroenterology* 2008; **134**: 21-28 [PMID: 18005960 DOI: 10.1053/j.gastro.2007.09.035]

11 **Chan AT**, Giovannucci EL, Meyerhardt JA, Schernhammer ES, Curhan GC, Fuchs CS. Long-term use of aspirin and nonsteroidal anti-inflammatory drugs and risk of colorectal cancer. *JAMA* 2005; **294**: 914-923 [PMID: 16118381 DOI: 10.1001/jama.294.8.914]

12 **Chan AT**, Manson JE, Feskanich D, Stampfer MJ, Colditz GA, Fuchs CS. Long-term aspirin use and mortality in women. *Arch Intern Med* 2007; **167**: 562-572 [PMID: 17389287 DOI: 10.1001/archinte.167.6.562]

13 **Rothwell PM**, Wilson M, Elwin CE, Norrving B, Algra A, Warlow CP, Meade TW. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. *Lancet* 2010; **376**: 1741-1750 [PMID: 20970847 DOI: 10.1016/S0140-6736(10)61543-7]

14 **Rothwell PM**, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. *Lancet* 2011; **377**: 31-41 [PMID: 21144578 DOI: 10.1016/S0140-6736(10)62110-1]

15 **Gann PH**, Manson JE, Glynn RJ, Buring JE, Hennekens CH. Low-dose aspirin and incidence of colorectal tumors in a randomized trial. *J Natl Cancer Inst* 1993; **85**: 1220-1224 [PMID: 8331682 DOI: 10.1093/jnci/85.15.1220]

16 **Cook NR**, Lee IM, Gaziano JM, Gordon D, Ridker PM, Manson JE, Hennekens CH, Buring JE. Low-dose aspirin in the primary prevention of cancer: the Women's Health Study: a randomized controlled trial. *JAMA* 2005; **294**: 47-55 [PMID: 15998890 DOI: 10.1001/jama.294.1.47]

17 **Baron JA**, Cole BF, Sandler RS, Haile RW, Ahnen D, Bresalier R, McKeown-Eyssen G, Summers RW, Rothstein R, Burke CA, Snover DC, Church TR, Allen JI, Beach M, Beck GJ, Bond JH, Byers T, Greenberg ER, Mandel JS, Marcon N, Mott LA, Pearson L, Saibil F, van Stolk RU. A randomized trial of aspirin to prevent colorectal adenomas. *N Engl J Med* 2003; **348**: 891-899 [PMID: 12621133 DOI: 10.1056/NEJMoa021735]

18 **Sandler RS**, Halabi S, Baron JA, Budinger S, Paskett E, Keresztes R, Petrelli N, Pipas JM, Karp DD, Loprinzi CL, Steinbach G, Schilsky R. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. *N Engl J Med* 2003; **348**: 883-890 [PMID: 12621132 DOI: 10.1056/NEJMoa021633]

19 **Logan RF**, Grainge MJ, Shepherd VC, Armitage NC, Muir KR. Aspirin and folic acid for the prevention of recurrent colorectal adenomas. *Gastroenterology* 2008; **134**: 29-38 [PMID: 18022173 DOI: 10.1053/j.gastro.2007.10.014]

20 **Benamouzig R**, Deyra J, Martin A, Girard B, Jullian E, Piednoir B, Couturier D, Coste T, Little J, Chaussade S. Daily soluble aspirin and prevention of colorectal adenoma recurrence: one-year results of the APACC trial. *Gastroenterology* 2003; **125**: 328-336 [PMID: 12891533 DOI: 10.1016/S0016-5085(03)00887-4]

21 **Cole BF**, Logan RF, Halabi S, Benamouzig R, Sandler RS, Grainge MJ, Chaussade S, Baron JA. Aspirin for the chemoprevention of colorectal adenomas: meta-analysis of the randomized trials. *J Natl Cancer Inst* 2009; **101**: 256-266 [PMID: 19211452 DOI: 10.1093/jnci/djn485]

22 **Burn J**, Bishop DT, Chapman PD, Elliott F, Bertario L, Dunlop MG, Eccles D, Ellis A, Evans DG, Fodde R, Maher ER, Möslein G, Vasen HF, Coaker J, Phillips RK, Bülow S, Mathers JC. A randomized placebo-controlled prevention trial of aspirin and/or resistant starch in young people with familial adenomatous polyposis. *Cancer Prev Res* (Phila) 2011; **4**: 655-665 [PMID: 21543343 DOI: 10.1158/1940-6207.CAPR-11-0106]

23 **Burn J**, Bishop DT, Mecklin JP, Macrae F, Möslein G, Olschwang S, Bisgaard ML, Ramesar R, Eccles D, Maher ER, Bertario L, Jarvinen HJ, Lindblom A, Evans DG, Lubinski J, Morrison PJ, Ho JW, Vasen HF, Side L, Thomas HJ, Scott RJ, Dunlop M, Barker G, Elliott F, Jass JR, Fodde R, Lynch HT, Mathers JC. Effect of aspirin or resistant starch on colorectal neoplasia in the Lynch syndrome. *N Engl J Med* 2008; **359**: 2567-2578 [PMID: 19073976 DOI: 10.1056/NEJMoa0801297]

24 **Burn J**, Gerdes AM, Macrae F, Mecklin JP, Moeslein G, Olschwang S, Eccles D, Evans DG, Maher ER, Bertario L, Bisgaard ML, Dunlop MG, Ho JW, Hodgson SV, Lindblom A, Lubinski J, Morrison PJ, Murday V, Ramesar R, Side L, Scott RJ, Thomas HJ, Vasen HF, Barker G, Crawford G, Elliott F, Movahedi M, Pylvanainen K, Wijnen JT, Fodde R, Lynch HT, Mathers JC, Bishop DT. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. *Lancet* 2011; **378**: 2081-2087 [PMID: 22036019 DOI: 10.1016/S0140-6736(11)61049-0]

25 **Ishikawa H**, Nakamura T, Kawano A, Gondo N, Sakai T. Chemoprevention of colorectal cancer in Japan: a brief introduction to current clinical trials. *J Gastroenterol* 2009; **44** Suppl 19: 77-81 [PMID: 19148798 DOI: 10.1007/s00535-008-2286-2]

26 **Chan AT**, Ogino S, Fuchs CS. Aspirin use and survival after diagnosis of colorectal cancer. *JAMA* 2009; **302**: 649-658 [PMID: 19671906 DOI: 10.1001/jama.2009.1112]

27 **Mahipal A**, Anderson KE, Limburg PJ, Folsom AR. Nonsteroidal anti-inflammatory drugs and subsite-specific colorectal cancer incidence in the Iowa women's health study. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 1785-1790 [PMID:

17035383 DOI: 10.1158/1055-9965.EPI-05-0674]
28 **Hayden M**, Pignone M, Phillips C, Mulrow C. Aspirin for the primary prevention of cardiovascular events: a summary of the evidence for the U.S. Preventive Services Task Force. *Ann Intern Med* 2002; **136**: 161-172 [PMID: 11790072 DOI: 10.7326/0003-4819-136-2-200201150-00016]
29 **United States Preventive Services Task Force.** Routine aspirin or nonsteroidal anti-inflammatory drugs for the primary prevention of colorectal cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2007; **146**: 361-364 [PMID: 17339621 DOI: 10.7326/0003-4819-146-5-200703060-00008]
30 **Funk CD**. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* 2001; **294**: 1871-1875 [PMID: 11729303 DOI: 10.1126/science.294.5548.1871]
31 **Smyth EM**, Grosser T, Wang M, Yu Y, FitzGerald GA. Prostanoids in health and disease. *J Lipid Res* 2009; **50** Suppl: S423-S428 [PMID: 19095631 DOI: 10.1194/jlr.R800094-JLR200]
32 **Smith WL**, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. *Annu Rev Biochem* 2000; **69**: 145-182 [PMID: 10966456 DOI: 10.1146/annurev.biochem.69.1.145]
33 **Patrono C**, Patrignani P, García Rodríguez LA. Cyclooxygenase-selective inhibition of prostanoid formation: transducing biochemical selectivity into clinical read-outs. *J Clin Invest* 2001; **108**: 7-13 [PMID: 11435450]
34 **Kang YJ**, Mbonye UR, DeLong CJ, Wada M, Smith WL. Regulation of intracellular cyclooxygenase levels by gene transcription and protein degradation. *Prog Lipid Res* 2007; **46**: 108-125 [PMID: 17316818 DOI: 10.1016/j.plipres.2007.01.001]
35 **Harper KA**, Tyson-Capper AJ. Complexity of COX-2 gene regulation. *Biochem Soc Trans* 2008; **36**: 543-545 [PMID: 18482003 DOI: 10.1042/BST0360543]
36 **Dixon DA**, Tolley ND, King PH, Nabors LB, McIntyre TM, Zimmerman GA, Prescott SM. Altered expression of the mRNA stability factor HuR promotes cyclooxygenase-2 expression in colon cancer cells. *J Clin Invest* 2001; **108**: 1657-1665 [PMID: 11733561]
37 **Wang D**, Dubois RN. Eicosanoids and cancer. *Nat Rev Cancer* 2010; **10**: 181-193 [PMID: 20168319 DOI: 10.1038/nrc2809]
38 **Patrono C**, Baigent C, Hirsh J, Roth G. Antiplatelet drugs: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest* 2008; **133**: 199S-233S [PMID: 18574266 DOI: 10.1378/chest.08-0672]
39 **Sharma NP**, Dong L, Yuan C, Noon KR, Smith WL. Asymmetric acetylation of the cyclooxygenase-2 homodimer by aspirin and its effects on the oxygenation of arachidonic, eicosapentaenoic, and docosahexaenoic acids. *Mol Pharmacol* 2010; **77**: 979-986 [PMID: 20194532 DOI: 10.1124/mol.109.063115]
40 **Dovizio M**, Bruno A, Tacconelli S, Patrignani P. Mode of action of aspirin as a chemopreventive agent. *Recent Results Cancer Res* 2013; **191**: 39-65 [PMID: 22893199 DOI: 10.1007/978-3-642-30331-9_3]
41 **Patrignani P**. Nonsteroidal anti-inflammatory drugs, COX-2 and colorectal cancer. *Toxicol Lett* 2000; **112-113**: 493-498 [PMID: 10720771 DOI: 10.1016/S0378-4274(99)00210-6]
42 **Warner TD**, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA, Vane JR. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. *Proc Natl Acad Sci USA* 1999; **96**: 7563-7568 [PMID: 10377455 DOI: 10.1073/pnas.96.13.7563]
43 **Ricciotti E**, Dovizio M, Di Francesco L, Anzellotti P, Salvatore T, Di Francesco A, Sciulli MG, Pistritto G, Monopoli A, Patrignani P. NCX 4040, a nitric oxide-donating aspirin, exerts anti-inflammatory effects through inhibition of I kappa B-alpha degradation in human monocytes. *J Immunol* 2010; **184**: 2140-2147 [PMID: 20065114 DOI: 10.4049/jimmunol.0903107]
44 **Patrignani P**, Filabozzi P, Patrono C. Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. *J Clin Invest* 1982; **69**: 1366-1372 [PMID: 7045161 DOI: 10.1172/JCI110576]
45 **Evangelista V**, Manarini S, Di Santo A, Capone ML, Ricciotti E, Di Francesco L, Tacconelli S, Sacchetti A, D'Angelo S, Scilimati A, Sciulli MG, Patrignani P. De novo synthesis of cyclooxygenase-1 counteracts the suppression of platelet thromboxane biosynthesis by aspirin. *Circ Res* 2006; **98**: 593-595 [PMID: 16484611 DOI: 10.1161/01.RES.0000214553.37930.3e]
46 **Pedersen AK**, FitzGerald GA. Dose-related kinetics of aspirin. Presystemic acetylation of platelet cyclooxygenase. *N Engl J Med* 1984; **311**: 1206-1211 [PMID: 6436696 DOI: 10.1056/NEJM198411083111902]
47 **Patrono C**, García Rodríguez LA, Landolfi R, Baigent C. Low-dose aspirin for the prevention of atherothrombosis. *N Engl J Med* 2005; **353**: 2373-2383 [PMID: 16319386 DOI: 10.1056/NEJMra052717]
48 **Eberhart CE**, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 1994; **107**: 1183-1188 [PMID: 7926468]
49 **Kutchera W**, Jones DA, Matsunami N, Groden J, McIntyre TM, Zimmerman GA, White RL, Prescott SM. Prostaglandin H synthase 2 is expressed abnormally in human colon cancer: evidence for a transcriptional effect. *Proc Natl Acad Sci USA* 1996; **93**: 4816-4820 [PMID: 8643486 DOI: 10.1073/pnas.93.10.4816]
50 **Kargman SL**, O'Neill GP, Vickers PJ, Evans JF, Mancini JA, Jothy S. Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. *Cancer Res* 1995; **55**: 2556-2559 [PMID: 7780968]
51 **Marnett LJ**, DuBois RN. COX-2: a target for colon cancer prevention. *Annu Rev Pharmacol Toxicol* 2002; **42**: 55-80 [PMID: 11807164]
52 **Sano H**, Kawahito Y, Wilder RL, Hashiramoto A, Mukai S, Asai K, Kimura S, Kato H, Kondo M, Hla T. Expression of cyclooxygenase-1 and -2 in human colorectal cancer. *Cancer Res* 1995; **55**: 3785-3789 [PMID: 7641194]
53 **Marnett LJ**. Aspirin and the potential role of prostaglandins in colon cancer. *Cancer Res* 1992; **52**: 5575-5589 [PMID: 1394181 DOI: 10.1146/annurev.pharmtox.42.082301.164620]
54 **Wang D**, Dubois RN. The role of COX-2 in intestinal inflammation and colorectal cancer. *Oncogene* 2010; **29**: 781-788 [PMID: 19946329 DOI: 10.1038/onc.2009.421]
55 **Nie D**, Honn KV. Cyclooxygenase, lipoxygenase and tumor angiogenesis. *Cell Mol Life Sci* 2002; **59**: 799-807 [PMID: 12088280 DOI: 10.1007/s00018-002-8468-9]
56 **Tsujii M**, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc Natl Acad Sci USA* 1997; **94**: 3336-3340 [PMID: 9096394]
57 **Fujita T**, Matsui M, Takaku K, Uetake H, Ichikawa W, Taketo MM, Sugihara K. Size- and invasion-dependent increase in cyclooxygenase 2 levels in human colorectal carcinomas. *Cancer Res* 1998; **58**: 4823-4826 [PMID: 9809985]
58 **Ogino S**, Kirkner GJ, Nosho K, Irahara N, Kure S, Shima K, Hazra A, Chan AT, Dehari R, Giovannucci EL, Fuchs CS. Cyclooxygenase-2 expression is an independent predictor of poor prognosis in colon cancer. *Clin Cancer Res* 2008; **14**: 8221-8227 [PMID: 19088039 DOI: 10.1158/1078-0432.CCR-08-1841]
59 **Sheehan KM**, Sheahan K, O'Donoghue DP, MacSweeney F, Conroy RM, Fitzgerald DJ, Murray FE. The relationship between cyclooxygenase-2 expression and colorectal cancer. *JAMA* 1999; **282**: 1254-1257 [PMID: 10517428 DOI: 10.1001/jama.282.13.1254]
60 **Fux R**, Schwab M, Thon KP, Gleiter CH, Fritz P. Cyclooxygenase-2 expression in human colorectal cancer is unrelated to overall patient survival. *Clin Cancer Res* 2005; **11**: 4754-4760 [PMID: 16000571 DOI: 10.1158/1078-0432.CCR-04-2586]

61 **Pugh S**, Thomas GA. Patients with adenomatous polyps and carcinomas have increased colonic mucosal prostaglandin E2. *Gut* 1994; **35**: 675-678 [PMID: 8200564 DOI: 10.1136/gut.35.5.675]

62 **Shao J**, Jung C, Liu C, Sheng H. Prostaglandin E2 Stimulates the beta-catenin/T cell factor-dependent transcription in colon cancer. *J Biol Chem* 2005; **280**: 26565-26572 [PMID: 15899904 DOI: 10.1074/jbc.M413056200]

63 **Balch CM**, Dougherty PA, Cloud GA, Tilden AB. Prostaglandin E2-mediated suppression of cellular immunity in colon cancer patients. *Surgery* 1984; **95**: 71-77 [PMID: 6229053]

64 **Chan AT**, Ogino S, Fuchs CS. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. *N Engl J Med* 2007; **356**: 2131-2142 [PMID: 17522398 DOI: 10.1056/NEJMoa067208]

65 **Fischer SM**, Hawk ET, Lubet RA. Coxibs and other nonsteroidal anti-inflammatory drugs in animal models of cancer chemoprevention. *Cancer Prev Res* (Phila) 2011; **4**: 1728-1735 [PMID: 21778329 DOI: 10.1158/1940-6207.CAPR-11-0166]

66 **Oshima M**, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF, Taketo MM. Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* 1996; **87**: 803-809 [PMID: 8945508 DOI: 10.1016/S0092-8674(00)81988-1]

67 **Chulada PC**, Thompson MB, Mahler JF, Doyle CM, Gaul BW, Lee C, Tiano HF, Morham SG, Smithies O, Langenbach R. Genetic disruption of Ptgs-1, as well as Ptgs-2, reduces intestinal tumorigenesis in Min mice. *Cancer Res* 2000; **60**: 4705-4708 [PMID: 10987272]

68 **Grosser T**, Fries S, FitzGerald GA. Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. *J Clin Invest* 2006; **116**: 4-15 [PMID: 16395396 DOI: 10.1172/JCI27291]

69 **Pradono P**, Tazawa R, Maemondo M, Tanaka M, Usui K, Saijo Y, Hagiwara K, Nukiwa T. Gene transfer of thromboxane A(2) synthase and prostaglandin I(2) synthase antithetically altered tumor angiogenesis and tumor growth. *Cancer Res* 2002; **62**: 63-66 [PMID: 11782360]

70 **Honn KV**. Inhibition of tumor cell metastasis by modulation of the vascular prostacyclin/thromboxane A2 system. *Clin Exp Metastasis* 1983; **1**: 103-114 [PMID: 6242706 DOI: 10.1007/BF00121490]

71 **Sample D**, Wargovich M, Fischer SM, Inamdar N, Schwartz P, Wang X, Do KA, Sinicrope FA. A dose-finding study of aspirin for chemoprevention utilizing rectal mucosal prostaglandin E(2) levels as a biomarker. *Cancer Epidemiol Biomarkers Prev* 2002; **11**: 275-279 [PMID: 11895877]

72 **Barnes CJ**, Hamby-Mason RL, Hardman WE, Cameron IL, Speeg KV, Lee M. Effect of aspirin on prostaglandin E2 formation and transforming growth factor alpha expression in human rectal mucosa from individuals with a history of adenomatous polyps of the colon. *Cancer Epidemiol Biomarkers Prev* 1999; **8**: 311-315 [PMID: 10207634]

73 **Hanif R**, Pittas A, Feng Y, Koutsos MI, Qiao L, Staiano-Coico L, Shiff SI, Rigas B. Effects of nonsteroidal anti-inflammatory drugs on proliferation and on induction of apoptosis in colon cancer cells by a prostaglandin-independent pathway. *Biochem Pharmacol* 1996; **52**: 237-245 [PMID: 8694848 DOI: 10.1016/0006-2952(96)00181-5]

74 **Liao X**, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, Imamura Y, Qian ZR, Baba Y, Shima K, Sun R, Nosho K, Meyerhardt JA, Giovannucci E, Fuchs CS, Chan AT, Ogino S. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. *N Engl J Med* 2012; **367**: 1596-1606 [PMID: 23094721 DOI: 10.1056/NEJMoa1207756]

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In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

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Books

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

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Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

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Conference paper

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Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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