ISSN 2220-3176 (online)

World Journal of *Clinical Infectious Diseases*

World J Clin Infect Dis 2016 August 25; 6(3): 28-60

Published by Baishideng Publishing Group Inc

WJCID

World Journal of Clinical Infectious Diseases

A peer-reviewed, online, open-access journal of clinical infectious diseases

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WJCID World Journal of Clinical Infectious Diseases

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Quarterly Volume 6 Number 3 August 25, 2016

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NAME OF JOURNAL
World Journal of Clinical Infectious Diseases

ISSN
ISSN 2220-3176 (online)

LAUNCH DATE
December 30, 2011

FREQUENCY
Quarterly

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8226 Regency Drive,
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E-mail: bpgoffice@wjgnet.com
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PUBLICATION DATE
August 25, 2016

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DOI: 10.5495/wjcid.v6.i3.28

World J Clin Infect Dis 2016 August 25; 6(3): 28-36
ISSN 2220-3176 (online)
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REVIEW

Challenges in management of recurrent and refractory *Clostridium difficile* infection

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Author contributions: Meehan AM, Tariq R and Khanna S planned and researched the topic; Meehan AM and Tariq R wrote the paper; Meehan AM and Khanna S revised the paper.

Conflict-of-interest statement: Sahil Khanna serves as a consultant to Rebiotix Inc and Summit Pharmaceuticals.

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Received: March 24, 2016
Peer-review started: March 24, 2016
First decision: April 15, 2016
Revised: May 5, 2016
Accepted: May 31, 2016
Article in press: June 2, 2016
Published online: August 25, 2016

Abstract

Clostridium difficile infection (CDI) is the most common nosocomial infection in the United States and is associated with a high mortality. One quarter of patients treated for CDI have at least one recurrence. Spore persistence, impaired host immune response and alteration in the gastrointestinal microbiome due to antibiotic use are factors in recurrent disease. We review the etiology of recurrent CDI and best approaches to management including fecal microbiota transplantation.

Key words: *Clostridium difficile* infection; Epidemiology; Outcomes; Treatment; Fecal microbiota transplantation

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Core tip: Recurrent *Clostridium difficile* infection (RCDI) is common and can be difficult to treat. Clostridia spores transmit disease. They are ubiquitous and hard to eradicate. The composition of the gut microbiome plays an essential yet poorly understood role in maintaining overall health, and in protecting against *Clostridium difficile* (*C. difficile*) infection. Antibiotic induced dysbiosis of the microbiome is a key contributor to RCDI. Here we review how *C. difficile* spores and alterations in the microbiome contribute to RCDI.

Meehan AM, Tariq R, Khanna S. Challenges in management of recurrent and refractory *Clostridium difficile* infection. *World J Clin Infect Dis* 2016; 6(3): 28-36 Available from: URL: http://www.wjgnet.com/2220-3176/full/v6/i3/28.htm DOI: http://dx.doi.org/10.5495/wjcid.v6.i3.28

INTRODUCTION

Clostridium difficile (*C. difficile*) is a gram positive, anaerobic, spore forming bacteria first associated with antibiotic-associated and pseudomembranous colitis in 1978[1,2]. Originally isolated from meconium and feces of newborn infants in 1935, it was dubbed "*Bacillus difficilis*" due to its poor culture growth characteristics[3]. Although

C. difficile culture is achievable now using Cycloserine Cefoxitin Fructose Agar media[4], the moniker remains apt, albeit for different reasons. A diagnosis of *Clostridium difficile* infection (CDI) adds considerably to healthcare cost, length of stay, complications and mortality[5,6].

CDI diagnosis is based on symptoms and toxin detection, and initial treatment involves oral metronidazole for mild-moderate cases or oral vancomycin if severe[7]. Both metronidazole and vancomycin lead to intestinal dysbiosis and impair "resistance to colonization" actually facilitating recurrence[8].

Recurrent CDI (RCDI) is defined as recurrence of clinical symptoms with a positive *C. difficile* stool test within 8 wk of symptom resolution[9]. Twenty to twenty five percent of CDI patients will have at least one recurrence[10] and subsequent risk can be as high as 40%-65%[11]. Reinfection *vs* relapse are indistinguishable clinically, however based on serotyping and PCR ribotyping up to 50% of patients recur with a strain that is different to the original one[12]. RCDI relates to spore production and persistence, the host immune response (or lack of it) to toxins, and alterations in the gut microbiome.

C. DIFFICILE SPORES: RESISTANCE AND PERSISTENCE

C. difficile spores are the agents of disease transmission[13]. They are ubiquitous and may survive on contaminated surfaces for months, possibly years[14-16]. *C. difficile* pathophysiology relates to spore exposure and ingestion, spore vegetation and toxin production in the setting of an altered host gut microbiome[17]. A healthy gut flora is protective against colonization and infection from *C. difficile*[18]. Asymptomatic colonization with toxin negative and positive strains has been described[8,19].

Anaerobic bacteria form spores when conditions are not conducive to growth (*i.e.*, starvation), specifically when deprived of carbon or nitrogen[16]. Clostridial spores are metabolically inactive (dormant) and impervious to most environmental assaults (except bleach)[7]. Anaerobic spore DNA is protected from damage by several mechanisms that have been established in related clostridial and bacteroides species and extrapolated to *C. difficile*. These include the fact that the spore core is anhydrous (water content 25%) and acidic (pH 6.5), which inhibits enzymatic activity and immobilizes most proteins[16]. There are high levels of ionic calcium-dipicolinic acid in the spore core, which forms a 1:1 complex with DNA. Deletion experiments suggest that saturation of DNA with α/β small acid soluble spore proteins (SASPs) is the dominant protective mechanism[20]. Mutants spores that lack α/β SASPs and calcium-dipicolinic acid lose viability rapidly during sporulation due to DNA damage[16].

Spores are the main vehicle of disease transmission, persistence and recurrence in CDI[14]. The environmental spore load necessary to infect 50% of mice after 1 h in one series of experiments was 5-10 spores/cm^2[21,22]. Spores shed through stool contaminate skin, bed clothes and even air, reaching 53-426 colony forming units/m^3 of air[15]. Mutants unable to produce Spo0A (a transcription regulatory protein essential for sporulation) do not persist or transmit disease in mice[23]. Thus elimination of spores can interrupt disease transmission. Presently this is most often pursued in the health care setting in the context of a known case (we don't as yet target spores in the community)[7]. Sodium hypochlorite (*i.e.*, bleach) is the most commonly used agent, with far UV light and vapor hydrogen peroxide also effective[14].

There are several additional issues of note. In murine gut *C. difficile* sporulates at a rapid rate - 56% relative to vegetative cells at 14 h post infection[24]. The murine colonic environment supports sporulation by phosphorylation of the master regulator Spo0A[14,23,25]. Presumably similar unknown triggers are present in the human gut.

Recent whole genome sequencing of CDI isolates in > 1200 patients with disease showed only 35% of cases were related to known cases, which suggests alternate routes of exposure (animals/food), outside of health care settings[26] (Presumably patients got the disease from spores in the community). The prevalence of asymptomatic carriage in hospital admission ranges from 7%-18%[27].

This has great clinical implications. Widespread community colonization with toxigenic *C. difficile* suggests that attempts to restrict spore spread only in the context of known exposure in healthcare settings may be insufficient. For meaningful interruption, universal modified contact precautions for all admissions may be necessary. Measures to prevent spore formation may alter the transmission cycle. Further study of the mechanism of spore formation may identify new targets. Thus far only fidaxomicin has been shown to decrease spore formation most likely by inhibiting transcription of sporulation genes[28]. Its high cost however precludes widespread use, as discussed below.

VEGETATIVE FORMS: TOXIN PRODUCTION AND CDI

Germination of spores to toxin producing vegetative forms can occur within minutes of exposure to specific triggers deemed germinants (*i.e.*, taurocholate)[14,16]. Taurocholate (a primary bile acid) is both necessary and sufficient to trigger *C. difficile* germination. L-glycine acts as a cogerminant[29]. In contrast, certain secondary bile acids, *i.e.*, deoxycholate can inhibit vegetative growth[30]. Secondary bile acids are derived by the action of endogenous flora on primary bile acids[31] and the relative ratio of each in the colon may determine spore/vegetative balance.

Toxins A (TcdA) and B (TcdB) and binary toxin (CDT) are the major virulence factors that contribute to pathogenesis[32]. Toxins A and B are multi-domain proteins that share a high degree of homology and comprise an N terminal catalytic domain with glucosyltransferase activity, a middle translocation domain and a C-terminal host cell binding region[33]. The toxin receptor remains unknown.

Both A and B are proinflammatory and cytotoxic and it is not clear if both are needed for pathogenesis[34]. Both alter the actin cytoskeleton, disrupt the epithelial barrier and cause apoptosis by glucosylation and inactivation of GTPases-Rac, Rho and Cdc42[35]. This induces mucosal damage and inflammation. Toxin expression derives from a 19.6 kb pathogenicity chromosomal locus (PaLoc) that encodes *TcdA* and *TcdB* in addition to *TcdR* (RNA polymerase sigma factor that positively regulates toxin expression), *TcdC* (putative negative regulator-deletion in 027 ribotype may increase toxin production), and *TcdE* (related to bacteriophage holins)[32,35]. The role of the toxins in the bacterial life cycle is unclear. Different *PaLoc* variants are called toxinotypes: 34 are described[36]. *PaLoc* has features of both stable integration and a mobile genetic element[37]. The CDT-binary toxin expressed in 027 ribotype ADP ribosylates G actin in target cells leading to protrusion bodies of microtubules that contact *C. difficile* and possibly increase colonization efficiency[38].

Toxigenic *C. difficile* causes disease: However colonization with toxigenic *C. difficile* can be asymptomatic[27]. After successful treatment many patients will continue to shed spores without manifesting disease. Colonization is a critical step in the pathogenic process and depends on adherence to gut epithelial cells by adhesion and flagellin proteins[39-41].

Colonization with non-toxin forming *C. difficile* may out-compete toxin forming *C. difficile*[27]. In one recent study, administration of nontoxigenic *C. difficile* spores (NCTD-M3) to patients after treatment of either first CDI episode or first recurrence, showed a 3-fold reduction (from 30% to 11%) in recurrent disease compared to placebo[42]. Patients given 10^7 spores/day for 7 d had the lowest recurrence rate (5%)[42]. The study does raise some concerns, primarily the possible acquisition of toxin containing PaLoc sequences by toxin negative strains, an event that has been shown to occur *in vitro*[43].

In theory, non-antibiotic toxin binders could ameliorate disease without disrupting intestinal flora. Cholestyramine, which binds toxin has been tried[44]. One difficulty is that it also binds vancomycin (as does colestipol and other anion exchange resins), complicating its use[45]. It can also bind bile salts and potentially stimulate *C. difficile* growth[46]. Given lack of efficacy data and possible harmful interactions use of cholestyramine or colestipol is not recommended.

Tolevamer, a polymer of styrene-sulfate that binds *C. difficile* toxin *in vitro*, was inferior to both metronidazole and vancomycin in 2 phase Ⅲ trials[47]. Only 44% of patients who took tolevamer had resolution of diarrhea or abdominal pain compared to 73% for metronidazole and 81% for vancomycin[47].

IMMUNE RESPONSE TO TOXINS AND CDI

Only half of hospitalized patients colonized with *C. difficile* develop CDI, and initial disease is associated with lack of anti-toxin A IgG[48]. The host immune response also plays a part in recurrent disease- patients with antibodies to toxin are less likely to relapse than those with undetectable toxin antibody[49,50]. Passive immunization by administration of intravenous immunoglobulin may have a role in patients with hypogammaglobulinemia[51,52], or in patients with severe disease[53].

Specific anti-toxin antibodies prevent mortality independent of antibiotic treatment. In one study a 3-fold reduction in relapse (25% to 7%) was seen when anti-toxin antibodies were used[54]. Data in animal models supports the efficacy of toxin-targeted vaccines[55]. Formalin inactivated toxin A/B (toxoid) protected hamsters from lethal *C. difficile* challenge[56]. Currently there are 2 vaccines in human trials. Sanofi Pasteur formalin inactivated toxins A/B vaccine was safe, well tolerated and immunogenic (generated antibodies to toxin)[57]. It is now in phase Ⅲ trial for primary prevention (https://clinicaltrials.gov/ct2/show/NCT01887912). An alternate approach involves a recombinant fusion protein of toxins A/B. A phase 1 trial of escalating doses of this recombinant is completed and results are pending (https://clinicaltrials.gov/ct2/show/NCT01296386).

There is some evidence of efficacy of vaccines in secondary prevention of RCDI[58], but more data is needed.

STANDARD ANTIMICROBIAL TREATMENT OF RCDI

Antimicrobial stewardship remains a key element of any RCDI management strategy. The reader is directed to other reviews for further discussion[59-61]. This review will focus on RCDI specific treatment.

Standard antimicrobial therapy targets the vegetative forms of *C. difficile*[7,52]. Spore vegetation and recurrent CDI are intricately linked. Favoring germination (by altering the germinant/sporulation ratio towards vegetation) would in theory allow eradication with antibiotics. Depending on antibiotic used however, this can also alter the microbiome and could increase the likelihood of relapse. Alternatively inhibiting germination, *i.e.*, by altering the gut flora towards secondary bile acids that inhibit vegetative forms[46] might also be a therapeutic option.

The use of vancomycin to treat CDI predates recognition of *C. difficile* as the causative agent of antibiotic associated colitis. First recurrence of CDI is treated with the same agent used for the initial episode. If clinically severe then vancomycin is used[7,52]. For second recurrence, pulsed and/or tapered vancomycin is recommended. Metronidazole is not used beyond the first recurrence due to possible cumulative neuropathy[62] (Table 1 is a summary of general clinical approach to RCDI).

Data supporting these recommendations is recognized as weak and poor quality with no corroborative randomized controlled trials.

Tedesco *et al*[63] reported on 22 patients treated for 21 d with a vancomycin taper and pulse and noted

Table 1 Management outline for recurrent *Clostridium difficile* infection[7]

General
Stop/minimize antibiotics (if possible, to allow gut flora to repopulate)
Rule out other causes of diarrhea, *i.e.*, post-infectious IBS
(check stool for C diff only in context of symptoms, not as test of cure)
Antibiotic treatment
Use the same antibiotic as initial regimen (depending on disease severity and response to initial treatment)[7,52]
Consider Vancomycin taper ± pulse[11]
Vancomycin followed by rifaximin chaser[67]
Fidaxomicin[80]
Probiotics
Probiotics with antibiotics may help[99]. Consider adding to last 2 wk of vancomycin pulse/taper and continue for 4 wk after
(caution in immunocompromised patients- may cause fungemia. Don't use in isolation. Not standardized, doses/active agents may vary)
Immunotherapy
Monoclonal antibody (neutralize toxin)[54]
IVIG[51]
Toxoid vaccine[58]
Non toxigenic strains[42]
Bacteriotherapy
Fecal microbiota transplant[111,114]

IBS: Irritable bowel syndrome; IVIG: Intravenous immunoglobulin.

no relapses (average follow-up 2-12 mo). In McFarland *et al*[11], 83 patients treated with 10-14 d course of vancomycin had an average relapse rate of 55% (range 42%-71%, depending on vancomycin dosing). Twenty-nine patients were treated with a vancomycin taper over an average of 21 d and 31% relapsed. If vancomycin taper was followed by vancomycin pulse (drug dosed every 48 or 72 h) then relapse decreased to 20% (10 patients). Lastly, 7 patients treated only with vancomycin pulse had 14% relapse[11]. The theory behind pulsed doses is to target vegetative forms of *C. difficile* but still allow restitution of the gut flora[11]. These numbers are small and the approach is not standardized. Oral vancomycin is also expensive: A 6 wk tapered course can cost hundreds of dollars[64].

Management of those who fail pulsed/tapered vancomycin is challenging.

ALTERNATIVE AGENTS FOR RCDI

Rifaximin is a synthetic rifamycin derivative that inhibits transcription[65]. It has little (< 0.4%) systemic absorption[65]. It is not used as monotherapy due to rapid emergence of resistance[66,67]. It has been used as an adjunct to vancomycin after 2 wk of standard treatment or taper[67]. Dosed at 400 mg BID for 2 wk after vancomycin taper, cure was described in 17/20 patients in 3 reports[67-69]. Recurrence rate was similar (15%) in a small (68 patients) RCT[70].

Fidaxomicin is the first macrolide antibiotic with an 18 membered macrocyclic lactone ring[71]. It is bactericidal and acts at an early step of RNA synthesis (it stops DNA strand separation)[72]. The *C. difficile* minimum inhibitory concentration is lower than that for vancomycin or metronidazolel[73]. A prolonged post antibiotic effect of at least 10 h allows twice daily dosing[74]. It is not absorbed systemically and has minimal effect on the gut microbiome. The effect on transcription inhibits both sporulation and toxin production[28,75]. The effect on sporulation may impact recurrences.

In vitro then and based on mechanism of action fidaxomicin should be an attractive option for RCDI. Indeed, in a phase 3 trial fidaxomicin was non inferior to vancomycin in terms of clinical cure[76]. Moreover, in the same study it strikingly decreased recurrence rates from 24%-25% to 13%-15%. Adverse event profiles were similar.

Subset analysis looking specifically at RCDI confirmed both the efficacy of fidaxomicin and decreased recurrence[77]. The stumbling block with fidaxomicin is the prohibitive cost ($140 per pill, 2800 for ten day course)[52].

Cadazolid, a novel hybrid antibiotic with a quinolone pharmacophore incorporated in an oxazolidinone ring has potent anti *C. difficile* activity and decreased propensity to induce antibiotic resistance[78,79]. It has a dual mechanism of action, both inhibiting translation and DNA synthesis[78,80]. Phase 1 studies with doses up to 3000 mg indicated the drug to be generally well tolerated with headache and diarrhea being most common SE.

A phase Ⅱ multi-center, double-blind, randomized study was conducted in 84 CDI patients. Cadazolid was dosed at 250, 500, or 1000 mg and deemed comparable or superior to vancomycin with respect to clinical and sustained cure rates[79,81]. Lower recurrence rates (18%-25% *vs* 50%) were noted for all doses[82]. Although there is no data as yet in RCDI, given decreased recurrence rate, and reported impact on spore production efficacy in RCDI is of significant interest.

GASTROINTESTINAL MICROBIOME: ROLE IN CDI

The adult gastrointestinal tract has 10^{14} bacterial cells from > 1000 different bacterial species[83,84], which comprise the microbiome, or gut flora. Composition varies depending on diet, age and health[85]. A "healthy" microbiome has a large number of different species of microorganisms with more of certain phyla, *i.e.*, *Firmicutes* and *Bacteroides* and less of others, *i.e.*, *Proteobacteria*[86]. Gut bacteria play critical roles in immunity, epithelial barrier function (resist pathogens) and nutrient absorption[87]. Any imbalance (in number, species, or composition) can distort this symbiosis leading to the converse, known as dysbiosis[88,89]. The microbiome varies between individuals but is generally stable over time[90].

C. difficile can be part of the normal microbiome[88], but is generally contained by other more dominant anaerobes. A healthy microbiome may protect against CDI in different ways. One may simply be due to numbers and competition for nutrients and mucosal niches[30].

Alternatively, the microbiome may elicit substances, *i.e.*, short chain fatty acids that actively inhibit *C. difficile*[91]. Normal intestinal flora primes a Myd88 TLR-5 dependent innate immune response which protects against CDI[92]. More recent data shows that certain bacteria (*i.e.*, *Clostridium schindens*) change the primary and secondary bile acids ratio[46].

The most common cause of alteration in the microbiome is antibiotic use, which can affect "mutualistic" interactions[93]. The gut microbiome in patients with *C. difficile* is indeed dysbiotic[94,95]. Probiotics have been used in an attempt to redress this.

Probiotics are preparations containing live microbial agents that may be beneficial to the host when ingested. They range from yoghurt to specific microbial extractions (*i.e.*, *lactobacillus*, *Saccharomyces boulardii*). Efficacy in RCDI may be multifactorial and comprise restitution of gut flora[96], specific anti *C. difficile* effect (*i.e.*, *S. boulardii* protease cleaves Toxin A)[96] and/or immune modulation[97].

At present preparations are not standardized or regulated, and may have no live organisms or organisms not listed on label[52]. There is risk of fungemia or bacteremia- even in immunocompetent hosts[98].

Staggered and tapered vancomycin with daily kefir (yoghurt) led to resolution of symptoms in 21/25 patients with RCDI[99]. This was a retrospective study and remains to be confirmed.

FECAL MICROBIOTA TRANSPLANTATION

Administration of exogenous fecal material *via* fecal microbiota transplant (FMT) to correct intestinal dysbiosis has been used successfully to treat CDI. FMT for pseudomembranous colitis was performed in 1950s by Eiseman *et al*[100] using fecal enemas. Successful use of FMT to treat CDI was reported in 1983[101]. A proof of principle study reported by Silverman *et al*[102] in 2010 described 7 patients with RCDI who self-administered fecal enemas at home. At an average of 14 mo follow-up there were no recurrences[102]. Brandt *et al*[103] reported long term follow-up of 77/94 patients administered colonoscopic FMT for RCDI with primary cure rate of 91% (resolution of symptoms without recurrence). Since then multiple case reports and small series have been published showing efficacy in CDI[102,104]. An open label randomized clinical trial comparing fecal transplant to vancomycin was stopped early when interim analysis showed that 94% patients in the transplant group had improvement of diarrhea compared to 31% in the vancomycin alone group[105]. FMT has been reported for more than 1000 cases worldwide with > 90% efficacy[106], including patients with severe CDI[107]. Current guidelines recommend FMT for 3rd recurrence (*i.e.*, after vancomycin taper)[7,52].

Also deemed "bacteriotherapy", FMT restores both the microbiome and favorable bile acid composition[31,108].

Barriers to mainstream use of fecal transplants have included general aversion to knowing ingestion of feces, technical issues with standardization of material (route of administration, donor, volume, preparation) and concern for transmission of disease/infection[109]. Donors are screened and stool tested for transmissible pathogens[110].

An attempt to standardize FMT involving frozen oral FMT capsules led to 90% clearance of diarrhea[111]. A recent trial from Canada directly compared efficacy of frozen- thawed *vs* fresh FMT administered *via* enema and showed equivalent outcomes (70%-75% overall cure)[112]. An alternative approach involved SER-109, a novel *Firmicutes* spore containing oral agent derived from healthy stool[113]. Thirty patients with RCDI received SER-109 after standard CDI antibiotic treatment. At 8 wk 29/30 patients showed clinical resolution and diversification of gut flora[113].

If borne out, these approaches would negate concerns for procedural risk, donor variability and disease transmission and allow standardization of transplanted material.

Many questions remain with respect to the microbiome and its role in RCDI. If indeed the main protective effect relates to bile acid composition then perhaps administration of favorable agents, *i.e.*, deoxycholate may suffice. Defined microbial systems (*i.e.*, a mixture of known specified microbes) have been used to treat CDI also[114]. The optimal composition remains to be defined. Current use of FMT is for those who have failed standard RCDI therapy. Use as first line therapy or indeed as prophylaxis in patients receiving antibiotics is possible. The role of microbiome modulation with FMT in other disease states ranging from obesity to multiple sclerosis[106] is being explored.

CONCLUSION

Recurrent/relapsing *C. difficile* remains a therapeutic challenge. *C. difficile* spores are the agents of persistence and disease and additional efforts to minimize spread are warranted. Further research on factors that affect sporulation and vegetation may yield additional therapeutic targets. The role of the gut microbiome remains mysterious; however it is clearly of great importance not only in RCDI, but in myriad disease states. FMT is an effective therapeutic modality, but long term follow-up is needed.

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P- Reviewer: García-Elorriaga G, Krishnan T, Moschovi MA
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Li D

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5495/wjcid.v6.i3.37

World J Clin Infect Dis 2016 August 25; 6(3): 37-60
ISSN 2220-3176 (online)
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REVIEW

Clinical research in febrile neutropenia in cancer patients: Past achievements and perspectives for the future

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Author contributions: Klastersky J contributed to historical background and introduction; Paesmans M contributed to risk prediction for complications and death; Klastersky J contributed to prevention according to risk; Aoun M contributed to empiric therapy according to risk; Georgala A contributed to emergence of resistant strains; Loizidou A contributed to persisting febrile neutropenia; Lalami Y contributed to cost issues; Dal Lago L contributed to febrile neutropenia at the extreme of age; Klastersky J and Aoun M contributed to conclusion.

Conflict-of-interest statement: None of the authors has any conflict of interest.

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Manuscript source: Invited manuscript

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Received: June 30, 2015
Peer-review started: July 6, 2015
First decision: September 30, 2015
Revised: April 25, 2016
Accepted: June 1, 2016
Article in press: June 3, 2016
Published online: August 25, 2016

Abstract

Febrile neutropenia (FN) is responsible for significant morbidity and mortality. It can also be the reason for delaying or changing potentially effective treatments and generates substantial costs. It has been recognized for more than 50 years that empirical administration of broad spectrum antibiotics to patients with FN was associated with much improved outcomes; that has become a paradigm of management. Increase in the incidence of microorganisms resistant to many antibiotics represents a challenge for the empirical antimicrobial treatment and is a reason why antibiotics should not be used for the prevention of neutropenia. Prevention of neutropenia is best performed with the use of granulocyte colony-stimulating factors (G-CSFs). Prophylactic administration of G-CSFs significantly reduces the risk of developing FN and consequently the complications linked to that condition; moreover, the administration of G-CSF is associated with few complications, most of which are not severe. The most common reason for not using G-CSF as a prophylaxis of FN is the relatively high cost. If FN occurs, in spite of prophylaxis, empirical therapy with broad spectrum antibiotics is mandatory. However it should be adjusted to the risk of complications as established by reliable predictive instruments such as the Multinational Association for Supportive Care in Cancer. Patients predicted at a low level of risk of serious complications, can generally be treated with orally administered antibiotics and as out-patients. Patients with a high risk of complications should be hospitalized and treated intravenously. A short period of time between the onset of FN and beginning of empirical therapy is crucial in those patients. Persisting fever in spite of antimicrobial therapy in neutropenic patients requires a special diagnostic attention, since invasive fungal infection is a possible cause for it and might require the use of empirical antifungal therapy.

Key words: Fever; Neutropenia; Prophylaxis; Algorithm; Cancer

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Core tip: The overall presentation of febrile neutropenia has considerably changed over the last 50 years. Prevention is now feasible with the use of granulocyte colony stimulating factors. If fever appears in a neutropenic patient, empirical therapy with broad spectrum antibiotics is mandatory; it should be adapted to the risk of severe complications that can be now predicted in individual patients using a reliable scoring system. Special situations such as persisting fever in neutropenic patients, the risk of invasive fungal infection and the management of older patients are crucial questions that are discussed as well as the issues linked to the high cost of prophylaxis and therapy.

Klastersky J, Paesmans M, Aoun M, Georgala A, Loizidou A, Lalami Y, Dal Lago L. Clinical research in febrile neutropenia in cancer patients: Past achievements and perspectives for the future. *World J Clin Infect Dis* 2016; 6(3): 37-60 Available from: URL: http://www.wjgnet.com/2220-3176/full/v6/i3/37.htm DOI: http://dx.doi.org/10.5495/wjcid.v6.i3.37

HISTORICAL BACKGROUND AND INTRODUCTION

In 1966, Bennett *et al*[1] showed convincingly that severe and/or protected neutropenia, in cancer patients, was associated with increased risks of severe infection. At that time, patients receiving chemotherapy (CT) were almost exclusively those with acute leukemia, a condition associated with severe bone marrow dysfunction. As a result of severe neutropenia, overwhelming infection-mainly caused by Gram-negative sepsis - was responsible for a mortality in the range of 90%, often precluding the completion of successful anti-leukemic therapy[2]. It was also observed at that time that mortality resulting from sepsis, in those severely neutropenic patients, was early after the onset of fever and that fever was often the only manifestation of the infection; this led to the concept of febrile neutropenia (FN), which was widely accepted as a significant clinical syndrome.

Today, the syndrome has become more heterogeneous; most patients with FN are receiving relatively less myelotoxic CT for solid tumors; as a consequence, the overall incidence of FN in CT-treated patients has dropped to 10% and the overall mortality, in cases of Gram-negative bacteremia, is about 20%[3]. At the same time, there has been a significant shift in the microbiological etiology of FN in neutropenic patients; gradually Gram-positive infections became more prevalent and, actually, Gram-positive and Gram-negative microorganisms are involved, as a cause of bacteremia in patients with FN, in 50% of the cases, respectively[3].

A major advance in the approach of FN has been the introduction of empirical broad spectrum antimicrobial therapy as soon as fever appeared in a neutropenic patient[4]. That concept that has never been challenged in a comparative trial, was then against the dogma of treating infection; however, it proved to be obviously so effective that it is still accepted as a paradigm for the management of FN today[5].

However, with the changing epidemiology of FN, it became obvious that all patients with FN probably had no longer the same risk of complications and death; this observation led to the search for prognostic factors of these complications and, consequently, with the possibility of prediction of that risk, to adjustments of empirical therapy. These aspects will be dealt with in details later in this paper. Finally, a major issue in CT treated cancer patients is the prevention of FN; these aspects will also be discussed in detail later.

NATURAL HISTORY OF FN

The severity of neutropenia - which directly influences the frequency of FN - is clearly related to the intensity of CT (number of agents and respective doses, as well as the myelotoxic potential of each component). However, the relationship between the type of CT and the risk of FN is far from being perfect. There are models that classify the common CT regimens according to the risk of FN as being low (< 10%), intermediate (10%-20%) or high (> 20%)[6,7] but their predictive values are far from being optimal because they do not take into account the factors linked to the patients and to the underlying disease(s) (cancer and co-morbidities) which can increase the risk of developing FN and result in different frequencies of FN with the use of the same type of CT. These factors, which also increase the risk of complications and death during an episode of FN, will be discussed later.

It has been shown, in patients with many different tumors (lymphoma, breast, colon, lung, ovary and others) that the risk of developing FN is maximal during the first cycle of CT and diminished afterwards[8]. While the precise reason for that is not known, the clinical implication is very clear: If a prophylaxis of FN exists (this will be discussed later), it should be applied from the first cycle of CT.

As shown in Table 1, FN is associated with a significant frequency of severe complications and deaths. These data are derived from a study of 2142 patients with FN registered in two observational studies conducted in different institutions and different countries[3]. It is shown that the type of underlying neoplasia, be it hematological malignancy or solid tumor, does not influence significantly the incidence of complications or deaths during episodes of FN; on the other hand, the presence of bacteremia significantly increases both morbidity and mortality. Unfortunately, bacteremia is not easy to predict on a clinical basis at the time of onset fever, although manifestations such as high fever, hypotension and thrombocytopenia are possible clues for it. It is also important to stress that the presence of a focal infection (*e.g.*, pneumonia or cellulitis) increases the

Table 1 Complications and death rates in patients with febrile neutropenia

	Complications (%)		Mortality (%)	
	Hemopathies	Solid tumors	Hemopathies	Solid tumors
No bacteremia	17	11	4	3
Bacteremia	30	35	9	13

Adapted from Klastersky *et al*[10].

risk of dying during an episode of FN; these focal infections are probably a surrogate for bacteremia but they also can lead to specific local complications by themselves[9]. Besides the severity of neutropenia (which is mainly influenced by the type of CT administrated) and the presence of bacteremia (which is difficult to predict) other factors influence significantly the risk of complications and death during an episode of FN. Among these factors, age (> 65 years) plays a critical role[10]. As shown recently, adverse events (including neutropenia) were more frequent in elderly patients[11]; the importance of prevention of severe neutropenia in elderly patients cannot be overemphasized.

Besides age and the other predisposing factors to complications and death, various comorbidities such as the stage of the neoplastic disease, poor nutrition, diabetes, chronic pulmonary disease, renal function impairment, and many others increase the morbidity and mortality of FN. Although the precise evaluation of the risk of FN associated with these various comorbidities, is not always easy to define, it is clear that it significantly increases with the number of comorbidities that are present in a patient[8,12].

Before finishing this introductory review of the past and present of FN, it is important to stress two important consequences of the development of FN in a patient. The first is the possible impact of FN on the following courses of CT as in some patients the dose of CT may be reduced or its timing modified, with possible reduction of the dose intensity, jeopardizing the efficacy of anticancer treatment; this might be particularly detrimental for patients treated with curative intent or in the adjuvant or neoadjuvant setting.

The second aspect to be stressed is that the cost of FN is substantial; it is estimated to be in the range of $16000 for each episode, with those episodes associated with complications or death being the most expensive[13]. Although these cost figures vary from country to country and from institution to institution, it is generally considered that they are underestimated, especially if all the expenses, including namely the social costs, are taken into account.

RISK PREDICTION FOR COMPLICATIONS AND DEATH

Past achievements

FN is a limiting factor for CT administration and requires prompt initiation of antimicrobial treatment. It is a possibly lethal complication with a mortality rate as high as 10% and associated costs are important especially if patients need to be hospitalized[14]. On the other hand, FN has long been recognized as a heterogeneous syndrome in terms of type and site of infection, further neutropenia duration, *etc*. Some patients at high risk may therefore be undertreated at the time of initiation of empiric treatment and some patients may be overtreated. Risk prediction is therefore an important issue with therapeutic implications: If correctly identified, low-risk patients may benefit from simplified therapy (oral therapy, outpatient treatment) and high-risk patients might benefit from more aggressive initial antimicrobial therapy and/or from early intensive care.

At least, two approaches can be considered to predict risk: One is to make use of clinical criteria defined alone without assessment of the possible interactions between them, the other is to integrate independent risk factors to produce a model predicting risk. Risk models have the following advantages: They only make use of the non-redundant information, they should produce objective and reproducible prediction, they have known characteristics. They however have drawbacks: They need to be validated, updated and tested in different settings. Nevertheless, we will focus our report on risk models only and for populations of adult patients.

When risk models are to be developed, an outcome has first to be defined: It might be development of bacteremia, development of invasive bacterial infection, response to empiric treatment, serious medical complication, death or death due to infection. This last endpoint is likely the most relevant one but due to its low frequency, developing a model for its occurrence is highly challenging due to sample size issues. The validated models have made use of a composite endpoint: Occurrence of a serious medical complication and/or death. Secondly, the clinical use for the model needs to be defined in order to optimize the model for the chosen goal.

Models developed to predict low-risk of serious medical complications and/or death

There are essentially two models that have been validated.

Talcott's model: The first one was developed and validated by Talcott *et al*[15]: It was derived, using clinical judgment, on a series of 261 febrile neutropenic episodes and firstly validated on a series of 444 episodes. Unfortunately, that model, although being reliable for predicting FN patients at low risk of complications (with an excellent positive predictive value but lacking from sensitivity), was not effective[16], as 9 patients out of 30 (30%) needed readmission. After that pilot study, a randomized clinical trial was initiated comparing management of patients with FN in-hospital or with early discharge. Planned sample size was 448 patients for showing an increase from 4% to 10% of the complication rate although an equivalence design (or a non-inferiority of the experi-

Table 2 Multinational Association for Supportive Care in Cancer scoring system

Characteristic	Weight
Burden of illness: No or mild symptoms	5
No hypotension	5
No chronic obstructive pulmonary disease	4
Solid tumor or no previous fungal infection	4
No dehydration	3
Burden of illness: Moderate symptoms	3
Outpatient status	3
Age < 60 yr	2

Points attributed to the variable "burden of illness" are not cumulative. The maximum theoretical score is therefore 26.

mental arm) would have been more convincing. The trial was closed for poor accrual after recruitment of 113 patients (66 in the in-hospital arm and 47 in the arm with early discharge). Complication rates were 9% *vs* 8%. Surprisingly, there was no evidence for improvement of patients' quality of life (QoL) in the experimental arm but costs were reduced with the home arm[17].

Multinational Association for Supportive Care in Cancer model: The Multinational Association for Supportive Care in Cancer (MASCC) risk-index score has been developed (Table 2) and its clinical prediction rule for identification of low-risk patients was first validated in the primary publication[18]. The event "occurrence of a serious medical complication" was precisely defined in the study protocol and can be found in[18]. The MASCC score has been, since 2002, accepted as a standard technique to predict low-risk of complications in patients with FN by the European Society of Medical Oncology[19] and by Infectious Diseases Society of America (IDSA)[20,21]. Indeed, several validation studies[22-28] were published and already tabulated in a review published in supportive care in cancer (Table 3)[29]. From this review, it should be stressed that the performance of the MASCC model decreases when haematological patients are present in the patients populations. The positive predictive value is > 90% when the score is used for patients with solid tumor but may decrease to 83% when haematological patients are eligible.

The MASCC model represents an improvement over the Talcott's classification[18]. The selected factors appear to be more specifically associated with the clinical severity of the FN episode rather than with the underlying cancer. A weakness of the model is that it includes a subjective assessment, burden of illness but all the attempts to substitute it with more objective factors failed. Hematological malignancy was not included in the final model. Neutropenia duration certainly plays a role too but cannot be reliably assessed at the onset of the febrile episode. The MASCC score is however not perfect, especially in patients with hematological patients. However, up to now, attempts to improve it did not lead to the development of validated models ready to use in clinical practice[30-32].

The use of the MASCC model to guide the management of a febrile neutropenic episode has been studied and includes the choice of the empiric regimen (intravenous, oral, monotherapy or combination) or the setting of treatment (in-hospital, in-hospital with early discharge or ambulatory) according to risk[33]. For instance, oral therapy has been shown to be safe in patients predicted at low-risk by the MASCC score[24,25,34-37] as well as a management including early discharge, expected to improve patients QoL, to reduce risk of nosocomial infections and costs, individual[24,38] studies as well as in meta-analyses[39,40]. Even, in hematological patients, outpatient treatment seems to be possible in patients who are clinically stable and defervesced[23]. It should be stressed however that low-risk prediction is not the only criterion for suitability for oral and/or ambulatory therapy as other factors need to be considered (like social factors and acceptance of home therapy by patients and their physicians).

Models developed to predict low-risk of serious medical complications and/or death

MASCC model: The MASCC model was developed to predict a low risk of serious complications and the threshold of 21 was chosen to optimize sensitivity for a targeted positive predictive value. However, the value of the score estimates the probability of complications and other thresholds could be considered when prediction of high-risk is the goal as the threshold of 21 is clearly associated to a too low sensitivity. Combining the data from 2 observational studies[41], overall complications rate was 79% and mortality rate was 36% in patients with a score < 15. However, no clinical prediction rule for predicting high-risk was proposed. Blot and Nitenberg[42] suggested to improve the performance of the MASCC score for high-risk prediction by repeating calculation of the severity score and by including number of organ dysfunction but they didn't propose any practical model. Some laboratory parameters have been suggested to be associated with poor outcome in patients with FN as thrombocytopenia and increased CRP[43], serum lactate[44,45], electrolytes abnormalities[46].

CISNE score: A Spanish team worked on the prediction of serious complications for patients with FN. In a first study, designed as a case-control study[28], they reviewed retrospectively 861 episodes of FN and matched patients who developed complications to patients who did not (3 controls for 1 case): They suggested that ECOG performance status $\geqslant$ 2, chronic obstructive pulmonary disease, chronic heart failure, stomatitis grade $\geqslant$ 2, monocyte count and stress hyperglycemia are factors associated to complications. From a subsequent data set of 1133 patients with FN and clinically stable 3 h after FN diagnosis, they derived, using logistic regression analysis, and validated a score predicting complications, ranging from 0 to 8 (Table 4)[47]. They defined low (score of 0) and intermediate risk (score of 1 or 2) *vs* high-risk (score > 2). The characteristics of CISNE score and MASCC

Table 3 Validation studies of Multinational Association for Supportive Care in Cancer score for predicting low-risk

Ref.	N episodes	Patients with hema-tological malignancy (%)	Predicted at low-risk (%)	Se (%)	Sp (%)	PPV (%)	NPV (%)
Klastersky *et al*[24], 2006	1003	55	72	79	56	88	40
Stratum of hematological tumors	549	100	70	77	51	84	40
Stratum of solid tumor patients	454	0	74	81	64	93	38
Uys *et al*[22], 2004	80	30	73	95	95	98	86
Cherif *et al*[23], 2006	279	100	38	59	87	85	64
Klastersky *et al*[24], 2006	611	43	72	78	54	88	36
Innes *et al*[25], 2008	100	6	90	92	40	97	20
Baskaran *et al*[26], 2008	116	100	71	93	67	83	85
Hui *et al*[27], 2011	227	20	70	81	60	86	52
Carmona-Bayonas *et al*[28], 2011[1]	169	0	?	94	36	NA	NA

[1]Selected patients population ("apparently" stable patients). The characteristics were calculated for a test aiming to identify low-risk patients and may then differ from the original publications. Due to the case-control design of the study, the rate of patients predicted at low risk as well as the negative and positive predictive values are meaningless. Se: Sensitivity; Sp: Specificity; PPV: Positive predictive value; NPV: Negative predictive value.

Table 4 CISNE score

Characteristic	Weight
ECOG performance status ⩾ 2	2
Stress induced hyperglycemia	2
Chronic obstructive pulmonary disease	1
Chronic cardiovascular disease	1
Mucositis NCI grade ⩾ 2	1
Monocytes < 200/μL	1

ECOG: Electrocorticogram; NCI: National cancer institute.

score (at the threshold of 21 chosen however to predict low-risk) for predicting high-risk are shown in Table 5. Although the overall misclassification rate is lower for MASCC than for CISNE, sensitivity for predicting high-risk is much better for CISNE score as well as negative predictive value. Positive predictive value is poor for both systems. The authors acknowledged the fact that a threshold of 21 for MASCC was not intended to predict high-risk but stated that CISNE score remains more performant at other thresholds than the MASCC score.

Perspectives

Many achievements were reached for predicting low-risk for FN and allowed to successfully adapt therapeutic strategy. There is however place for improvement, especially for increasing the positive predictive value overall and certainly for patients with hematological malignancies. Further research may include further investigation of laboratory parameters, investigation genetic predisposition for infection development or monitoring of intermediate-risk patients with early repeated measurements of risk scores of whom we don't know the value. The situation is more challenging for identifying patients at high-risk. The CISNE score was only very recently proposed and its usefulness for improving patients outcome remains to be demonstrated. Clinical trials should be conducted to assess the value of "aggressive" empiric therapy or the use of early intensive care. Due to the relative low frequency of complications, further achievements in this area will be possible only thanks to large international collaboration studies that should be strongly encouraged.

Table 5 Characteristics of CISNE score and Multinational Association for Supportive Care in Cancer score for predicting high-risk

	CISNE	MASCC
Predicting high risk, complications	118	53
Predicting low risk, no complications	747	853
Predicting high risk, no complications	234	128
Predicting low risk, complications	34	99
	1133	1133
Se	0.78	0.35
Sp	0.76	0.87
PPV	0.34	0.29
NPV	0.96	0.90
Miscl rate	0.24	0.20

High-risk of prediction: CISNE > 2, MASCC < 21. Se: Sensitivity; Sp: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; MASCC: Multinational Association for Supportive Care in Cancer.

PREVENTION OF FN

As has been stated in the introduction, FN is associated with serious medical complications; moreover, it can jeopardize the effectiveness of CT and represents significant extra-cost. Although, the incidence of FN and the frequency of associated complications have decreased significantly over the last 50 years, FN remains a major medical problem in patients receiving CT, especially in view of the large numbers of patients receiving CT today all over the world. It is estimated that 10% of these patients will develop FN and that 10% of them will die as a result of it; which means that eventually 1% of the patients receiving CT die as a consequence of neutropenia, a figure which is appalling for patients treated with a curative intent or in the adjuvant or neoadjuvant setting[1].

The first attempts to prevent FN in CT-treated patients has been done with antimicrobials (first non-absorbable antibiotics and later, co-trimoxazole) with some success, but also with the observation of the emer-

gence of resistant strains that limited soon or later the efficacy of that approach[2,3].

Recently, fluoroquinolones have been broadly used for that prophylaxis. Once again, most studies showed that fluoroquinolones reduced the incidence of infection and the infection-related mortality in neutropenic patients but at the expense of emergence of quinolone-resistant strains[4]. This should at the end make the prophylaxis useless; moreover, these strains jeopardize the use of fluoroquinolones as a therapy of FN, in low risk patients, as will be discussed elsewhere. For all those reasons, the use of antimicrobials, including fluoroquinolones, should be discouraged. Guidelines from American Society for Clinical Oncology limit the use of antibacterial prophylaxis to patients at high risk for FN; others recommend avoidance of such practices for the prevention of FN[5].

The use of granulocyte-colony stimulating factors (G-CSF)[1]; this approach is highly effective, without virtually any short-term side effects; on the other hand, more problematic is the cost of such a prophylaxis and this is clearly a limiting factor for a large scale use today. Two pivotal studies have established the effectiveness of primary prophylaxis with either filgrastim[6] or pegfilgrastim[1]. Pegfilgrastim differs from filgrastim by its prolonged time of action, as the polyethylene glycol tail added to the filgrastim molecule, prevents it from being excreted through the kidneys; the elimination of pegfilgrastim depends only on its inactivation by the rising numbers of neutrophils. Therefore, pegfilgrastim can be administered as a single injection after CT, whereas filgrastim requires daily injections and periodic granulocyte level monitoring until neutrophil recovery (usually 7 to 10 doses). This makes pegfilgrastim use easier for the patient and the physician, but an injection of pegfilgrastim costs at least twice as much as a full course (10 administrations) of filgrastim.

Several meta-analyses have confirmed the efficacy of G-CSF for the prevention of FN in CT-treated patients, and have shown that mortality associated with FN could be reduced[8,9].

Is pegfilgrastim more effective than filgrastim in preventing FN? A recent meta-analysis suggests that it might be the case[10]. However, outside clinical trials, it appears that in the community oncology practice, despite that filgrastim is often given later and for shorter times than officially recommended, no major differences are seen between the efficacy of pegfilgrastim and filgrastim[11,12].

The current recommendations, namely those proposed by European Organization for Research and Therapy of Cancer (EORTC)[13] state that patients with a > 20% risk of developing FN should receive G-CSF primary prophylaxis and those with a risk < 10% should not. Patients with an intermediary risk (10%-20%) should be evaluated for further risk factors, such as age > 65 years, advanced disease and various comorbidities (as discussed previously in the introductory section); if present, those factors should lead to a more liberal use of G-CSF in that group of patients. The general use of algorithm in the use of G-CSF in neutropenic patients for primary prophylaxis of FN is indicated in Figure 1.

The official recommendation to pay attention to age and other comorbidities for deciding to use G-CSF a risk of FN < 20% is an important step towards a better protection of more patients against the adverse consequences of FN. Actually, most of the patients receiving CT today have a < 20% risk of developing FN, as indicated in Figure 2; applying strictly the initial rule allowing primary prophylaxis with G-CSF only in patients with a risk > 20%, would have without protection a substantial number of patients[48]. The introduction of criteria such as age and comorbidities in patients with an intermediary risk, allows to extend the potential benefit of primary prophylaxis to more patients.

A further issue might be the optimal management of patients with a risk < 10%. It has been shown that the efficacy of primary prophylaxis is actually better in patients with a lower risk of developing FN when compared to those with a higher risk[8]. In that context, and in a retrospective analysis, it has been found that a reduced dose of filgrastim (300 μg on day 8 and 12), after a CT carrying a 7% risk of FN in patients with breast cancer, was similarly effective as a full course of filgrastim[49]. Of course, these stimulating observations need confirmatory prospective trials, to see whether it might be appropriate to propose primary prophylaxis with reduced doses, especially if there are other risk factors (*e.g.*, age and comorbidities) or if CT is given with a curative intent or in an adjuvant or neo-adjuvant context[50]. In that context, it should be stressed that, under "real life" conditions, there is wide variation in the patterns of G-CSF utilization by practicing oncologists. A recent study indicates that despite guidelines, the use of G-CSF has not been consistent. Wide variations in overuse, underuse and misuse are very common, which means possibly that physicians might perceive the usefulness of administering G-CSF, even if the guidelines are not strictly followed; alternatively, it might mean that present guidelines do not always fit clinical practice[51].

Cost is the main problem for a possible extension of the use G-CSF for primary prophylaxis of FN[51]; it is difficult to accept, on ethical grounds, that the administration of a potentially life-saving procedure is based merely on economic conditions. Moreover, the trade-off used in these early - but influential studies - is controversial, as it was based mainly on the cost for hospitalization for FN, which is definitely not the only aspect of the cost of an episode of FN. For all those reasons, the balance between the cost and the benefits of primary prophylaxis with G-CSF of FN needs to be reevaluated[50,52].

A potential solution to the limiting effect of cost on the more liberal use of G-CSF might come from the introduction of biosimilars to filgrastim or pegfilgrastim[53]. Several of such preparations have been approved in Europe and are proposed at lower prices than the original products. Thus, a combination of modified schedule of administration, tailoring the dose to the clinical needs, and a price reduction might make G-CSF prophylaxis for

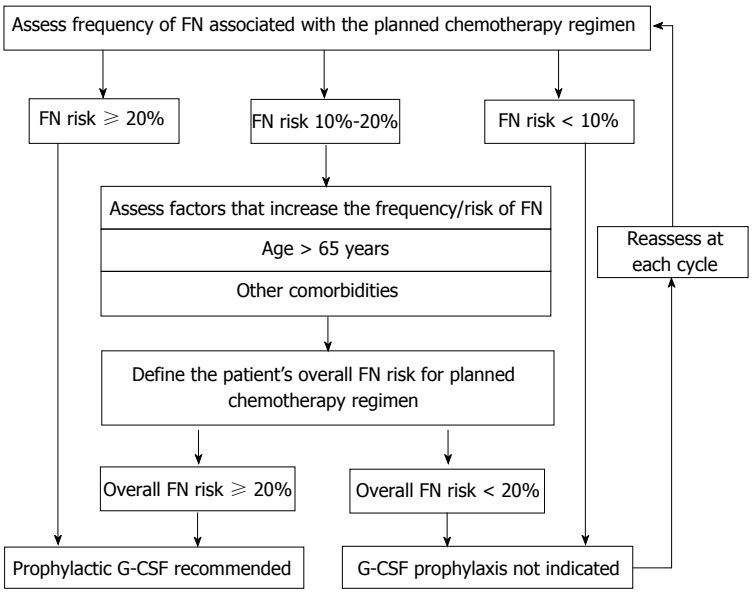

Figure 1 Algorithm to decide primary prophylactic granulocyte colony-stimulating factor usage. Adapted from European Organization for Research and Treatment of Cancer Guidelines. Data taken from[13]. FN: Febrile neutropenia; G-CSF: Granulocyte colony-stimulating factor.

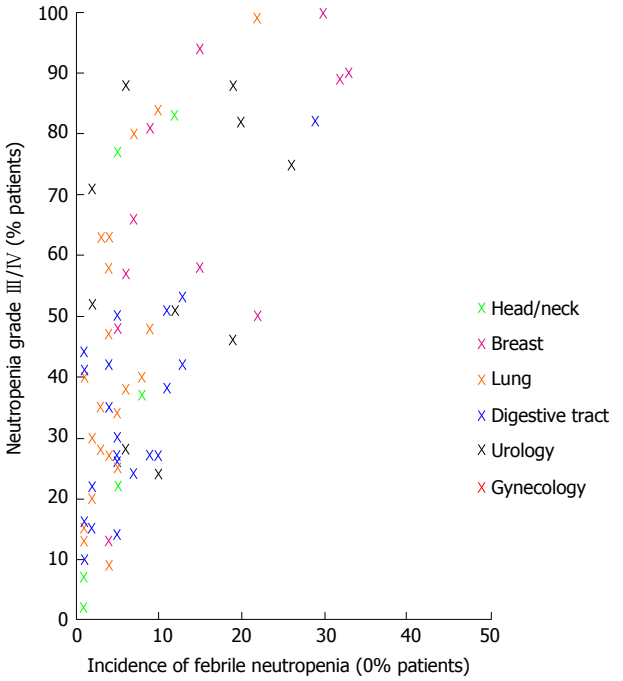

Figure 2 Relationship between the occurrence of febrile neutropenia and the severity of granulocytopenia.

FN available to more patients. Once again, it should be emphasized that new paradigms need to be based on adequately conducted clinical trials.

EMPIRIC THERAPY ACCORDING TO RISK

The elements of the management of FN have been a matter of intense research, improvement and refinement over the years (Table 6).

In the late 80's, there was a general perception that all neutropenic patients do not have the same risk of developing life-threatening complications. Not all neutropenic patients need hospitalization and intravenous antibiotics until resolution. Talcott *et al*[54] reported the first work that tried to assess the risk of adverse outcome during a neutropenia. However, the Talcott's criteria lack sensitivity (30%) and in the early 2000's, the MASCC developed an index scoring system that allows the selection of low-risk patients with good sensitivity (80%) and specificity (71%)[18]. The MASCC index has been tested in several independent trials[22,23] and is the most widely used in adult population. Thus progressively, a risk-adapted strategy for the management of FN was implemented.

Empiric treatment of low-risk patients

The major objective of identifying low-risk patients is to develop a strategy of management that decreases the costs, improves the QoL while maintaining safety. Over the time, there was an evolution in the different strategies used as well as in the selection criteria of low-risk patients. One of the first strategies consisted in early discharge to continue intravenous antibiotics on an outpatient basis and was tested successfully in two pilot trials[16,55] and in a randomized multicenter study including 80 adults[56]. In the second one, ambulatory intravenous antibiotics were given from the onset of FN. Once-daily dosing regimens such as ceftriaxone alone or combined with aminoglycoside are the most practical. Using such a strategy, a German multicenter study reported a hospital readmission rate of 24% for persisting fever or clinical deterioration[57].

The third one, a step-down strategy from inpatient intravenous antibiotics to oral antibiotics with early discharge has the advantage of allowing a period of observation and assessment of microbiology results which is critical for safety. The oral antibiotic therapy selected was

Table 6 Major elements of the management of febrile neutropenia over time

60's	High mortality (> 90%) in FN with gram-negative bacilli bacteremia
	Establishing the concept of empiric antibiotic therapy
70's	Anti-pseudomonal penicillins plus aminoglycoside combination as empiric therapy of choice
	Oral non resorbable antimicrobials (aminoglycosides, glycopeptides, polymyxines, colimycin, in different combinations with nystatin), for intestinal flora suppression
80's	Establishing empirical antifungal therapy
	Oral trimethoprim-sulfamethoxazole (or nalidixic acid and fluoroquinoles for prophylaxis in HM
	Assessment of risk factors predicting complications: Talcott's criteria
90's	Monotherapy supplanted combination
	Ambulatory management first with IV antibiotics (ceftriaxone + aminoglycoside) and then with oral fluoroquinolones
2000's	Refinement of risk assessment: MASCC score
	Risk-adapted therapy

FN: Febrile neutropenia; HM: Hematologic malignancies; MASCC: Multinational Association for Supportive Care in Cancer.

a combination of ciprofloxacin and amoxicillin/clavulanate and was used successfully in two non-randomized trials including low risk patients with hematological malignancies[23,58]. Finally, giving oral antibiotics from the onset of FN to low-risk patients, with early discharge, is probably the strategy that best meets the objectives of reducing costs and improving QoL[59]. Because of their high oral bioavailability, good tolerance and bactericidal activity particularly against GNB[60], fluoroquinolones either alone or in combination with anti-Gram-positive agents such as clindamycin[61] or amoxicillin/clavulanate[62], have been the mainstay oral therapy. A first step was to establish the safety of an oral regimen given from onset of FN. This has been accomplished through the achievement of two randomized trials comparing ciprofloxacin plus amoxycillin/clavulanate with either ceftazidime[63] or ceftriaxone plus amikacin[64], in an inpatient setting. More recently, once daily oral moxifloxacin 400 mg monotherapy has been shown to be equivalent to the standard[38]. Concern has been raised about the limited activity of moxifloxacin against Pseudomonas aeruginosa (*P. aeruginosa*). However, the frequency of this organism in the population of solid tumors or lymphoma at low risk FN is very uncommon and should be assessed locally. In this trial XV of the EORTC, 59% of patients could be discharged early with only 5% readmission rate for clinical deterioration and other medical complications.

Several studies have assessed the role of oral antibiotics given from onset of FN with immediate discharge without hospitalization for observation[60,65-68]. All excluded patients with acute leukemia and hematopoietic stem cell transplantation. Patients should be able to ingest and tolerate oral antibiotics with the first dose being tested at the emergency room. A close follow-up is undertaken with phone calls and a visit every other day until resolution. Figure 3 summarizes some of the elements that may help in the management of patients with FN at low risk.

Despite the increasing resistance of Gram-negative bacteria to fluoroquinolones over time, their efficacy in empiric oral therapy for low-risk patients does not seem to be affected. On one hand, the rate of failure because of fluoroquinolone resistance is not higher in the recent trials as compared to older ones and on the other hand, the incidence of GNB bacteremia is low. However, epidemiological variations between institutions may exist and a careful monitoring is recommended.

Empiric treatment of high-risk patients with FN

Inpatient management with parenteral broad-spectrum antibiotics is the standard care of FN patients at high-risk. A β-lactam agent active against GNB including *P. aeruginosa* remains the central core of empiric therapy. However, the increasing resistance of GNB over the years has made the β-lactam choice much more challenging[69]. There are many geographical differences in the epidemiology of microbial resistance and it is more likely that the local epidemiology than any global data, for the selection of initial for empiric therapy[70]. Until the 90's, this choice was mainly influenced by one risk which was *P. aeruginosa* resistance to the different β-lactams.

Nowadays, this choice depends on too many risks. The risk of ESBL producing GNB especially *K. pneumoniae* and *E. coli*, risk of a MDR non-fermenter such as *P. aeruginosa*, *Acinetobacter baumanii* or *S. maltophilia*, risk of carbapenemase producing pathogen in addition to the risk of MRSA, VRE and anaerobes (see epidemiological section). Any delay in the early adequate therapy is associated with an increased mortality[71,72]. Therefore, defining risk factors for MDR pathogens, in neutropenic patients, is determinant for empiric antibiotic selection and outcome. The risk factors for MDR pathogens identified include prior exposure to broad-spectrum antibiotics, the severity of underlying disease such as in acute myelocytic leukemia, and the presence of medical comorbidities, as well as the presence of urinary catheter[73]. However, these are quite common to allow a specific selection of the patients who ultimately develop an infection due to MDR pathogens. ESBL-GNB or VRE stool colonization was associated with subsequent bacteremia due to the same pathogen in a prospective study[74] in hematological malignancy patients, with a RR of 4.5 for ESBL-GNB (95%CI: 2.89-7.04) and a RR of 10.2 for VRE (95%CI: 7.87-13.32).

Thus, surveillance cultures should be reassessed and validated prospectively for both infection control purposes and selection of β-lactam empiric therapy. Patients who are not at risk of ESBL-GNB infection will receive therapy with piperacillin/tazobactam or cefepime or ceftazidime, while patients at risk of ESBL-GNB, will receive upfront a carbapenem[74]. Anti-anaerobic coverage is indicated for necrotizing gingivitis, typhlitis and peri-anal abscess[19,75]; piperacillin/tazobactam and carbapenems are, however, active against the majority of anaerobe[76]. In case of allergy to penicillin, aztreonam combined with a glycopeptide is an acceptable alternative.

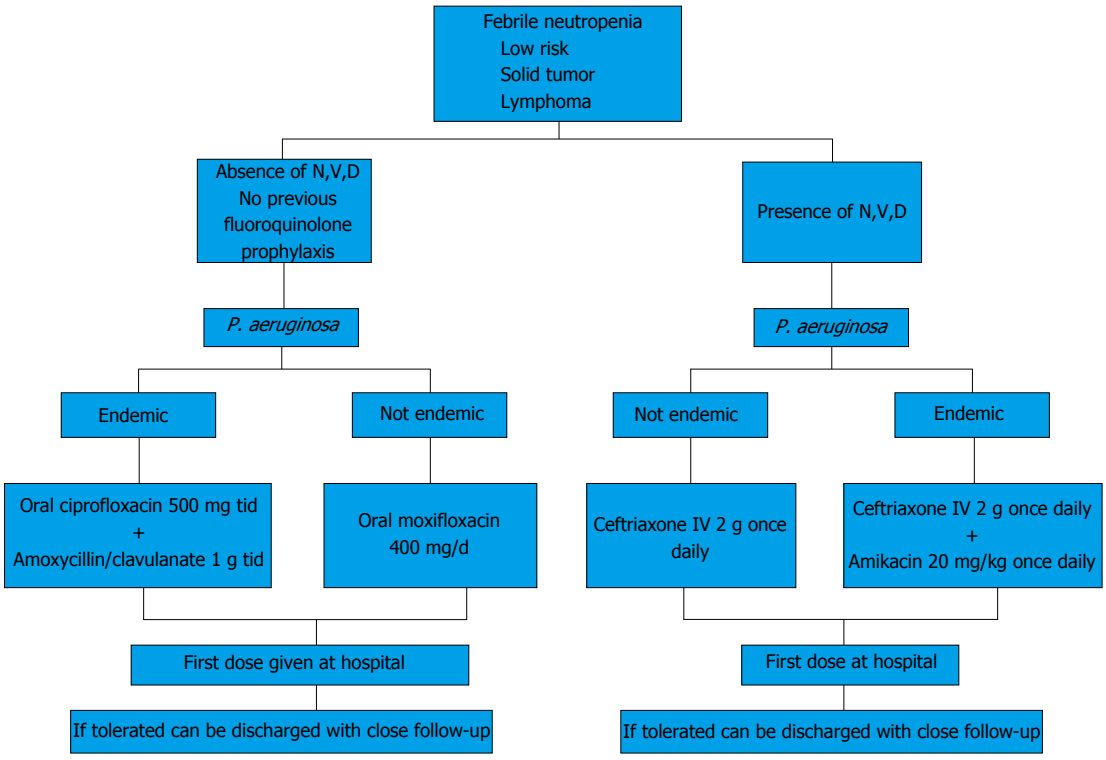

Figure 3 Decision tree for the administration of antibiotic therapy to low-risk patients with febrile nerutropenia. N: Nausea; V: Vomiting; D: Diarrhea; *P. aeruginosa*: Pseudomonas aeruginosa.

A combination therapy with an aminoglycoside has no advantage and is more toxic than monotherapy[77,78]. However, for the subgroup of patients with signs of sepsis or septic shock, the mortality is unacceptably high, especially when empiric therapy proves to be inadequate[79]. In such conditions, a combination with an aminoglycoside for a limited duration up to 3 d, seems reasonable[80,81].

In institutions where MDR non-fermenters such as *P. aeruginosa* or *Acinetobacter baumanii* or *carbapenemase*-producers enterobacteriae are endemic, combination with colistin has been advocated[82]. Empiric addition of a glycopeptide didn't show benefit in reducing treatment failure, in gram-positive infections[83]. However, addition of empiric glycopeptide under certain circumstances, is indicated such as in patients already colonized by MRSA, if MRSA is endemic in the institution, in the presence of folliculitis, furonculosis or catheter-related cellulitis and if viridans group *Streptococci* penicillin-resistance is prevalent[75].

In allogeneic hematopoietic stem cell transplant patients (HSCT) colonization by vancomycin-resistant enterococci (VRE) and T-cell depletion are important risk factors for VRE bacteremia[84]. In such patients, early empiric combination with linezolid or high-dose daptomycin (> 6 mg/kg per day) is justified[85,86]. Figure 4 provides indications for the selection of empiric therapy in high-risk patients with GN.

EMERGENCE OF RESISTANT STRAINS

The discovery and clinical use of antibiotics was officially initiated in 1936 with sulfonamides and followed in the 1940s with penicillin and streptomycin; a whole new era of anti-infective drugs was inaugurated with successful treatment of previous lethal diseases. The dream started fraying when the first resistant strains against sulfonamides[87], penicillin[88-90] and streptomycin appeared[90].

The exhilaration accompanying the modern antibiotics was over by the early 2000s; antimicrobial resistance emerged as part of the adaptive mechanisms deployed by micro-organisms (bacteria, fungi, viruses and parasites) in order to survive in a stressful environment (inside and outside the hospital). Bacteria developed successful resistance strategies through the last 6 decades. On the other hand, microbiologists and clinicians faced the ESKAPE concept: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumanii, Pseudomonas aeruginosa, Enterobacteriaceae*[91] and new comers such as *Mycobacterium tuberculosis*, HIV, *Aspergillus sp*. and malaria; very few antimicrobials were active against these bugs and the new drugs were even less designed, developed or available for human use.

In the narrow field of FN, complicating aggressive CT regimens, prophylaxis by oral antibiotics[92], broad-spectrum early antibiotherapy[75] and optimal supportive treatment[13] are well-established attitudes in order to decrease mortality and morbidity due to FN. These attitudes have to be revised and adapted in order to face the ESKAPE bugs and to continue to use antimicrobials to treat severe infections jeopardizing the prognosis of potentially curable malignant diseases.

The resistance related to antibiotics is a natural phenomenon associated to the evolution of bacterial life and the genes of resistance are frequently issued

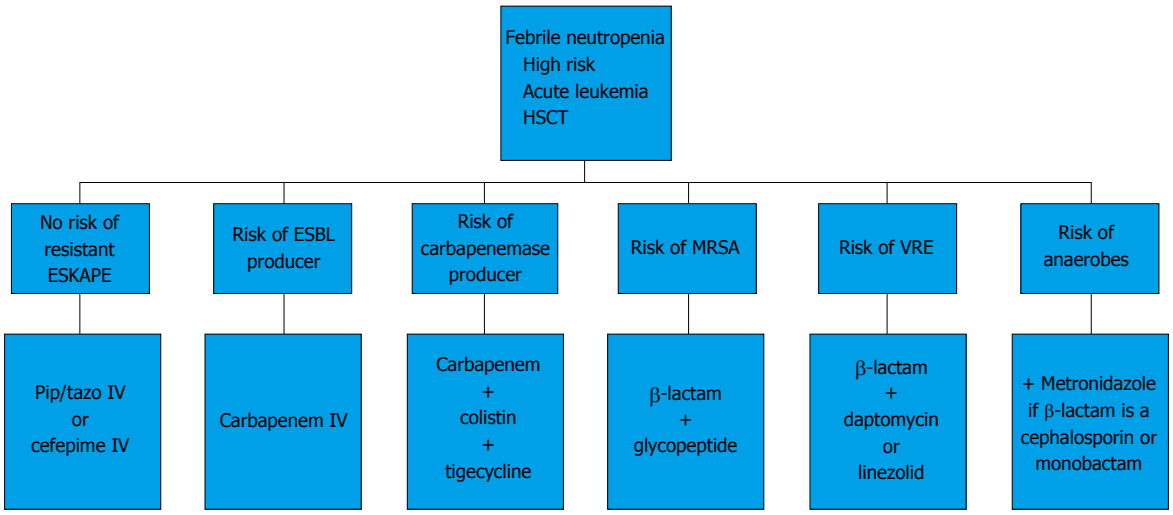

Figure 4 Decision tree for administration of antibiotics to high-risk patients with febrile neutropenia. ESKAPE: *E. coli*, *S. aureus*, *Klebsiella sp. Acinetobacter sp*, *P. aeruginosa*, *Enterococcus sp*; ESBL: Extended-spectrum β-lactamase; MRSA: Methicillin-resistant *S. aureus*; VRE: Vancomycin-resistant enterococci; HSCT: Hematopoietic stem cell transplant patients; *P. aeruginosa*: Pseudomonas aeruginosa.

from essential genes. Evidence exists that these genes pre-existed the era of antibiotics and they probably developed in antibiotic producing bacteria[93,94]. Bacteria, especially those of commensal and environmental flora use the mechanisms of resistance in order to survive in nature[95,96]. Antibiotics create a strong selective pressure on bacteria and create favorable conditions for the development of resistance; resistance to antibiotics is the final product of a complex process including multiple genetic maneuvers.

These genetic maneuvers include 3 levels. The first level is the point mutations (micro-evolutionary change) that occur in in a nucleotide base pair; the point mutations will create alterations in enzyme substrate specificity or the target site of an antibiotic, interfering with its activity. The second level of genomic variability (macro-evolutionary change) in bacteria results in massive modifications (inversions, duplications, insertions, deletions, or transpositions) of large portions of DNA as a single event. Specialized genetic elements called integrons, transposons, or insertion sequences generate these massive rearrangements independently from the rest of bacterial genome[95]. The third level of genetic variability is due to the acquisition of foreign DNA carried by plasmids, bacteriophages, isolated sequences of DNA and transposable genetic elements from other bacteria. The further inheritance of foreign DNA will contribute to enhance genetic variability of bacteria and increase their capacity to respond to selection pressures such as the use of antimicrobials[93].

Bacteria develop antibiotic resistance through (at least) eight different mechanisms: Enzymatic alteration (β-lactamases, extended-spectrum β-lactamases, carbapenemases), decreased permeability (outer/inner membrane permeability), efflux, alteration of the target site, protection of the target sight, overproduction of the target, bypass of the inhibited process and bind-up of the antibiotic. All classes of antibiotics may be affected *via* different mechanisms. The use of old (polymyxins, metronidazole) and new (linezolid, tigecycline) antibiotics when antibacterial resistance became important led to the apparition of resistant strains against these drugs, *via* the same mechanisms deployed against traditional antibiotics. Additionally to these mechanisms, bacteria may associate different mechanisms of antibiotic resistance resulting to MDR (multiple drug resistance)/Pan-resistance strains. In 2005, Deplano *et al*[97] described a Belgian out-break of Pan-resistant *Pseudomonas aeruginosa* (89% of the isolates belonged to serotype O:11). The Pan-resistance was due to the overexpression of AmpC chromosomal β-lactamases conferring resistance to multiple β-lactam antibiotics associated to the mutational loss of OrpD porin, conferring resistance to imipenem and the upregulation of the MexXY efflux system which exports fluoroquinolones, tetracycline, aminoglycosides and antipseudomonal β-lactam molecules[97]. Methodical transfer of multiple-resistance elements located on mobile genetic elements (transposons, plasmids) can help bacteria to acquire MDR/Pan-resistance[98,99]. The capacity of bacteria to seize numerous antibiotic resistance genes is illustrated by resistance integrons, which can insert resistance gene cassettes into their *attI* integration site and are often found on transposons carried on plasmids, with obviously endless recombinant capacity[100].

Moving in the inner circle of the ESKAPE bugs and their impact on the management of FN is strewn with pitfalls. Understanding the various mechanisms leading to resistance and being acquainted with the established epidemiological profiles will permit the quick and right choice of (empirical) antibiotic treatment in the advent of fever during neutropenia.

The *Enterococcus faecium* is actual the most important pathogen (among the *Enterococcus* sp.) in hospital acquired infections, followed by the *Enterococcus faecalis*. Enterococci are less virulent than other Gram-positive cocci and usually occur in the context of polymicrobial

infection in debilitated patients. The acquisition of resistance (to multiple antibiotics including vancomycin; VRE) allowed the emergence of superinfections in immunocompromised patients[101]. Acute outbreaks are usually monoclonal[101] and the hands of health workers spread Enterococci among patients. Patients may be colonized with *E. faecium* on the gastrointestinal tract and thus serve as a reservoir; adequate identification and management of these patients are the only way to prevent transmission to other patients and subsequent outbreaks[102]. Resistant strains to vancomycin (and to teicoplanin) appear when the production of peptidoglycan precursors is modified and therefor present a weak affinity for glycopeptides; Van A and VaB are the most frequent phenotypes associated to glycopeptide resistance[103]. Admission to intensive care and length of hospitalization, prior use of broad spectrum antibiotics, severity of illness and exposure to other patients colonized with VRE are well known factors for developing colonization/infection to VRE. Linezolid and daptomycin constitute the main therapeutic issues, but controlled trials lack actually[104].

The *Staphylococcus aureus* is well-known to be resistant to natural penicillins since the mid 40's; resistance to methicillin (a penicillinase-resistant penicillin) was first described in the mid 60's while the resistance to vancomycin was first reported in the mid-90's (Figure 1). The *mec* A gene, as part of the mobile genetic element named staphylococcal cassette chromosome is responsible for the synthesis of the penicillin-binding protein, PBP2a, located in the bacterial membrane and being able to catalyze the transpeptidation reactions of peptidoglycan during cell wall construction; it's an inducible protein and under the effect of regulatory genes implicated to its transcription (*mec R1*, *mecI, blaZ*, BlaR1 and BlaI), resistance towards β-lactams is observed[105,106]. The β-lactamases genes (*blaZ*, *BlaR1* and *Bla*) can produce hydrolyzing enzymes targeting the β-lactam ring[106]. Broad use of vancomycin provoked the emergence of intermediate (VISA)/resistant (VRSA) strains[107,108]. The mechanism of resistance in VISA is related to a thickening of the wall cell containing dipeptides that trap vancomycin and thus decrease the amount of drug directed against intracellular targets[109]. The mechanism of resistance in VRSA is related to a plasmid transfer containing the *van*A gene from Enterococci to *Staphylococcus aureus*[110]. While precise guidelines about treatment of MRSA infections exist[111], treatment against VISA/VRSA is mainly based on experimental trials using daptomycin, quinupristin-dalfopristin and linezolid[112,113].

The *Klebsiella pneumonia* and the *Enterobacteriaceae* represent the major providers of extended-spectrum β-lactamases (ESBLs) and carbapenemases. ESBLs include enzymes that have derived from narrow spectrum β-lactamases (TEM-1, TEM-2, SHV-1) or from chromosomally encoded β-lactamases produced by *Kluyvera sp*. (CTX-M type ESBLs)[114]. The broad use of carbapenems for serious infections due to ESBLs-producing bacteria selected the carbapenemases (mainly OXA-48, KPC, VIM, NDM); these plasmid-acquired enzymes hydrolyze most β-lactams including cabapenems. Their spread all over the world is spectacular[115,116] and worry about the outcome of serious infections due to these germs is more than real as therapeutic armamentarium is reduced to colistin, aminoglycosides and tigecycline. The detection of carbapenemases should be triggered when the *Enterobacteriaceae* have resistance or reduced susceptibility to carbapenems[117], while screening (stool, anal swabs) should be performed during outbreaks and endemic scenarios[116]. Mortality is mainly evaluated among bloodstream infections: It may vary from 39% to 53% but remains unacceptably high[74,118,119]. Well-identified risk factors (in multivariate analysis models) are the age of patient, APACHE Ⅱ (Ⅲ) score at infection onset, inappropriate antimicrobial therapy, onset of bacteremia while in the intensive care unit and malignancy; combination of antibiotics were more efficient than monotherapy and the emergence of strains resistant to colistin is already described[74,118-120].

The *Acinetobacter baumanii* and the *Pseudomonas aeruginosa* are the most popular and the most implicated in serious infections within immunocompromised patients between non-fermentative Gram-negative bacilli. Broad-spectrum empiric antibiotics always include coverage against Pseudomonas aeruginosa, in the setting of FN[75], while Acinetobacter baumanii is related to serious infections in the intensive care unit (ICU)[121]. *Pseudomonas aeruginosa* may acquire genes encoding a tremendous amount of β-Lactamases such as the OXA and PSE type β-Lactamases, KPC and the metallo-β-Lactamases. The metallo-β-Lactamases can induce resistance to all β-Lactam antibiotics (including carbapenems and excepting aztreonam) and the β-Lactamase inhibitors are inefficient; worst, the genes coding for theses enzymes may be linked to genes inducing resistance to other anti-pseudomonas drugs[122]. Nonetheless the most common mechanism of resistance to carbapenems is the loss of an outer-membrane protein called OrpD, following a mutation[123]. Other mechanisms such as upregulation of efflux pumps, outer-membrane impermeability, enzymatic alterations of the antibiotics and the 16S ribosomal RNA methylation may lead resistance to all class of anti-pseudomonas drugs including aminoglycosides[122-124]. The *Acinetobacter baumanii* infections occur more often in the ICU and the burn units and neutropenic patients seem to avoid reasonably this pathogen[69]. Besides intrinsic resistance (cephalosporinase: *bla* ADC, (OXA-69), *Acinetobacter baumanii* may acquire genes encoding different β-lactamases/carbapenemases; these enzymes are OXA-type β-lactamases (OXA-23) and metallo-β-lactamases (IMP, VIM, GIM, SPM)[125]. Fluoroquinolones are neutralized when point mutations in the in the quinolone resistance determining region of DNA gyrase gene occur[126] and upregulated efflux pumps may contribute to fluoroquinolone resistance. Aminoglycoside resistance results when enzymes capable of modyfing aminoglycosides are produced: Aph A6 3'-aminoglycoside phosphotransferase type Ⅵ will inactivate amikacin[126] and adenyltransferases (aadA1,

Table 7 Possible causes of fever in high risk neutropenic patients unresponsive to broad spectrum antimicrobials[139]

Infectious causes	Frequency
Fungal infections responding (40%)/resistant (5%) to empiric ATB	45%
Bacterial Infections (cryptic foci, biofilm, resistant organism)	10%
Toxoplasma gondii, mycobacteria, legionella, mycoplasma, chl.pneumoniae	5%
Viral infections (HSV, CMV, EBV, HHV6, VZ, parainfluenza, RSV, influenza)	5%
Graft *vs* host disease in hematopoietic stem cell transplantation	10%
Undefined (drug, toxic effects of chemotherapy, antitumor response, undefined pathogens)	25%

HSV: Herpes simplex virus; CMV: Cytomegalovirus; EBV: Epstein-Barr virus; HHV6: Human herpesvirus 6; RSV: Respiratory syncytial virus.

aadB) or acetyltranferases (aacC1, aacC2) will neutralize gentamycin and tobramycin[126,127]. Unfortunately, upregulated efflux pumps of the AdeABC type induced resistance to tigecycline[128].

Despite fascinating progress in treating serious bacterial diseases performed in the last century and since the discovery of penicillin, the emergence of resistant strains is the major threat in the 21st century. Frail patients undergoing sophisticated treatments (transplantations, CT, immunotherapy) for complex diseases such as cancer, autoimmune conditions are exposed to a supplementary risk of complications due to non-treatable bacterial infections[129,130].

The economic impact of infections due to resistant bacteria is well-known: The length of hospitalization is longer, the hospital charges are higher and the mortality/morbidity are increased[131,132]. The infection control team and the antimicrobial stewardship programs seem to be the most promising tools in fighting against resistant strains in the lack of new antibacterials; implementation of strategies preserving antibacterials may is the future in modern medicine if we don't want to lose the progress achieved in the past decades. Management of FN needs to be carefully thought in the advent of these disturbing elements and close collaboration with specialized teams in controlling infectious diseases is the only way to bring through the ESKAPE pathogens[98].

PERSISTING FN

Definition

Persistent febrile neutropenia (PFN) is FN that does not resolve in spite of the empirical administration of broad-spectrum antibacterial agents. It can concern 30%-40% of the patients presenting FN. The diagnosis of PFN requires at least 5 d of therapy in patients with haematological malignancy, including HSCT[133-135] but only 2 d in solid tumours[75,136], probably due to different immune response. Patients with haematological malignancies are usually more seriously ill, than patients with solid cancers[137].

Etiology of PFN

The most frequent cause of fever in high risk neutropenic patients unresponsive to broad spectrum antimicrobials is fungal infection (45%), followed by bacterial, viral infections, toxoplasmosis, drugs, toxic effects of CT and antitumor response (Table 7)[137].

Diagnostic approach

PFN for more than 3 d should prompt a thorough search for a source of infection. PFN with neutropenia lasting more than 7 d in high-risk hematological patients should lead to an evaluation for invasive fungal infection with a chest CT scan looking after pulmonary nodules or nodular pulmonary infiltrates and early assessment with bronchoscopy, bronchoalveolar lavage with cultures/stains, a sinus CT scan[75] and a regular *Aspergillus* galactomannan antigen testing and/or β-D-glucan detection. Repeated imaging may be required in patients with persistent pyrexia.

Procalcitonin (PCT) monitoring can be useful, a delayed PCT peak higher than 500 mg/mL suggest the early diagnosis of invasive fungal disease and PCT decrease reflects response to antifungal therapy[138].

Diarrhea, if present, should be assessed by analyzing a stool sample for *C. difficile* toxin. An abdominal CT may be helpful for the diagnosis of neutropenic enterocolitis[139]. Surveillance of IV catheters for possible skin bloodstream breakthrough infection is also indicated[75].

An evaluation for viral infections, by herpesviridae (Herpes, Varicella Zoster, HHV6, HHV8), Cytomegalovirus, Epstein Barr, but also respiratory virus, as guided by the local epidemiology (respiratory syncytial virus, influenza, parainfluenza) is recommended especially in high risk hematological patients. Eventually, exclusion of other non-infectious sources of recurrent or persistent fever like drugs, thrombophlebitis, cancer, resorption of hematoma is warranted[75].

Prospective trials are presently ongoing to evaluate the utility and cost-effectiveness of PET/CT in identifying sites of infection in cancer patients with PFN without an obvious source, in order to improve targeted therapy.

Therapeutic attitude

Modifications to the initial empirical antibiotic regimen should be guided[75] firstly by possible changes of the clinical stability, without a source of infection detected; in hemodynamically stable and asymptomatic patients, watchful waiting and re-evaluation for new possible infection is indicated, while in hemodynamically unstable patients, the antimicrobial regimen should be broadened to target drug-resistant bacteria. Delaying appropriate antibiotic therapy for such pathogens, is associated with increased mortality[140].

Unusual infections should be considered, particularly in the context of a rising C-reactive protein (CRP), in such cases proceeding to imaging of chest and abdomen is advisable. Sometimes the investigations may be directed by clinical findings[4,141].

Therapeutic approach for fungal infections

Empiric antifungal therapy should be considered in high-risk neutropenic patients who PFN after four to seven days and without identified source for the fever[75]. The incidence of fungal infection (especially *Candida* or *Aspergillus sp.*) rises after patients have experienced more than 7 d of PFN. In 1970s, already several studies have shown that invasive fungal infections were a common cause of PFN (9%-37.5%)[142-146] and was associated with significant mortality (69%)[145].

The IDSA guidelines recommend lipid formulation of amphotericin B, caspofungin, voriconazole, or itraconazole as suitable options for empiric antifungal therapy in PFN. The choice of the initial antifungal agent may vary based on epidemiology and local susceptibility patterns[133], toxicity and the cost of the antifungals.

Resolution of fever occurs in approximately 40%-50% of patients given empirical antifungal therapy[143,144,147,148], but such a successful outcome does not prove that the patient had indeed an occult fungal infection, since slow responses to empiric antibacterial therapy can occur.

Fluconazole can be given as first-line treatment provided that the patient is at low risk of invasive aspergillosis, has not received an azole antifungal as prophylaxis and local epidemiological data suggest low rates of azole-resistant *Candida*[19].

Liposomal amphotericin B or an echinocandin antifungal such as caspofungin are appropriate first-line treatments in high risk patients with PNF without an obvious site of infection and also in patients already exposed to an azole or known to be colonized with non-albicans *Candida*[19].

Addition of the newer antifungal agents active against possible azole-resistant *Candida sp*. Is also recommended, if the patient has been already treated with fluconazole prophylaxis.

In patients with nodular pulmonary infiltrates, invasive mold infection should be strongly suspected and prompt assessment with bronchoscopy, bronchoalveolar lavage for cultures and galactomannan testing should be performed; in those patients a preemptive treatment with voriconazole or a lipid formulation of amphotericin B is indicated.

PFN receiving anti-mold prophylaxis should be treated with a different class of antifungal than the one used for prophylaxis, in order to avoid cross resistance. The usual sensitivity and resistance of the common fungi are indicated in Table 8[149-151].

Pre-emptive antifungal therapy implies a diagnostic workup with chest and/or sinus computed tomography, serum galactomannan and/or β-D-glucan to evaluate fungal infections in patients with PFN[133]; that approach has been proposed in order to reduce unnecessary use of empirical antifungal therapy, associated toxicity and high cost[147]. Patients receiving pre-emptive antifungals are more likely to present a documented invasive fungal infection (IFI) compared to patients receiving empirical therapy by the time the antifungal agent is started[152].

Paediatric population with PFN are also at high risk for IFI. Prospective monitoring of serum galactomannan twice per week in high-risk hospitalized children for early diagnosis of invasive aspergillosis is probably indicated.

Computed tomography (CT) of the lungs and targeted imaging of other clinically suspected areas of infection, as well as other investigations, such as BAL and *trans*-bronchial or *trans*-thoracic biopsy are indicated in the case of pulmonary lesions[153]. CT of the sinuses is proposed in children of at least 2 years, although imaging during prolonged FN can be inconclusive and symptoms of sinonasal IFD in children are scarce[154,155].

Particular entities of PFN

Recurrent or recrudescent fever refers to a new episode of fever after an initial resolution of fever with antimicrobial therapy when the patient remains neutropenic[155]. This is relatively common, but it has not been adequately studied. Bacterial and fungal infections are common causes of this syndrome (around 30%)[156,157]. The various guidelines do not separate recurrent/recrudescent fever from persistent fever, although these two may be clinically and etiologically different.

Engraftment fever (myeloid reconstitution syndrome) consists of a new onset or worsening of inflammatory and/or infectious process, in temporal relationship to neutrophil recovery after aplasia[157,158]. This has to be differentiated from superinfection or the immune reconstitution syndrome. The engraftment syndrome is a diagnosis of exclusion, which presents particularly in the setting of stem cell transplantation (autologous or allogeneic) consisting in fever, rash and pulmonary infiltrates originally and is usually treated with corticosteroids when severe.

ECONOMIC AND COST ISSUES RELATED TO FN

General considerations and perspectives for clinical practice

Treatment of FN usually requires several days of hospitalization, diagnostic procedures, administration of intravenous empiric broad-spectrum antibiotics and hematopoietic growth factors[159,160]. Thus, such medical management is resource intensive. It is not surprising that FN has a considerable economic impact, particularly in the inpatient setting[51,161].

Our understanding of such a problematic issue is mainly derived from several seminal United States retrospective economic analyses, highlighting average costs per hospitalization for FN management, ranging from $18880 to $22086 (€15000-€24000). The direct costs for outpatient management were considerably lower, at $985 per episode. Patients with hematological malignancies usually have much higher hospitalization costs associated with each episode than those with solid tumors ($US23000-38600 *vs* $US7598-14900)[162-165]. In a recent review, a large variation in estimation among the cost of illness studies in lymphoma patients experiencing

Table 8 Usual sensitivity and resistances of fungi against the different antifungals[149-151]

Antifungal classes	Antifungal agent	Common resistances	Common sensitivity
Polyenes	Amphotericine B:	Candida lusitaniae	Candida
	Deoxycolate	Trichosporon	Aspergillus
	Liposomal	Fusarium	Zygomycetes
	Lipid complex	Scedosporium	
	Colloidal dispersion	Aspergillus terreus	
	5 Fluorocytosine	Zygomycetes	Candida
		Scedosporium	Torulopsis
		Fusarium	T. glabrata
		Cryptococcus	Cryptococcus
		Candida	Phialophora
			Cliadosporium
			Exophiala
Triazoles	Fluconazoles	Aspergillus	Candida albicans and others
		Candida kruzei	Candida glabrata[1]
		Candida glabrata	Cryptococcus neoformans
		Zygomycetes	Blastomyces dermatitidis
			Coccidioides
			Histoplasma capsulatum
	Itraconazole	Aspergillus niger	As itraconazole + Aspergillus flavus
		Aspergilus terreus	Aspergillus fumigatus
		Zygomycetes	Candida kruzei
		Mucor	Trichophyton
		Fusarium solani	
		Penicillium	
	Voriconazole	Zygomycetes	As itraconazole + Aspergillus niger
		Sisyrinchium inflatum	Aspergillus tereus
		Fusarium oxysporum, penicillium, Schedosporium apiospermum	
	Posaconazole	Trichosporon asahii	As voriconazole + Trichophyton
			Zygomycetes
Echinocandins	Caspofungin	Cryptococcus	
	Micafungin	Zygomycetes	
	Anidulafungin	Fusarium	
		Paecilomyces lilacinus	
		Trichosporon	
		Schedosporium	
		prolificans	
		Schedosporium inflatum	
		Candida parapsilosis	

[1]Are not always sensible to the antifungals.

FN have been reported, ranging from $5819 to $34756 (2013 $) per episode of FN[166]. It seems now well established that such previous exclusive estimations, based on hospitalization, may have underestimated costs by as much as 40% by ignoring important costs occurring after hospital discharge[167].

Similar trends with a different cost burden degree were observed in western European developed countries, with smaller studies providing estimates of the average charge for FN-related hospitalization ranging from €2619 in Spain to €4931 in France[168,169]. In a recent study conducted in Ireland, the mean cost per FN episode in the inpatient setting was estimated to be €8915[170]. It should be noted that results of cost-effectiveness studies may differ greatly across different countries and health care systems. Future cost evaluation studies should compare the cost of FN and intervention costs within the same health care system, and not between countries, so as to determine more accurately if the intervention is cost-effective.

Furthermore, results of studies that were conducted may not be directly applicable to other settings. Moreover, literature data based on clinical trials may carry the risk of representing care in overselected populations rather than "real life" practice. Many potential factors account for the large variation in estimating the cost of FN, such as the year of pricing, the perspective employed, and the cost estimation approach used. The public health care system is unique for each country, with different standards of care as well as different costing of health care resources.

Since FN is an acute condition, and typically produces temporary complete disability, the cost involved from the patient time lost from work was initially thought to be non-significant[171]. Thereafter, such indirect costs, including costs associated with patient work loss, caregiver work loss, paid caregiver and/or non-revenue-generating support centers, were estimated with great variations between studies, ranging from 11% to 44% of the total cost of FN management[161,166,172]. Future studies should place greater emphasis on improving the accuracy of providing a clearer description of these indirect costs.

The major economic impact of neutropenic complications is mainly related to the cost of hospitalization and the associated length of stay (LOS). In a retrospective analysis, it has been demonstrated that one-third of patients hospitalized for more than 10 d account for 78% of the total cost. The average LOS decreased over time by 10% while the cost per day increased by 28%, raising the total cost per episode of FN by 13%. The mean LOS was longer for patients with leukemia (19.0 d) compared to patients with lymphoma and solid tumors[51]. A recent publication on subpopulations of FN admissions with breast cancer in the United States between 2009 and 2011, showed, despite a shorter LOS than previously reported (5.7 d *vs* 8.0 d, $P < 0.05$), a significantly higher mean hospital charge ($ 37087)[173] than prior observation from former observations from Kuderer and colleagues ($ 12372)[8], suggesting that FN related hospitalizations continue to account for highly significant care expenditure.

Low risk patients generally have short hospitalizations and account for a relatively small proportion of the overall costs associated with FN[174]. There is also strong evidence suggesting that costs of in-hospital treatment are greater than the costs of ambulatory care for FN[166,175]. Therefore, strategies that support FN outpatient treatment may have important clinical and economic impacts[16,18,61]. However, these patients may have been selected for outpatient treatment because of their lower risk for complications. Future prediction risk models should not only include risk factors of FN to be considered for use of prophylactic therapies but also the predictors of higher cost of FN as well. Currently, the MASCC scoring system is widely used to prognosticate the severity of FN among cancer patients[18]. However, there is room to improve the sensitivity and specificity of the prognostic model. Considered that the management strategies of low-risk and high-risk FN are different, improving the current prognostic model to predict the severity of FN is worth to further explore in future studies.

Undoubtedly, recombinant G-CSFs represent a major clinical achievement[8]. Meta-analyses, which have shown that pegfilgrastim performs as well as or better than filgrastim in reducing FN rates for patients undergoing CT[176]. Consistently, several studies evaluated the relative cost effectiveness of pegfilgrastim, and showed that any incremental costs are justifiable given the clinical outcomes[177-180].

As already said, it is possible that these economic considerations have been the main incentive for international guidelines, justifying the use of primary prophylaxis, at a risk level > 20%[13,181,182]. However, considering only the cost of hospitalization for setting such threshold may not be optimal. Such guidelines do not consider all aspects of value in cancer patients, namely clinical impacts on QoL and mostly, potential effect of completing full dose CT therapeutic plan, with subsequent disease control and impact on survival, especially in the curative setting.

Both filgrastim and pegfilgrastim are expensive ($2600 and $3500 respectively for full treatment per cycle), and their economic burden is inseparable from the economics of FN. These agents will allow a greater relative dose intensity, less dose-delays and thereby, greater costs associated with the use of CT agents. Their high cost should be balanced not only against the cost of FN but also to the impact on increased clinical outcomes, such as QoL and survival. However, the exact economic benefits of such FN prophylaxis are not completely understood and established, mainly due to the lack of consistency in general use of G-CSFs among physicians. Indeed, under- and over-prophylaxis with G-CSFs remain a reality, being the consequences of either a bad knowledge and clinical applications of the guidelines, or the willingness for clinicians to overprotect their patients undergoing CT. It has been suggested that G-CSFs are underused for CT regimens with high risk of FN, and overused for those associated with low risk[183].

Actually, the risk of development of FN is not always easily determined on the basis of the type and dose of CT, and still many patients with a risk < 20% still develop FN, with a rate of complications similar to that of patients with a high risk[184]. Moreover, it seems that efficacy of G-CSF prophylaxis might be better in populations with low risk of FN ($\leqslant$ 10%)[8]. Current guidelines will have to be revisited to allow a larger number of patients to have access to primary prophylaxis, without compromising cost efficacy. Hence, other prophylaxis strategies have been explored, including in particular, limitation of primary prophylaxis to the first two cycles of CT only[185] or shorter duration of G-CSF primary prophylaxis (2 *vs* 7 daily injections)[49], but with reports of conflicting and ambiguous results in the literature. Further studies are needed and will be performed in this specific topic.

The great majority of previous large FN trials considered hematological malignancies, lymphomas, breast and lung cancers. Other groups, such head and neck cancer patients, may deserve special attention, because they truly represent a high risk group in terms of age, co-morbidities and aggressiveness of multimodal therapies. In this group, platinum and taxane-containing regimens (*i.e.*, induction TPF) have a reported FN incidence ranging from 5.2%-20%[186,187] and therefore, they are not considered as high risk to have access to primary prophylaxis with G-CSFs. It is now established and recognized that patients considered for clinical trials (with shorter therapy durations) are usually well selected (usually excluding high risk such elderly patients), and could be different from those unselected and managed in real-life daily clinical practice among the community setting.

A recent retrospective analysis from a Japanese group reported a 41%, 25% and 33% incidence of FN in the first, second and third cycles of taxane and platinum-based CT regimens. G-CSF was used in 58 out of 71 patients (82%) during the first cycle, but exclusively therapeutically and not prophylactically following health insurance rules for G-CSFs in Japan[188]. Their relative dose intensity was around 80% of other reports. Tube

feeding, diabetes mellitus and presence of CT-related gastrointestinal adverse effects (such mucositis, diarrhea and emesis) were significant predictors of FN. In this analysis, 62% and 70% of the patients had received prior CT and radiation respectively. The major interest of this retrospective analysis, and despite several limitations, is to show the much higher risk of FN in community setting than in clinical trials in a very specific group of tumors with high needs. Further investigations are needed for a better management and prophylaxis of FN in head and neck cancer patients.

Finally, a more comprehensive consideration of value should encompass not only the cost, but also potential survival benefit, QoL and equity between patients. More affordable G-CSFs, QoL through the use of biosimilars, might influence our prescribing to prevent FN in the future[189,190]. Several studies have demonstrated that the biosimilar G-CSF is equivalent in terms of efficacy and safety when compared against native G-CSF[191-193]. Although we dispose of encouraging clinical and safety outcomes, there is still a need for longer follow-up studies to confirm the safety, efficacy as well as cost effectiveness of these biosimilars.

FN AT THE EXTREME OF AGE (DAL LAGO L)

Elderly population

Due to the ageing, European population aged 65 years and older is projected to increase, leading to even older patients with cancer[194].

There is a paucity of evidence-based data for cancer management in older patients because of the underrepresentation in studies. Indeed, many clinical trials have tended to exclude older individuals, either on the basis of age alone, comorbidity, or both[195]. Consequently data about anti-cancer treatments are extrapolated from results in younger population, with a risk of overtreatment and/or complications such as FN following CT. Indeed, many clinical trials have tended to exclude older individuals, either on the basis of age alone, comorbidity, or both. The explanation for this situation is complex and associated with a biased approach by both physicians[196]. However, we do know that older patients are just as likely as younger ones to participate in clinical trials if given the opportunity.

Older age as risk factor for FN

Particular consideration should be given to the high risk of FN in elderly patients (aged 65 and over). Primary prophylaxis of FN is currently indicated for a risk > 20% of FN, but FN is more often complicated in older patients, even if the theoretical risk of FN is < 20%[13].

In a phase Ⅲ randomized trial in 509 metastatic breast cancer patients who received first-line CT with doxorubicin or a pegylated liposomal formulation. One of the risk factors for FN was advanced age[197].

FN prophylaxis

Elderly cancer patients cannot tolerate standard doses of CT but should probably benefit more from prophylaxis because of the frequency and severity of myelosuppressive complications.

One of the first randomized studies that demonstrated the benefit of primary prophylaxis of FN during CT evaluated the incidence of FN and related events in 852 older cancer patients (≥ 65 years of age) with either solid tumors or non-Hodgkin's lymphoma receiving pegfilgrastim; the administration of pegfilgrastim resulted in a significantly lower incidence of FN for both solid tumor and NHL patients compared with reactive use[198].

Cooper *et al*[9] meta-analysis of GCS-F for FN prophylaxis following CT demonstrated that there was no clear difference in GCS-F effectiveness in studies restricting to elderly population. Indeed, Lyman *et al*[51] meta-analysis of 59 individual randomized controlled trials involving nearly 25000 patients with solid tumors or lymphoma demonstrated significant reductions in all-cause mortality over the period of 2 years follow-up with GCS-F-supported CT (RR = 0.93), independent of the age group[17].

In a phase Ⅲ randomized trial of 175 NSCLC patients randomly assigned to CT with or without addition of G-CSF to antibiotic prophylaxis, it was shown a decreased incidence of FN with the addition of G-CSF, and older age was related to the risk of FN in cycle 1[199].

Phase Ⅲ results of 779 patients with ovarian cancer treated with carboplatin or cisplatin/paclitaxel were retrospectively analyzed according to feasibility, toxicity, and QoL in patients aged < 70 or ≥ 70 years; 13% of patients were aged ≥ 70 years. Toxicities were comparable between elderly and younger patients, except for FN (5% *vs* < 1%, $P = 0.005$)[200].

FN complications

It is therefore important to identify patients at risk for complications if FN appears using instruments like the MASCC score). This score identifies age 65 or older as an important risk factor for disease burden in case of FN[18].

Perspectives

Risk factors of CT toxicity (for example FN) other than chronological age should be identified and evaluated, as that chronologic age is often different from physiologic age. The next step in geriatric oncology will be to implement ongoing predictive models for CT toxicity that integrate patient age, and characteristics of the tumor and its treatment as well as laboratory values and overall geriatric assessment[201,202]. This might allow to better selection of patients who will benefit of primary GCS-F prophylaxis of FN.

CONCLUSION

During the past 50 years, FN prognosis has dramatically changed as a result of better supportive care in patients

with cancer and namely the use of empirical broad spectrum anti-microbial therapy. Nonetheless, FN is still diagnosed in 10% of the CT-treated patients and is responsible overall for a 10% mortality without taking into account the morbidity resulting from FN and the possible negative effect on cancer therapy.

A major advance in the management of FN has been the stratification of the population of patients with FN for the risk of complications and death. Using validated reliable predictive instruments, such as the MASCC score, it is possible to identify a population of "low risk" patients who can benefit from simplified and less expensive therapeutic approaches (*e.g.*, orally administered anti-microbial therapy and early home return).

Although the MASCC scoring index has been widely accepted, there is still room for improving its effectiveness, especially in some subset of the FN population, namely in patients with hematological malignancies. Similarly, attempts to improve the performance of the score by adding to it, some biological parameters are promising. Although the MASCC score can identify patients at high risk of complications during FN, more precise prediction of such patients is needed, to make possible earlier and closer monitoring of those patients who present still a high rate of death and complications, mainly because of uncontrolled sepsis. New paradigms for the diagnosis and management of non-low-risk patients with FN are urgently needed.

A major advance in the management of FN has been the introduction of the GCSFs, which efficacy for the prevention of CT-associated has been demonstrated beyond any doubt: 50%-80% of such episodes can now be avoided. Unfortunately GCSFs are expensive and this has led to restrictive algorithms for their use, to balance the cost of the prophylaxis and that of the management of FN; these considerations usually do not take into account the effect of FN on the well-being (QoL) of the patients. It is highly desirable that future research focuses on the definition of subset of patients who could benefit from GCSF prophylaxis, taking into account not only the type of CT used, but also many comorbid conditions making FN more common and more debilitating.

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P- Reviewer: Pavlovic M, Yoshida M
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Li D

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