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EDITORIAL

Ability of community-based prostate cancer screening to target an appropriate and underserved population

Jared Moss, Eric Heidel, Jason Johnson, Chase Powell, Erin Dittrich, Susan Rawn, Paul D Terry, Mitchell Goldman, W Bedford Waters, Wesley M White

Jared Moss, W Bedford Waters, Wesley M White, Division of Urologic Surgery, the University of Tennessee Medical Center, Knoxville, TN 37920, United States
Eric Heidel, Jason Johnson, Chase Powell, Erin Dittrich, Susan Rawn, Paul D Terry, Mitchell Goldman, Department of Surgery, the University of Tennessee Medical Center, Knoxville, TN 37920, United States
Paul D Terry, Departments of Public Health and Surgery, the University of Tennessee, Knoxville, TN 37996, United States

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Correspondence to: Paul D Terry, PhD, MPH, FACE, Departments of Public Health and Surgery, the University of Tennessee, 1914 Andy Holt Ave., HPER 390, Knoxville, TN 37996, United States. pdterry@utk.edu
Telephone: +1-865-9741108

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Abstract

Screening is not universally beneficial due to over- and under-diagnosis, and false positives that beget additional testing and associated adverse events and expense. We examined data from all men who participated in a mass community prostate cancer screening between May 2009 and September 2010. The data contained information regarding patient demographics, family history of prostate cancer, lower urinary tract symptoms, prior history of prostate cancer, most recent digital rectal examination, and the presence of an established relationship with a physician. Current American Urological Association screening recommendations were then applied to determine the appropriateness of our outreach effort. A total of 438 men (mean age 66.5 years) underwent screening. A total of 106 (24.2%) patients in our study met contemporary criteria for screening. Of these men, the vast majority was well educated, well insured, and well informed about the need for prostate cancer screening. Based on these data, mass community-based prostate cancer screening does not appear to identify and screen at-risk men. Future efforts at mass screening should more carefully target men most likely to benefit.

Key words: Prostate cancer; Screening; Outcomes; Prostate specific antigen; Community health

Core tip: Mass prostate specific antigen-based prostate screening is used throughout the world as a means of reducing prostate cancer morbidity and mortality. However, a large proportion of men who underwent mass screening in our region were, in hindsight, not appropriate candidates for screening. Given the recent warnings of the United States Preventative Services Task Force and American Urological Association regarding the over-diagnosis of prostate cancer, it is incumbent on urologists, hospitals, and public health agencies to critically examine the role of screening practices, recognizing both the potential for community benefit

and of harm from inappropriate screening.

Moss J, Heidel E, Johnson J, Powell C, Dittrich E, Rawn S, Terry PD, Goldman M, Waters WB, White WM. Ability of community-based prostate cancer screening to target an appropriate and underserved population. *World J Methodol* 2015; 5(2): 10-12 Available from: URL: http://www.wjgnet.com/2222-0682/full/v5/i2/10.htm DOI: http://dx.doi.org/10.5662/wjm.v5.i2.10

INTRODUCTION

Prostate cancer is the second most common malignancy diagnosed in American men with an annual estimated incidence of approximately 240000[1]. The introduction of prostate specific antigen (PSA) screening has effected a stage migration that has led to earlier diagnoses and the perception of improved survival[2]. Recently, the United States Preventative Services Task Force suggested that PSA-based screening is unnecessary and potentially harmful in some groups of men[3]. Consistent with the Task Force, the American Urological Association (AUA) currently discourages the common practice of "mass" screening[4].

OUR EXPERIENCE WITH MASS PROSTATE CANCER SCREENING

Our institution has previously offered PSA-based prostate screening to our community without adherence to any specific guidelines. Therefore, we retrospectively examined the nature of our prior screenings to determine if our outreach efforts were targeting appropriate screening candidates and/or an underserved population.

Prostate cancer screening was offered to all men in the Knoxville, Tennessee metropolitan area. Through mass mailings, social media, and traditional media formats, men were invited to one of nearly 10 geographically distributed screening locations. All participants provided information regarding demographics, baseline prostate health, family history of prostate cancer, prior screening, and access to either an urologist or primary care physician. Participants then underwent PSA testing and a digital rectal examination (DRE) by a board certified urologist. Results of the DRE were categorized as normal, abnormal concerning or highly suspicious for cancer, or enlarged consistent with benign prostatic hyperplasia. PSA values were forwarded to the screening provider and compared to examination results, and the patient was either (1) advised to undergo routine screening once a year; or (2) encouraged to follow-up on his "abnormal results" for additional confirmatory testing.

We retrospectively examined demographic data from these patient-reported forms, called patients individually to confirm accuracy of these results, and applied current best practice screening guidelines based on AUA recommendations[4]. Men aged 55-69 years were considered appropriate screening candidates, whereas men outside of this age range and/or those who had undergone prior screening within one year and/or men with a prior diagnosis of prostate cancer were considered poor screening candidates. African-American men and/or men with a family history of prostate cancer within a first-degree relative were considered appropriate candidates for screening. The Statistical Package for the Social Sciences version 21 (Armonk, NY: IBM Corp) was used to calculate frequency and cross-tabulation statistics to assess characteristics of the dataset. The University of Tennessee Graduate School of Medicine Institutional Review Board approved the study.

Between May 1, 2009 and September 30, 2010, 438 men underwent PSA-based prostate cancer screening. The mean age of the cohort was 66.5 years (age range 37-91). In this cohort, 98% were Caucasian, 16% reported family history of prostate cancer, and 27.1% reported attendance at a similar screening event within the past 12 mo. In addition, 97.6% and 95.3% reported an understanding of the need for annual PSA and DRE, respectively. Two-thirds of screened individuals had completed some form of higher education (greater than a high school degree, reflecting a better-educated population than the more general regional population. Approximately 95% of the cohort maintained health insurance at the time of screening. In all, 87.3% of the total cohort reported an established relationship with a primary care physician. Finally, nearly 97% were educated about the need for an annual PSA and DRE, and at least 80% of patients had attended mass screening at some point in the past. Taken as a whole, men participating in our mass prostate cancer screening represent a well educated, insured population; relatively few of those men were deemed to be appropriate for screening.

DISCUSSION

We found that a large proportion of men who underwent screening in our cohort were not appropriate candidates for screening. Further, most of the men who "met criteria" for screening based on age, ethnicity, and/or family history, were well educated, well insured, and well informed; hence, men most in need of screening were largely absent from our mass screening effort.

Screening is not universally beneficial due to the overdiagnosis of potentially indolent disease, false positives that beget additional testing and associated adverse events and expense, and poor sensitivity that may lead to underdiagnosis and a false sense of security. Given the criticism centered on PSA-based screening and "mass screening" in particular, it is incumbent on urologists, hospitals, and public health agencies to critically examine the role of screening

practices, recognizing both the potential for community benefit and of harm from inappropriate screening.

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REFERENCES

1 **Smith RA**, Manassaram-Baptiste D, Brooks D, Cokkinides V, Doroshenk M, Saslow D, Wender RC, Brawley OW. Cancer screening in the United States, 2014: a review of current American Cancer Society guidelines and current issues in cancer screening. *CA Cancer J Clin* 2014; **64**: 30-51 [PMID: 24408568 DOI: 10.3322/caac.21212]

2 **Schröder FH**, Hugosson J, Roobol MJ, Tammela TL, Ciatto S, Nelen V, Kwiatkowski M, Lujan M, Lilja H, Zappa M, Denis LJ, Recker F, Berenguer A, Määttänen L, Bangma CH, Aus G, Villers A, Rebillard X, van der Kwast T, Blijenberg BG, Moss SM, de Koning HJ, Auvinen A. Screening and prostate-cancer mortality in a randomized European study. *N Engl J Med* 2009; **360**: 1320-1328 [PMID: 19297566 DOI: 10.1056/NEJMoa0810084]

3 **Moyer VA**. Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2012; **157**: 120-134 [PMID: 22801674 DOI: 10.7326/0003-4819-157-2-201207170-00459]

4 **Carter HB**, Albertsen PC, Barry MJ, Etzioni R, Freedland SJ, Greene KL, Holmberg L, Kantoff P, Konety BR, Murad MH, Penson DF, Zietman AL. Early detection of prostate cancer: AUA Guideline. *J Urol* 2013; **190**: 419-426 [PMID: 23659877 DOI: 10.1016/j.juro.2013.04.119]

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EDITORIAL

Recurrent urinary tract infections in children: Preventive interventions other than prophylactic antibiotics

Kishor Tewary, Hassib Narchi

Kishor Tewary, Department of Pediatrics, University Hospitals of North Midlands, Stoke-on-Trent, ST4 6QG Staffordshire, United Kingdom
Hassib Narchi, Department of Pediatrics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, PO Box 17666, United Arab Emirates

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Correspondence to: Hassib Narchi, MD, FRCPCH, Department of Pediatrics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, PO Box 17666, United Arab Emirates. hassib.narchi@uaeu.ac.ae
Telephone: +971-37-137414
Fax: +971-37-672022

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Abstract

Urinary tract infection (UTI) is one of the most common childhood infections. Permanent renal cortical scarring may occur in affected children, especially with recurrent UTIs, leading to long-term complications such as hypertension and chronic renal failure. To prevent such damage, several interventions to prevent UTI recurrences have been tried. The most established and accepted prevention at present is low dose long-term antibiotic prophylaxis. However it has a risk of break through infections, adverse drug reactions and also the risk of developing antibiotic resistance. The search is therefore on-going to find a safer, effective and acceptable alternative. A recent meta-analysis did not support routine circumcision for normal boys with no risk factors. Vaccinium Macrocarpon (cranberry), commonly used against UTI in adult women, is also effective in reducing the number of recurrences and related antimicrobial use in children. Sodium pentosanpolysulfate, which prevents bacterial adherence to the uroepithelial cells in animal models, has shown conflicting results in human trials. When combined with antibiotic, *Lactobacillus acidophilus* (LA-5) and *Bifidobacterium*, by blocking the *in vitro* attachment of uropathogenic bacteria to uroepithelial cells, significantly reduce in the incidence of febrile UTIs. Deliberate colonization of the human urinary tract of patients with recurrent UTI with *Escherichia-coli* (*E. coli*) 83972 has resulted in subjective benefit and less UTI requiring treatment. The non-pathogenic *E. coli* isolate NU14 DeltawaaL is a candidate to develop live-attenuated vaccine for the treatment and prevention of acute and recurrent UTI. Diagnosing and treating dysfunctional elimination syndromes decrease the incidence of recurrent UTI. A meta-analysis found the lack of robust prospective randomized controlled trials limited the strength of the established guidelines for surgical management of vesicoureteral reflux. In conclusion, several interventions, other than antibiotic prophylaxis, for the prevention of recurrent UTI have been tried and, although showing some promise, they do not provide so far a definitive effective answer. Finding suitable alternatives still requires further high quality research of those seemingly promising interventions.

Key words: Kidney; Recurrence/prevention; Urinary tract infections; Vesico-ureteral reflux; Vaccinium macrocarpon; Circumcision; Vaccination; Constipation; Lactobacillus

Core tip: Antibiotic prophylaxis against urinary tract infection recurrences is associated with adverse drug reaction and development of resistance. Although showing some promise, alternative interventions, such as Vaccinium Macrocarpon (cranberry), *Lactobacillus* and Probiotics, circumcision, surgical management of vesicoureteral reflux, deliberate colonization of the urinary tract with *Escherichia-coli* (*E. coli*) 83972, treating constipation and dysfunctional voiding, administration of synthetic substitutes that reproduce natural surface glycosaminoglycan(s) anti-adherence effect on uroepithelial cells and *E. coli* isolate NU14 DeltawaaL as a candidate for developing a live-attenuated vaccine, do not provide so far a definitive effective answer. Further high quality research is still required.

Tewary K, Narchi H. Recurrent urinary tract infections in children: Preventive interventions other than prophylactic antibiotics. *World J Methodol* 2015; 5(2): 13-19 Available from: URL: http://www.wjgnet.com/2222-0682/full/v5/i2/13.htm DOI: http://dx.doi.org/10.5662/wjm.v5.i2.13

INTRODUCTION

Urinary tract Infection (UTI) is one of the most common childhood infections[1,2], affecting round 1.7% of boys and 8.4% of girls by the age of seven years[3]. A third to one half of affected children will suffer from at least one recurrence[4]. While in infancy this infection is mostly secondary to hematogenous dissemination, it usually occurs as an ascending infection in older children, where the common organism involved include Gram negative bacteria such as *Escherichia-coli* (*E. coli*), *Klebsiella*, *Proteus*, *Enterobacter*, *Pseudomonas* and *Serratia* species[5]. Permanent renal cortical scarring may occur in 15%-65% of affected children[4], especially in recurrent UTI and its long-term complications include hypertension and chronic renal failure which may result in end stage renal disease[6]. Contrary to previous beliefs, acquired renal scarring correlates best with recurrent UTI rather than the presence of vesicoureteral reflux (VUR). In a bid to prevent long-term damage with recurrent UTI several interventions have been tried, aiming at one or more of the factors that facilitate the development of UTI.

These factors include: (1) microbial growth in the urogenital tract; (2) foreskin facilitating peri-meatal bacterial growth; (3) bacterial adhesion to the uroepithelial cells; (4) bioflora favoring pathogenic urobacteria; (5) insufficient urothelial cytokine secretion; (6) urinary stasis; and (7) vesicoureteral reflux. The interventions considered included therefore: (1) long term antibiotic prophylaxis; (2) circumcision in male children; (3) cranberry and glycosaminoglycans; (4) lactobacillus and probiotics products; (5) vaccination; (6) management of dysfunctional elimination syndrome; and (7) vesicoureteral reimplantation[4].

PREVENTIVE INTERVENTIONS

Antibiotic prophylaxis

This is currently the most established and accepted prevention of UTI recurrences at present[7]. However, this policy is of limited efficacy with little or no clinical benefit at all in various trials[8-10]. Although compliance to treatment is reported to be 91%, only 31% of children have antibiotic metabolites identified in the urine[11]. Furthermore, the risk of break through infections is estimated to be between 25% and 38%[12,13]. Antibiotic usage is not without risk. Approximately 10% of children on long-term antibiotic therapy develop adverse reactions that range from common gastrointestinal symptoms to bone-marrow suppression and rarely Stevens Johnson Syndrome[14,15]. Also worrisome is the growing evidence of antibiotic resistance developing with excessive use of long-term antibiotics[16].

Considering all those concerns with antibiotic prophylaxis, there is a growing need to re-evaluate the other suggested alternative interventions to prevent UTI recurrences. We look at the currently available evidence behind these alternative interventions.

Circumcision

Although for a long time the absolute indications for circumcision have been phymosis secondary to xerotica obliterans and recurrent balanoposthitis, prevention of UTI in boys has been added after a brief report that showed that the foreskin was a risk factor for UTI in male infants[17]. Several studies have since supported the benefits of neonatal circumcision, especially as the complication rate was found to be only 1.6%, consisting mainly of haemorrhage, inappropriate penile appearance, ring impaction or stenosis[18]. When compared to 3000 uncircumcised newborns where the rate of UTI is 2%, no UTI was found in any of the other 3000 circumcised neonates up to 15 mo after the procedure[18]. Circumcision significantly reduced the incidence of UTI in male children by 90%[19,20], however it is limited to one particular group of sex, and the incidence of UTI in boys is only 1% of total UTI population. With previous studies suggesting that uropathogen's attachment to the foreskin, by providing a environment for bacterial colonisation, made the foreskin a risk factor for UTI, a study was conducted on children with low grade VUR and showed that, when compared to antibiotic prophylaxis alone, circumcision associated with antibiotic prophylaxis resulted in a significant decrease in bacterial colonisation rate[21]. In a cohort of infants with antenatal hydronephrosis, circumcision

provided a significant reduction in the frequency of UTI frequency when comparing the periods before and after circumcision[22].

The support for neonatal circumcision to prevent recurrent UTI is still being challenged. In a study of ritually circumcised Jewish male neonates, there was a high prevalence of UTI, suggesting that the procedure puts the infants at an increased risk of UTI[23]. It has been suggested that the differences in UTI incidence between circumcised and non-circumcised boys is not due to the procedure, but could instead be attributed to several confounding factors such as prematurity, low birth weight, perinatal anoxia, lack of breast feeding, poor hygienic practices, low parental education, prenatal maternal UTI, history of a UTI in a first degree relative, history of fever in the mother at the time of delivery, previous infections, previous course of antibiotics, method of urine collection, and diagnostic standards used[24]. Furthermore, no effect on the incidence of postoperative UTI was found with circumcision performed during anti-reflux surgery[25].

To clear the confusion, a meta-analysis of 12 studies on circumcision and UTI prevention was conducted and, although the procedure seemed to be more beneficial to boys with recurrent UTI (only 11 needed to prevent 1 UTI) and boys with grade III or more VUR (four needed to prevent one UTI), it was calculated that overall, 111 circumcisions would be required to prevent one UTI, costing £55000 in the United Kingdom. The study concluded that a decision to carry our routine circumcision for normal boys with no risk factors was not supported by that meta-analysis[26]. In addition, although, in its policy on circumcision in 1989, the American of Paediatrics concluded that newborn circumcision decreased the rate of UTI from 1% to 0.1%, it modified the guideline in 1999 stating that routine circumcision was not necessary in all newborns[27].

Preventing bacterial adhesion to the uroepithelial cells

Vaccinium macrocarpon (Cranberry): Vaccinium macrocarpon, also called large cranberry, American cranberry and bearberry, is a cranberry of the subgenus *Oxycoccus* and genus *Vaccinium*. It is one of the most commonly used and acceptable preventive agent against UTI in adult women, and has also been tried in pediatric age groups where it was associated with a much better compliance than oral antibiotics and without significant side effects[15,28].

The mechanism of action of cranberry resides in the action of proanthocyanidine it contains on mannose-resistant P-fimbriated *E. coli*, strains that cause cystitis and pyelonephritis[29]. The proanthocyanidine containing "A" type linkage prevents the adhesion of proteinaceous fibres or fimbriae [heteropolymeric fibers carrying a Gal (alpha 1-4) Gal-specific PapG adhesin at its distal end and located on the bacterial cell] to the specific carbohydrate receptors on uro-epithelial cells[30-32]. This effect occurs at a concentration as low as 75 ug/mL[33].

Data on the effectiveness of cranberry in the prevention of UTI in adults is encouraging but still incomplete. In premenopausal women, while cranberry juice did not significantly reduce UTI risk compared with placebo, the reduction in urinary P-fimbriated *E. coli* strains supported the biological plausibility of its activity[34]. In renal transplant patients, a combination of cranberry juice and L-methionine reduces by more than 50% the incidence of UTI and also decreases the prevalence of symptomatic pyuric patients[35]. A Cochrane review of 10 good quality randomised controlled trials in over 1000 women suggests that cranberry juice decreases the number of symptomatic UTIs over a 12-mo period[36].

The evidence of efficacy is still less clear in children. Some studies have shown promising results in paediatric UTI prevention[37]. However, in a double-blind randomized placebo-controlled trial involving 255 children, while cranberry juice did not significantly reduce the number of children who experienced a recurrence of UTI, it was effective in reducing the actual number of recurrences and related antimicrobial use[38]. A recent Cochrane review showed that while cranberry juice decreases the number of symptomatic UTIs in women, there is still lack of such evidence in children[36]. It is also likely that its acidic nature reduces its palatability in children[36].

Glycosaminoglycans and sodium pentosanpolysulfate: The transitional epithelial cells at the surface of the urinary bladder secrete and bind to their surfaces one or more glycosaminoglycans that markedly reduce the ability of microorganisms to adhere to the mucosa, a prerequisite to cause a UTI[39]. Comparing the prevalence of UTI in intact mucin deficient rabbit bladders with those treated with sodium pentosanpolysulfate (PSP, a similar but synthetic substitute for the surface glycosaminoglycan), UTI were more frequent in mucin deficient bladders after exposure to bacteria. This suggests that the natural surface glycosaminoglycan(s) and the synthetic substitutes that reproduce their antiadherence effect appear to be protecting factors[40]. In human trials, the results have been conflicting. While no significant effect of sodium PSP was found compared to placebo in patients with interstitial cystitis and painful bladder disease[41], another study in patients with interstitial cystitis has shown a significant benefit from treatment with sodium PSP[42]. So far, no studies on its role in preventing UTI in children have been performed.

Bioflora modification

These alternatives are based on two mechanisms: Competitive exclusion and bacterial interference.

Competitive exclusion-lactobacillus and probiotics: The interest in studying probiotics for the prevention of UTI started after an animal study where the injection of five strains of periurethral uropathogenic organisms into the urinary bladder of female rats and then instilling an isolate of *Lactobacilli casei* GR1 from the urethra of a healthy woman resulted in decreased the development

of UTI by 84% up to 60 d later[43]. This experiment was underpinned by the concept of competitive exclusion as indigenous bacteria block the *in vitro* attachment of uropathogenic bacteria to human uroepithelial cells.

Women with recurrent UTI are believed to have pre-existing alterations in their normal vaginal microflora resulting in depletion of hydrogen peroxide (H_2O_2) containing Lactobacili which are protective against infections. Restoration of normal vaginal microflora through the use of (H_2O_2) containing lactobacillus probiotic has been investigated to test the hypothesis that it confers a protective effect against recurrent UTI in women. Intravaginal administration of Lactobacilli Crispatus suppository was established to be safe[44]. Vaginal suppositories of *Lactobacillus Crisptatus* reduced by 50% the incidence of UTI in 50 pre-menopausal women[45]. Oral probiotic yogurt, prepared from inoculating of *Lactobacillus acidophilus* (LA-5) and *Bifidobacterium* in heated milk, combined with antibiotic, was compared to antibiotic prophylaxis alone for prevention of UTI and it resulted in a significant reduction in the incidence of febrile UTIs in the third year of administration (although there was no difference in the first two years)[46]. Several studies challenged the benefit of *Lactobacillus*: it did not provide significant prevention of UTI In sick neonates in a neonatal intensive care unit[47]. And, when compared to *Vaccinium Macroponam, it* was not more effective in preventing UTI[48].

Bacterial interference: Animal studies showing the interference of *E. coli* with the growth of *Pseudomonas aeruginosa* in the bladder of male Wistar rats[49] raised the theory of bacterial interference as a therapeutic option. This was supported by the finding that asymptomatic bacteriuria, especially with *E. coli* 83972 (associated with symptom-free colonizations for long periods of time) protects against recurrent UTI. This observation has prompted clinical trials with deliberate colonization of the human urinary tract of patients with recurrent UTI which has resulted in a subjective benefit and in less UTI requiring treatment in colonized patients[50]. Similarly, UTI commonly occurs in patients with spinal cord injury as their bladder, particularly in the presence of an indwelling bladder catheter, can become colonized by a variety of organisms, including benign colonizing bacteria which are often left untreated because they may provide some protection against symptomatic infection with more pathogenic microbes. As a result, intentional colonisation of the neurogenic bladder in patients with spinal cord injury with a non-pathogenic strain of *E. coli* such as *E. coli 83972*[51-53] or *E. coli HU21117*[54] was attempted and was shown to reduce the risk of symptomatic UTI with pathogenic *E. coli* in these patients and was safe.

Vaccination

As deletion of the O antigen ligase gene, waaL, from the uropathogenic *E. coli* isolate NU14 results in a strain that stimulates urothelial cytokine secretion, NU14 DeltawaaL was tested as a vaccine for UTI in mice *via* instillation into the bladder as was shown to protect mice against challenge with a broad range of clinical uropathogenic *E. coli* isolates and produced immunity that lasted 8 wk. It is therefore a candidate live-attenuated vaccine for the treatment and prevention of acute and recurrent UTI by caused by uropathogenic *E. coli*[55,56]. Human trials have not been performed so far.

Voiding habits

Non-neurogenic neurogenic bladder, first described in 1986[57], is a disorder of functional bladder obstruction causing urinary retention and altered bladder anatomy that may lead to upper urinary tract dilatation and scarring[58]. Dysfunctional elimination syndromes are functional bowel and/or bladder disorders, including bladder instability, inability to effectively empty the bladder, infrequent voiding enuresis, UTI, incontinence, constipation or other voiding symptoms. They are common and often unrecognized in children with primary VUR[59]. Girls with recurrent UTI are more likely to have a high degree of dysfunctional elimination[60]. These syndromes are associated with delayed VUR resolution and an increased rate of breakthrough urinary tract infection, which may require ureteral reimplantation surgery[59]. These problems are not only important during childhood, but they may also have a negative impact on bladder and bowel function later life[61]. Objective assessment of symptoms severity, is required for screening and diagnosis purposes, confirmation of treatment results and follow up. It might also be useful for screening purposes[62].

Diagnosing and treating constipation as well as dysfunctional voiding are required to treat this condition[63]. Correcting constipation has been shown to decrease in the incidence of recurrent UTI[64]. In children with dysfunctional elimination, treating constipation with polyethylene glycol 3350 is successful, lacks significant side effects and is associated with good compliance and persistent constipation is associated with decreased resolution of voiding symptoms[65]. Biofeedback is an effective, non-invasive method of treating dysfunctional elimination syndrome with 80% success rate[66]. Children-directed biofeedback is also promising[67] and animated biofeedback, with pelvic floor muscle exercises, coordination of breathing and pelvic floor muscle contractions has been shown to be beneficial in improving dysfunctional elimination[68]. Sacral neuromodulation has been suggested for children with dysfunctional elimination syndrome whose symptoms are refractory to maximum medical therapy but should be cautiously used as it carries a significant risk of complications[69].

Ureteral re-implantation

The role of surgical treatment of VUR in the prevention

of UTI recurrences is well documented. Ureteral reimplantation in 205 infants (180 boys and 25 girls) with primary VUR reduced the frequency of febrile UTI reduced from 0.23538 before surgery to 0.00894 and 0.00081 per patient per month at six and 12 mo after surgery respectively, with no development of renal scarring on DMSA scan[70]. Several studies on a large number of children have shown absence of significant difference in renal growth between surgical ureteral re-implantation and medically treated children with primary VUR, both in previously scarred and in normal kidneys up to 10 years later, and, although pyelonephritis occurred significantly less often in surgically treated children, there was no significant difference in glomerular filtration rate nor in the development of hypertension[71-73]. A systematic meta-analysis was carried out by the Vesicoureteral Reflux Guideline Update Committee of the American Urological Association established to update the management of primary vesicoureteral reflux in children. A total of 2028 articles were reviewed, data were extracted from 131 articles including a total of 17972 patients. Guidelines for managing vesicoureteral reflux in children were issued but the lack of robust prospective randomized controlled trials limited the strength of these guidelines[74].

CONCLUSION

Several interventions, other than antibiotic prophylaxis, for the prevention of recurrent UTI have been tried and, although showing some promise, they do not provide so far a definitive effective answer.

Cranberry juice appears to be a promising and safe alternative with no serious adverse events. However its efficacy remains questionable in the pediatric population. Few studies are available on probiotics, but their efficacy is still debated for UTI prevention. Circumcision, a largely popular choice in certain countries, lacks good quality studies to prove its safety, and effectiveness. It was found to be particularly useful for children with low grade VUR and antenatal hydronephrosis, but the presence of many confounding factors requires further larger good quality studies to establish its efficacy. Glycosaminoglycan and sodium pentosanpolysulfate, found to be useful in animal models, have not been tested yet in humans. The benefit of surgical interventions, such as ureteral re-implantation, is confined to a particular group of patients, and the statistical significance of its efficacy remains questionable. Although improving voiding habits is certainly a beneficial approach, its effectiveness in isolation remains unproven. Vaccination is an attractive emerging option, but high quality large randomised controlled trials in humans are needed to look for its efficacy in UTI prevention.

Finding suitable alternatives to oral long-term antibiotic prophylaxis for UTI prevention still requires further high quality research of those seemingly promising interventions.

REFERENCES

1 **Foxman B**, Brown P. Epidemiology of urinary tract infections: transmission and risk factors, incidence, and costs. *Infect Dis Clin North Am* 2003; **17**: 227-241 [PMID: 12848468]

2 **Stull TL**, LiPuma JJ. Epidemiology and natural history of urinary tract infections in children. *Med Clin North Am* 1991; **75**: 287-297 [PMID: 1996034]

3 **Hellström A**, Hanson E, Hansson S, Hjälmås K, Jodal U. Association between urinary symptoms at 7 years old and previous urinary tract infection. *Arch Dis Child* 1991; **66**: 232-234 [PMID: 2001110]

4 **Paintsil E**. Update on recent guidelines for the management of urinary tract infections in children: the shifting paradigm. *Curr Opin Pediatr* 2013; **25**: 88-94 [PMID: 23241875 DOI: 10.1097/MOP.0b013e32835c14cc]

5 **Zorc JJ**, Kiddoo DA, Shaw KN. Diagnosis and management of pediatric urinary tract infections. *Clin Microbiol Rev* 2005; **18**: 417-422 [PMID: 15831830 DOI: 10.1128/CMR.18.2.417-422.2005]

6 **Jacobson SH**, Eklöf O, Eriksson CG, Lins LE, Tidgren B, Winberg J. Development of hypertension and uraemia after pyelonephritis in childhood: 27 year follow up. *BMJ* 1989; **299**: 703-706 [PMID: 2508881]

7 **NICE guidelines**. Urinary tract infection in children. CG54, 2007. [accessed 2014 Oct 12]. Available from: URL: http://www.nice.org.uk/guidance/CG54

8 **Montini G**, Rigon L, Zucchetta P, Fregonese F, Toffolo A, Gobber D, Cecchin D, Pavanello L, Molinari PP, Maschio F, Zanchetta S, Cassar W, Casadio L, Crivellaro C, Fortunati P, Corsini A, Calderan A, Comacchio S, Tommasi L, Hewitt IK, Da Dalt L, Zacchello G, Dall'Amico R. Prophylaxis after first febrile urinary tract infection in children? A multicenter, randomized, controlled, noninferiority trial. *Pediatrics* 2008; **122**: 1064-1071 [PMID: 18977988 DOI: 10.1542/peds.2007-3770]

9 **De Cunto A**, Pennesi M, Salierno P. Re: Antibiotic prophylaxis for the prevention of recurrent urinary tract infection in children with low grade vesicoureteral reflux: results from a prospective randomized study: G. Roussey-Kesler, V. Gadjos, N. Idres, B. Horen, L. Ichay, M. D. Leclair, F. Raymond, A. Grellier, I. Hazart, L. De parscau, R. Salomon, G. Champion, V. Leroy, V. Guigonis, D. Siret, J. B. Palcoux,S. Taque, A. Lemoigne, J. M. Nguyen and C. Guyot. J Urol 2008; 179: 674-679. *J Urol* 2008; **180**: 2258-2259 [PMID: 18804797 DOI: 10.1016/j.juro.2007.09.090]

10 **Williams G**, Craig JC. Prevention of recurrent urinary tract infection in children. *Curr Opin Infect Dis* 2009; **22**: 72-76 [PMID: 19532083 DOI: 10.1097/QCO.0b013e328320a885]

11 **Bollgren I**. Antibacterial prophylaxis in children with urinary tract infection. *Acta Paediatr Suppl* 1999; **88**: 48-52 [PMID: 10588271]

12 **Tamminen-Möbius T**, Brunier E, Ebel KD, Lebowitz R, Olbing H, Seppänen U, Sixt R. Cessation of vesicoureteral reflux for 5 years in infants and children allocated to medical treatment. The International Reflux Study in Children. *J Urol* 1992; **148**: 1662-1666 [PMID: 1433584]

13 Prospective trial of operative versus non-operative treatment of severe vesicoureteric reflux in children: five years' observation. Birmingham Reflux Study Group. *Br Med J* (Clin Res Ed) 1987; **295**: 237-241 [PMID: 2888509]

14 **Karpman E**, Kurzrock EA. Adverse reactions of nitrofurantoin, trimethoprim and sulfamethoxazole in children. *J Urol* 2004; **172**: 448-453 [PMID: 15247700 DOI: 10.1097/01.ju.0000130653.74548.d6]

15 **Uhari M**, Nuutinen M, Turtinen J. Adverse reactions in children during long-term antimicrobial therapy. *Pediatr Infect Dis J* 1996; **15**: 404-408 [PMID: 8724061]

16 **Allen UD**, MacDonald N, Fuite L, Chan F, Stephens D. Risk factors for resistance to "first-line" antimicrobials among urinary tract isolates of Escherichia coli in children. *CMAJ* 1999; **160**: 1436-1440 [PMID: 10352632]

17 **Wiswell TE**, Smith FR, Bass JW. Decreased incidence of urinary tract infections in circumcised male infants. *Pediatrics* 1985; **75**:

901-903 [PMID: 3991278]
18 **Simforoosh N**, Tabibi A, Khalili SA, Soltani MH, Afjehi A, Aalami F, Bodoohi H. Neonatal circumcision reduces the incidence of asymptomatic urinary tract infection: a large prospective study with long-term follow up using Plastibell. *J Pediatr Urol* 2012; **8**: 320-323 [PMID: 21115400 DOI: 10.1016/j.jpurol.2010.10.008]
19 **Bader M**, McCarthy L. What is the efficacy of circumcision in boys with complex urinary tract abnormalities? *Pediatr Nephrol* 2013; **28**: 2267-2272 [PMID: 23400859 DOI: 10.1007/s00467-013-2410-2]
20 **Shaikh N**, Morone NE, Bost JE, Farrell MH. Prevalence of urinary tract infection in childhood: a meta-analysis. *Pediatr Infect Dis J* 2008; **27**: 302-308 [PMID: 18316994 DOI: 10.1097/INF.0b013e31815e4122]
21 **Gücük A**, Burgu B, Gökçe İ, Mermerkaya M, Soygür T. Do antibiotic prophylaxis and/or circumcision change periurethral uropathogen colonization and urinary tract infection rates in boys with VUR? *J Pediatr Urol* 2013; **9**: 1131-1136 [PMID: 23721792 DOI: 10.1016/j.jpurol.2013.04.014]
22 **Kose E**, Yavascan O, Turan O, Kangin M, Bal A, Alparslan C, Sirin Kose S, Kuyum P, Aksu N. The effect of circumcision on the frequency of urinary tract infection, growth and nutrition status in infants with antenatal hydronephrosis. *Ren Fail* 2013; **35**: 1365-1369 [PMID: 23992538 DOI: 10.3109/0886022X.2013.828263]
23 **Toker O**, Schwartz S, Segal G, Godovitch N, Schlesinger Y, Raveh D. A costly covenant: ritual circumcision and urinary tract infection. *Isr Med Assoc J* 2010; **12**: 262-265 [PMID: 20929075]
24 **Van Howe RS**. Effect of confounding in the association between circumcision status and urinary tract infection. *J Infect* 2005; **51**: 59-68 [PMID: 15979493 DOI: 10.1016/j.jinf.2004.07.003]
25 **Kwak C**, Oh SJ, Lee A, Choi H. Effect of circumcision on urinary tract infection after successful antireflux surgery. *BJU Int* 2004; **94**: 627-629 [PMID: 15329127 DOI: 10.1111/j.1464-410X.2004.05014.x]
26 **Singh-Grewal D**, Macdessi J, Craig J. Circumcision for the prevention of urinary tract infection in boys: a systematic review of randomised trials and observational studies. *Arch Dis Child* 2005; **90**: 853-858 [PMID: 15890696 DOI: 10.1136/adc.2004.049353]
27 **Shapiro E**. American academy of pediatrics policy statements on circumcision and urinary tract infection. *Rev Urol* 1999; **1**: 154-156 [PMID: 16985788]
28 **Craig JC**, Simpson JM, Williams GJ, Lowe A, Reynolds GJ, McTaggart SJ, Hodson EM, Carapetis JR, Cranswick NE, Smith G, Irwig LM, Caldwell PH, Hamilton S, Roy LP. Antibiotic prophylaxis and recurrent urinary tract infection in children. *N Engl J Med* 2009; **361**: 1748-1759 [PMID: 19864673 DOI: 10.1056/NEJMoa0902295]
29 **Roberts JA**, Kaack MB, Fussell EN. Bacterial adherence in urinary tract infections: preliminary studies in a primate model. *Infection* 1989; **17**: 401-404 [PMID: 2693359]
30 **Beachey EH**. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surface. *J Infect Dis* 1981; **143**: 325-345 [PMID: 7014727]
31 **Roberts JA**, Marklund BI, Ilver D, Haslam D, Kaack MB, Baskin G, Louis M, Möllby R, Winberg J, Normark S. The Gal(alpha 1-4)Gal-specific tip adhesin of Escherichia coli P-fimbriae is needed for pyelonephritis to occur in the normal urinary tract. *Proc Natl Acad Sci USA* 1994; **91**: 11889-11893 [PMID: 7991552]
32 **Howell AB**, Reed JD, Krueger CG, Winterbottom R, Cunningham DG, Leahy M. A-type cranberry proanthocyanidins and uropathogenic bacterial anti-adhesion activity. *Phytochemistry* 2005; **66**: 2281-2291 [PMID: 16055161 DOI: 10.1016/j.phytochem.2005.05.022]
33 **Foo LY**, Lu Y, Howell AB, Vorsa N. The structure of cranberry proanthocyanidins which inhibit adherence of uropathogenic P-fimbriated Escherichia coli in vitro. *Phytochemistry* 2000; **54**: 173-181 [PMID: 10872208]
34 **Stapleton AE**, Dziura J, Hooton TM, Cox ME, Yarova-Yarovaya Y, Chen S, Gupta K. Recurrent urinary tract infection and urinary Escherichia coli in women ingesting cranberry juice daily: a randomized controlled trial. *Mayo Clin Proc* 2012; **87**: 143-150 [PMID: 22305026 DOI: 10.1016/j.mayocp.2011.10.006]
35 **Pagonas N**, Hörstrup J, Schmidt D, Benz P, Schindler R, Reinke P, van der Giet M, Zidek W, Westhoff TH. Prophylaxis of recurrent urinary tract infection after renal transplantation by cranberry juice and L-methionine. *Transplant Proc* 2012; **44**: 3017-3021 [PMID: 23195017 DOI: 10.1016/j.transproceed.2012.06.071]
36 **Goldman RD**. Cranberry juice for urinary tract infection in children. *Can Fam Physician* 2012; **58**: 398-401 [PMID: 22499815]
37 **Afshar K**, Stothers L, Scott H, MacNeily AE. Cranberry juice for the prevention of pediatric urinary tract infection: a randomized controlled trial. *J Urol* 2012; **188**: 1584-1587 [PMID: 22910239 DOI: 10.1016/j.juro.2012.02.031]
38 **Salo J**, Uhari M, Helminen M, Korppi M, Nieminen T, Pokka T, Kontiokari T. Cranberry juice for the prevention of recurrences of urinary tract infections in children: a randomized placebo-controlled trial. *Clin Infect Dis* 2012; **54**: 340-346 [PMID: 22100577 DOI: 10.1093/cid/cir801]
39 **Parsons CL**, Pollen JJ, Anwar H, Stauffer C, Schmidt JD. Antibacterial activity of bladder surface mucin duplicated in the rabbit bladder by exogenous glycosaminoglycan (sodium pentosanpolysulfate). *Infect Immun* 1980; **27**: 876-881 [PMID: 6155340]
40 **Parsons CL**. Prevention of urinary tract infection by the exogenous glycosaminoglycan sodium pentosanpolysulfate. *J Urol* 1982; **127**: 167-169 [PMID: 6173492]
41 **Holm-Bentzen M**, Jacobsen F, Nerstrøm B, Lose G, Kristensen JK, Pedersen RH, Krarup T, Feggetter J, Bates P, Barnard R. A prospective double-blind clinically controlled multicenter trial of sodium pentosanpolysulfate in the treatment of interstitial cystitis and related painful bladder disease. *J Urol* 1987; **138**: 503-507 [PMID: 2442415]
42 **Fritjofsson A**, Fall M, Juhlin R, Persson BE, Ruutu M. Treatment of ulcer and nonulcer interstitial cystitis with sodium pentosanpolysulfate: a multicenter trial. *J Urol* 1987; **138**: 508-512 [PMID: 2442416]
43 **Reid G**, Chan RC, Bruce AW, Costerton JW. Prevention of urinary tract infection in rats with an indigenous Lactobacillus casei strain. *Infect Immun* 1985; **49**: 320-324 [PMID: 3926645]
44 **Czaja CA**, Stapleton AE, Yarova-Yarovaya Y, Stamm WE. Phase I trial of a Lactobacillus crispatus vaginal suppository for prevention of recurrent urinary tract infection in women. *Infect Dis Obstet Gynecol* 2007; **2007**: 35387 [PMID: 18288237 DOI: 10.1155/2007/35387]
45 **Stapleton AE**, Au-Yeung M, Hooton TM, Fredricks DN, Roberts PL, Czaja CA, Yarova-Yarovaya Y, Fiedler T, Cox M, Stamm WE. Randomized, placebo-controlled phase 2 trial of a Lactobacillus crispatus probiotic given intravaginally for prevention of recurrent urinary tract infection. *Clin Infect Dis* 2011; **52**: 1212-1217 [PMID: 21498386 DOI: 10.1093/cid/cir183]
46 **Mohseni MJ**, Aryan Z, Emamzadeh-Fard S, Paydary K, Mofid V, Joudaki H, Kajbafzadeh AM. Combination of probiotics and antibiotics in the prevention of recurrent urinary tract infection in children. *Iran J Pediatr* 2013; **23**: 430-438 [PMID: 24427497]
47 **Dani C**, Biadaioli R, Bertini G, Martelli E, Rubaltelli FF. Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. A prospective double-blind study. *Biol Neonate* 2002; **82**: 103-108 [PMID: 12169832]
48 **Kontiokari T**, Sundqvist K, Nuutinen M, Pokka T, Koskela M, Uhari M. Randomised trial of cranberry-lingonberry juice and Lactobacillus GG drink for the prevention of urinary tract infections in women. *BMJ* 2001; **322**: 1571 [PMID: 11431298]
49 **Watanabe K**, Sakai Y, Takezaki T, Ogawa A. Bacterial interference in mixed infection in the rat bladder. *Urol Int* 1988; **43**: 2-6 [PMID: 3131942]
50 **Sundén F**, Håkansson L, Ljunggren E, Wullt B. Bacterial interference--is deliberate colonization with Escherichia coli 83972 an alternative treatment for patients with recurrent urinary tract infection? *Int J Antimicrob Agents* 2006; **28** Suppl 1: S26-S29 [PMID: 16843646 DOI: 10.1016/j.ijantimicag.2006.05.007]
51 **Darouiche RO**, Hull RA. Bacterial interference for prevention of urinary tract infection: an overview. *J Spinal Cord Med* 2000; **23**: 136-141 [PMID: 10914355]

52 **Darouiche RO**, Donovan WH, Del Terzo M, Thornby JI, Rudy DC, Hull RA. Pilot trial of bacterial interference for preventing urinary tract infection. *Urology* 2001; **58**: 339-344 [PMID: 11549475]

53 **Darouiche RO**, Thornby JI, Cerra-Stewart C, Donovan WH, Hull RA. Bacterial interference for prevention of urinary tract infection: a prospective, randomized, placebo-controlled, double-blind pilot trial. *Clin Infect Dis* 2005; **41**: 1531-1534 [PMID: 16231269 DOI: 10.1086/497272]

54 **Darouiche RO**, Green BG, Donovan WH, Chen D, Schwartz M, Merritt J, Mendez M, Hull RA. Multicenter randomized controlled trial of bacterial interference for prevention of urinary tract infection in patients with neurogenic bladder. *Urology* 2011; **78**: 341-346 [PMID: 21683991 DOI: 10.1016/j.urology.2011.03.062]

55 **Billips BK**, Yaggie RE, Cashy JP, Schaeffer AJ, Klumpp DJ. A live-attenuated vaccine for the treatment of urinary tract infection by uropathogenic Escherichia coli. *J Infect Dis* 2009; **200**: 263-272 [PMID: 19522648 DOI: 10.1086/599839]

56 **Moriel DG**, Schembri MA. Vaccination approaches for the prevention of urinary tract infection. *Curr Pharm Biotechnol* 2013; **14**: 967-974 [PMID: 24372245]

57 **Hinman F**. Nonneurogenic neurogenic bladder (the Hinman syndrome)--15 years later. *J Urol* 1986; **136**: 769-777 [PMID: 3761428]

58 **Hinman F**. Urinary tract damage in children who wet. *Pediatrics* 1974; **54**: 143-150 [PMID: 4847848]

59 **Koff SA**, Wagner TT, Jayanthi VR. The relationship among dysfunctional elimination syndromes, primary vesicoureteral reflux and urinary tract infections in children. *J Urol* 1998; **160**: 1019-1022 [PMID: 9719268]

60 **Mingin GC**, Hinds A, Nguyen HT, Baskin LS. Children with a febrile urinary tract infection and a negative radiologic workup: factors predictive of recurrence. *Urology* 2004; **63**: 562-565; discussion 565 [PMID: 15028458 DOI: 10.1016/j.urology.2003.10.055]

61 **Bower WF**, Yip SK, Yeung CK. Dysfunctional elimination symptoms in childhood and adulthood. *J Urol* 2005; **174**: 1623-1627; discussion 1627-1628 [PMID: 16148668]

62 **Tokgöz H**, Tan MO, Sen I, Ilhan MN, Biri H, Bozkirli I. Assessment of urinary symptoms in children with dysfunctional elimination syndrome. *Int Urol Nephrol* 2007; **39**: 425-436 [PMID: 17308873 DOI: 10.1007/s11255-006-9062-0]

63 **Halachmi S**, Farhat WA. Interactions of constipation, dysfunctional elimination syndrome, and vesicoureteral reflux. *Adv Urol* 2008; 828275 [PMID: 18604297 DOI: 10.1155/2008/828275]

64 **Neumann PZ**, DeDomenico IJ, Nogrady MB. Constipation and urinary tract infection. *Pediatrics* 1973; **52**: 241-245 [PMID: 4578713]

65 **Erickson BA**, Austin JC, Cooper CS, Boyt MA. Polyethylene glycol 3350 for constipation in children with dysfunctional elimination. *J Urol* 2003; **170**: 1518-1520 [PMID: 14501649 DOI: 10.1097/01.ju.0000083730.70185.75]

66 **Desantis DJ**, Leonard MP, Preston MA, Barrowman NJ, Guerra LA. Effectiveness of biofeedback for dysfunctional elimination syndrome in pediatrics: a systematic review. *J Pediatr Urol* 2011; **7**: 342-348 [PMID: 21527216 DOI: 10.1016/j.jpurol.2011.02.019]

67 **Feng WC**, Churchill BM. Dysfunctional elimination syndrome in children without obvious spinal cord diseases. *Pediatr Clin North Am* 2001; **48**: 1489-1504 [PMID: 11732126]

68 **Kajbafzadeh AM**, Sharifi-Rad L, Ghahestani SM, Ahmadi H, Kajbafzadeh M, Mahboubi AH. Animated biofeedback: an ideal treatment for children with dysfunctional elimination syndrome. *J Urol* 2011; **186**: 2379-2384 [PMID: 22019033 DOI: 10.1016/j.juro.2011.07.118]

69 **Dwyer ME**, Vandersteen DR, Hollatz P, Reinberg YE. Sacral neuromodulation for the dysfunctional elimination syndrome: a 10-year single-center experience with 105 consecutive children. *Urology* 2014; **84**: 911-917 [PMID: 25096339 DOI: 10.1016/j.urology.2014.03.059]

70 **Matsumoto F**, Tohda A, Shimada K. Effect of ureteral reimplantation on prevention of urinary tract infection and renal growth in infants with primary vesicoureteral reflux. *Int J Urol* 2004; **11**: 1065-1069 [PMID: 15663676 DOI: 10.1111/j.1442-2042.2004.00967.x]

71 **Shimada K**, Matsui T, Arima M, Ikoma F. Clinical analysis of the small kidney associated with VUR. *Int Urol Nephrol* 1988; **20**: 231-238 [PMID: 3403190]

72 **Olbing H**, Hirche H, Koskimies O, Lax H, Seppänen U, Smellie JM, Tamminen-Möbius T, Wikstad I. Renal growth in children with severe vesicoureteral reflux: 10-year prospective study of medical and surgical treatment: the International Reflux Study in Children (European branch). *Radiology* 2000; **216**: 731-737 [PMID: 10966703 DOI: 10.1148/radiology.216.3.r00au35731]

73 **Weiss R**, Duckett J, Spitzer A. Results of a randomized clinical trial of medical versus surgical management of infants and children with grades III and IV primary vesicoureteral reflux (United States). The International Reflux Study in Children. *J Urol* 1992; **148**: 1667-1673 [PMID: 1433585]

74 **Peters CA**, Skoog SJ, Arant BS, Copp HL, Elder JS, Hudson RG, Khoury AE, Lorenzo AJ, Pohl HG, Shapiro E, Snodgrass WT, Diaz M. Summary of the AUA Guideline on Management of Primary Vesicoureteral Reflux in Children. *J Urol* 2010; **184**: 1134-1144 [PMID: 20650499 DOI: 10.1016/j.juro.2010.05.065]

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EDITORIAL

Costimulatory blockade: A novel approach to the treatment of glomerular disease?

Pasquale Esposito, Teresa Rampino, Antonio Dal Canton

Pasquale Esposito, Teresa Rampino, Antonio Dal Canton, Department of Nephrology, Dialysis and Transplantation, Fondazione IRCCS Policlinico San Matteo and University of Pavia, 27100 Pavia, Italy

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Correspondence to: Pasquale Esposito, MD, PhD, Department of Nephrology, Dialysis and Transplantation, Fondazione IRCCS Policlinico San Matteo and University of Pavia, Piazzale Golgi 19, 27100 Pavia, Italy. pasqualeesposito@hotmail.com
Telephone: +39-382-503883
Fax: +39-382-503883

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Abstract

Costimulatory pathways (Cluster of differentiation 28, tumor necrosis factor-related, adhesion and T Cell Ig- and mucin-domain molecules) regulating the interactions between receptors on the T cells and their ligands expressed on several cell types, have a key role in controlling many immunological and non immunological processes. Indeed, accumulating evidence indicate that these molecules are involved in the pathogenesis of numerous conditions, such as allograft rejection, atherosclerosis, rheumatoid arthritis, psoriasis and renal diseases, including glomerulonephritis. Primary or secondary (*i.e.*, associated with infections, drugs or systemic diseases, such as systemic lupus erythematosus, diabetes, *etc*.) glomerulonephritis represent a group of heterogeneous diseases with different pathogenic mechanisms. Since costimulatory molecules, in particular CD80 and CD40, have been found to be expressed on podocytes in the course of different experimental and clinical glomerulonephritis, costimulation has been thought as a new therapeutic target for patients with glomerular diseases. However, although experimental data suggested that the blockade of costimulatory pathways is effective and safe in the prevention and treatment of glomerular diseases, clinical trials reported contrasting results. So, at this moment, there is not a strong evidence for the general use of costimulatory blockade as an alternative treatment strategy in patients with primary or secondary glomerulonephritis. Here, we critically discuss the current data and the main issues regarding the development of this innovative therapeutic approach.

Key words: Costimulation; Glomerulonephritis; Cluster of differentiation 80; Cytotoxic T-lymphocyte-associated antigen-4; Lupus nephritis; Abatacept; Proteinuria; Podocytes

Core tip: Glomerulonephritis refer to a group of renal disorders, primary or secondary to infections, drugs or systemic diseases, characterized by inflammation within the glomerulus. Among glomerular diseases there is a great clinical, histological and prognostic heterogeneity

and several different pathogenetic mechanisms have been implied. Current standard treatments include steroids and cytotoxic agents, which present important side effects and an unsatisfactory remission rate. Therefore, experimental and clinical research is addressed to the development of alternative therapies. Here, we critically discuss new therapeutic opportunities provided by the use of agents acting on the modulation of costimulatory pathways.

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COSTIMULATORY PATHWAYS

Costimulatory pathways regulating the interactions between receptors on the T cells and their ligands expressed on several cell types (including immunocompetent cells, fibroblasts, endothelial cells, *etc.*) play a crucial role in the modulation of immunological and non-immunological processes[1].

In particular, costimulation is essential for the full activation of naïve T cells after antigen-specific recognition, and without costimulation the T cell-antigen interaction results in anergy[2].

Different costimulatory families [Cluster of differentiation 28 (CD28), tumor necrosis factor (TNF)-related, adhesion and T Cell Ig- and mucin-domain (TIMs) molecules], characterized by structural and functional analogies, have been described. These molecules can interact with each other either up- or down-regulating T cell activation[3] (Table 1).

Among the identified costimulatory molecules, the best characterized are the CD28:B7 and the TNF-related families. The CD28:B7 family includes the following receptor-ligand pairs: CD28/CTLA4:CD80/CD86, induced costimulatory molecules (ICOS:ICOSL) and the programmed death-1 pathway (PD-1:PD-L1/PD-L2)[4]. CD28 is a disulfide-bound molecule that belongs to the immunoglobulin superfamily and is constitutively expressed on T cells[5].

Its interaction with CD80 (B7.1) and CD86 (B7.2), expressed on the surface of antigen-presenting cells (APCs), leads to the full activation of T cells[6]. Conversely cytotoxic T-lymphocyte-associated antigen-4 (CTLA4), a structural homologous of CD28 with a higher avidity for CD80 and CD86, acts as a negative regulator of T cells[7].

TNF superfamily comprises: CD40:CD40L, OX40:OX40L, CD30:CD30L, CD27:CD70, CD137:CD137L, *etc*. CD40 is mainly expressed on B-cells, but also on monocytes, dendritic cells, endothelial cells, smooth muscle cells and fibroblasts[8]. The engagement of CD40 with its ligand, CD40L (CD154), leads to B cell expansion and differentiation and it is decisive in the regulation of APCs and dendritic cells functions[9]. It is important to underline that costimulatory molecules, expressed by a broad variety of cells, seem to be involved in the pathogenesis of numerous conditions, such as atherosclerosis, rheumatoid arthritis, psoriasis and renal diseases, including allograft rejection and glomerulonephritis[10-14].

The insights regarding the contribution the costimulatory molecules in these conditions has not only allowed elucidating important regulatory mechanisms, but has also provided novel targets for therapeutic interventions[15].

Table 1 Immunomodulatory effects of costimulation pathways

Family	Ligand	Receptor	Effects on immune cells
CD28	CD80 (B7.1)/	CD28	+
	CD86 (B7.2)	CTLA-4	-
	ICOSL	ICOS	+
	PDL1	PD-1	-
TNF-related	CD40	CD40L (CD154)	+
	OX-40	OX-40L	+
Adhesion molecules	ICAM-1	LFA-1	+
TIM	TIM4/9	TIM1/3	+/-

Costimulatory pathways may influence immune response through stimulatory (+) or inhibitory (-) signals. Ligands may be present on antigen-presenting cells, including B-lymphocytes and dendritic cells, but also on muscle, endothelial, fibroblast, platelets and epithelial-derived cells. Receptors are mainly expressed on T-cells[48]. CTLA-4: Cytotoxic T-lymphocyte-associated antigen-4; ICOS: Induced costimulatory molecule; PD-1: Theprogrammeddeath-1; LFA-1: Lymphocyte function-associated antigen 1; ICAM-1: Intracellular adhesion molecule 1; TIM: T cell Ig and mucin.

COSTIMULATION AND GLOMERULONEPHRITIS

Glomerulonephritis refer to a group of renal disorders, primary or secondary to infections (human immunodeficiency virus, hepatitis C virus, *etc.*), drugs and systemic diseases (for example, systemic lupus erithematosus-SLE, cancer and diabetes), characterized by inflammation within the glomerulus[16].

Among glomerular diseases there is a great clinical, histological and prognostic heterogeneity and several different pathogenic mechanisms are implied, including podocyte damage, immunoglobulin deposition and immune cell infiltration[17]. During the last years growing evidence suggest a role for costimulatory molecules also in this specific setting.

In particular, CD80 expression has been detected in podocytes, which integrity is essential to maintain a regular glomerular function[18].

Indeed, in experimental models of genetic, drug-induced, immune-mediated and bacterial toxin-induced kidney diseases, CD80 overexpression on podocytes might be harmful for glomerular permeability, disturbing the slit diaphragm and down-regulating podocytes-β1

integrin activation, finally leading to the development of proteinuria and loss of renal function[19,20]. The crucial role of CD28:CD80 pathway in the pathogenesis of glomerular diseases is also confirmed by the evidence that CD80 knockout mice present an attenuated form of proliferative glomerulonephritis, associated with a significant reduction of renal tissue lesions[21].

Moreover, the use of monoclonal antibodies targeting CD28 or CTLA-4 was effective in treating and preventing different forms of experimental nephritis, including lupus-like nephritis[22]. Interestingly, similar results were also found in human glomerulonephritis. In particular, a significant increase in CD80 podocyte expression and urinary excretion has been reported in patients with minimal change disease (MCD) in relapse compared to those in remission or with focal segmental glomerulosclerosis (FSGS)[23,24]. Similarly, patients with proliferative lupus nephritis present a strong podocyte surface expression of CD80[20].

Beyond CD28:CD80 pathway, also costimulatory molecules of TNF-related family, *i.e.,* CD40:CD154, have been found expressed in renal tissue in the course of both experimental and human glomerular diseases. CD40 was isolated in murine models of proteinuric disease, such as membranous glomerulonephritis, lupus nephritis and necrotizing nephritis[25]. Moreover, glomerular and tubular CD40 expression was up-regulated in human lupus nephritis and in other inflammatory renal diseases, being associated with the presence of CD40L+ mononuclear cells[26]. Furthermore, the inhibition of CD40 pathway through the administration of a CD40-Ig fusion protein or anti-CD40L antibodies prevented the development of proteinuric kidney diseases in mice[27,28].

COSTIMULATORY BLOCKADE AS A NOVEL TREATMENT FOR GLOMERULONEPHRITIS

As a consequence of the role of costimulation in the pathogenesis of several pathological conditions, costimulatory blockade has been thought as a new rational therapeutic approach[29]. Therefore different strategies, mainly based on the design of specific monoclonal antibodies (mAbs) interfering with these critical pathways, have been tested. However, the clinical development of the majority of these new strategies is currently suspended for safety concerns.

This is, for example, the case of anti-CD40L mAb, which although effective in the prevention of glomerular diseases and renal allograft rejection in murine and primate experimental models, significantly increased the occurrence of thromboembolic events[27,30-32]. More severe complications occurred during the development of anti-CD28 mAbs. Indeed, six healthy volunteers enrolled in a phase I clinical trial and treated with a humanized superagonistic anti-CD28 mAb, developed a life-threatening systemic inflammation due to massive cytokine release, determining the complete abandon of this approach[33]. A more promising strategy- the only one that has found clinical applications so far- seems to be the development of CTLA-4 immunoglobulin fusion proteins. These proteins are composed by an extracellular portion of human CTLA4 plus a Fc part of human IgG1, which, binding CD80 and C86 with high avidity, prevent CD28 ligation, acting as potent inhibitors of CD28:CD80/CD86 pathways[34,35]. Abatacept, which has been approved by FDA for the treatment of rheumatoid arthritis in 2005, and its derivate, Belatacept, belong to this category of drugs.

Belatacept has been extensively studied mainly in the experimental and clinical setting of renal transplantation.

Belatacept was evaluated in 2 open-label, randomized, multicenter, controlled phase 3 studies: the Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial (BENEFIT) and BENEFIT-EXT ("extended criteria").

These studies showed that Belatacept was non-inferior to Cyclosporine in terms of patient and graft survival, being associated to a better graft function and a reduced incidence of chronic nephropathy[36,37]. Hence, although the administration of belatacept was not exempt from adverse effects, in 2011 it was approved by Food and Drug Administration (FDA) as the first costimulatory blocker for use in renal transplantation[38].

Regarding the specific setting of primary and secondary glomerulonephritis, instead, only Abatacept has been used in clinical studies with discordant results.

Two recent randomized trials investigated the safety and efficacy of Abatacept in addition to standard treatments in patients with lupus nephritis.

A twelve months blind multicentre trial, performed by Furie *et al*[39], enrolled 298 patients with active lupus nephritis and proteinuria, randomized to receive corticosteroids and Mycophenolate mofetil in association with Abatacept (30 mg/kg loading for 3 mo, followed by 10 mg/kg), Abatacept (10 mg/kg) or placebo. The authors found that the treatment with Abatacept was associated with a reduction of antiDNA antibody, C3 and C4 levels and proteinuria. However, there were not significant differences in the time to reach a complete response and in the proportion of subjects with confirmed complete response after 52 wk of follow-up among the three groups.

Similar results have been reported by Askanase *et al*[40] who evaluated the efficacy of Abatacept *vs* placebo added to a standard treatment regimen with Cyclophosphamide followed by Azathioprine in 134 patients with active lupus nephritis. They also found no significant differences between the groups in terms of number of patients reaching and/or maintaining complete or partial response.

So, even if previous studies reporting the strong expression of podocyte CD80 in human proliferative lupus nephritis appeared promising, the results of these clinical trials have unexpectedly called into question the

utility of Abatacept in patients with SLE.

Abatacept has been also studied in patients with primary glomerulonephritis.

In a recent paper, Yu *et al*[41] tested Abatacept in 5 patients with FSGS (4 with recurrent FSGS after kidney transplantation and 1 with primary FSGS) who presented positive CD80 (B7.1) immunostaining of podocytes in kidney-biopsy specimens.

After treatment with Abatacept all these patients presented a partial or complete remission, expressed as a significant reduction of serum creatinine and/or proteinuria. Interestingly, the authors provided also a rationale for the beneficial effects of Abatacept, demonstrating that the drug *in vitro* blocks podocyte migration and stabilizes β1-integrin activation in podocytes[41].

Although exciting, these results have been criticized for several important methodological issues[42,43]. First of all, it should be considered that the 4 patients with recurrent FSGS underwent intensive plasmapheresis, aimed to remove putative circulating permeability factors. Thus, it is not possible to recognize if the disease remission was due to this treatment independently of the use of Abatacept. Moreover, subsequent reports arose doubt about the immunostaining techniques used to detect CD80 in renal tissue, highlighting the lack of any negative controls. In particular, Larsen *et al*[44] tested the presence of CD80 in 60 renal biopsy specimens from patients with different proteinuric glomerular diseases with two immunostaining methods (immunoperoxidase and immunofluorescence). The authors found that for both staining techniques and in all cases, CD80 was undetectable within podocytes. The presence of so contrasting results among experimental and clinical trials raises doubt about the potential role of Abatacept in patients with proteinuric glomerulonephritis[45].

To be thorough, it has to point out that the efficacy of Abatacept in the treatment of MCD has been recently reported in a single case[46].

Considering the overall above reported data, we might infer that, although the podocyte CD80 pathway seems to have an important role in some proteinuric glomerular diseases, clinical results suggest that current therapeutic strategies do not warrant a satisfactory control of glomerulonephritis.

CONCLUSION

The critical analysis of the currently available data suggests some conclusions: (1) costimulatory pathways might be implied in the pathogenesis of glomerulonephritis, especially the forms associated with proteinuria and nephrotic syndrome; (2) the development of drugs targeted to block costimulation is of great potential utility, also considering that the current available therapeutic options are limited[47]; (3) clinical trials have shown insufficient or, at least, contrasting effects of this kind of approach in the achievement of therapeutic targets and disease remission.

So, it appears clear that further molecular, cellular and clinical studies, including the design and evaluation of new drugs and exploration of new pathways, should be performed before considering costimulatory blockade as a valid alternative treatment in the general population of patients with glomerulonephritis.

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REFERENCES

1 **Bretscher PA**. A two-step, two-signal model for the primary activation of precursor helper T cells. *Proc Natl Acad Sci USA* 1999; **96**: 185-190 [PMID: 9874793 DOI: 10.1073/pnas.96.1.185]

2 **Schwartz RH**. T cell anergy. *Annu Rev Immunol* 2003; **21**: 305-334 [PMID: 12471050 DOI: 10.1146/annurev.immunol.21.120601.141110]

3 **Magee CN**, Boenisch O, Najafian N. The role of costimulatory molecules in directing the functional differentiation of alloreactive T helper cells. *Am J Transplant* 2012; **12**: 2588-2600 [PMID: 22759274 DOI: 10.1111/j.1600-6143.2012.04180.x]

4 **Greenwald RJ**, Freeman GJ, Sharpe AH. The B7 family revisited. *Annu Rev Immunol* 2005; **23**: 515-548 [PMID: 15771580 DOI: 10.1146/annurev.immunol.23.021704.115611]

5 **Keir ME**, Sharpe AH. The B7/CD28 costimulatory family in autoimmunity. *Immunol Rev* 2005; **204**: 128-143 [PMID: 15790355]

6 **McAdam AJ**, Schweitzer AN, Sharpe AH. The role of B7 co-stimulation in activation and differentiation of CD4+ and CD8+ T cells. *Immunol Rev* 1998; **165**: 231-247 [PMID: 9850864 DOI: 10.1111/j.1600-065X.1998.tb01242.x]

7 **Alegre ML**, Frauwirth KA, Thompson CB. T-cell regulation by CD28 and CTLA-4. *Nat Rev Immunol* 2001; **1**: 220-228 [PMID: 11905831 DOI: 10.1038/35105024]

8 **Mach F**, Schönbeck U, Sukhova GK, Bourcier T, Bonnefoy JY, Pober JS, Libby P. Functional CD40 ligand is expressed on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for CD40-CD40 ligand signaling in atherosclerosis. *Proc Natl Acad Sci USA* 1997; **94**: 1931-1936 [PMID: 9050882 DOI: 10.1073/pnas.94.5.1931]

9 **van Kooten C**, Banchereau J. CD40-CD40 ligand. *J Leukoc Biol* 2000; **67**: 2-17 [PMID: 10647992]

10 **Andersson J**, Libby P, Hansson GK. Adaptive immunity and atherosclerosis. *Clin Immunol* 2010; **134**: 33-46 [PMID: 19635683 DOI: 10.1016/j.clim.2009.07.002]

11 **Esposito P**, Dal Canton A. CD40/CD40L and cardiovascular risk in patients on haemodialysis: a role for soluble CD40? *Nephrol Dial Transplant* 2011; **26**: 2414-2415; author reply 2414-2415 [PMID: 21565946 DOI: 10.1093/ndt/gfr227]

12 **Liu MF**, Kohsaka H, Sakurai H, Azuma M, Okumura K, Saito I, Miyasaka N. The presence of costimulatory molecules CD86 and CD28 in rheumatoid arthritis synovium. *Arthritis Rheum* 1996; **39**: 110-114 [PMID: 8546719 DOI: 10.1002/art.1780390115]

13 **Paukkonen K**, Naukkarinen A, Horsmanheimo M. The development of manifest psoriatic lesions is linked with the invasion of CD8 + T cells and CD11c + macrophages into the epidermis. *Arch Dermatol Res* 1992; **284**: 375-379 [PMID: 1363190 DOI: 10.1007/BF00372065]

14 **Esposito P**, Grosjean F, Rampino T, Libetta C, Gregorini M, Fasoli G, Marchi G, Sileno G, Montagna F, Dal Canton A. Costimulatory pathways in kidney transplantation: pathogenetic role, clinical significance and new therapeutic opportunities. *Int Rev Immunol* 2014; **33**: 212-233 [PMID: 24127878 DOI: 10.3109/08830185.2013.829470]

15 **Linsley PS**, Nadler SG. The clinical utility of inhibiting CD28-mediated costimulation. *Immunol Rev* 2009; **229**: 307-321 [PMID:

19426230 DOI: 10.1111/j.1600-065X.2009.00780.x]
16 **Hebert LA**, Parikh S, Prosek J, Nadasdy T, Rovin BH. Differential diagnosis of glomerular disease: a systematic and inclusive approach. *Am J Nephrol* 2013; **38**: 253-266 [PMID: 24052039 DOI: 10.1159/000354390]
17 **Couser WG**. Pathogenesis of glomerular damage in glomerulonephritis. *Nephrol Dial Transplant* 1998; **13** Suppl 1: 10-15 [PMID: 9507491 DOI: 10.1093/ndt/13.suppl_1.10]
18 **Schwartz MM**. The role of podocyte injury in the pathogenesis of focal segmental glomerulosclerosis. *Ren Fail* 2000; **22**: 663-684 [PMID: 11104157 DOI: 10.1081/JDI-100101955]
19 **Clement LC**, Liu G, Perez-Torres I, Kanwar YS, Avila-Casado C, Chugh SS. Early changes in gene expression that influence the course of primary glomerular disease. *Kidney Int* 2007; **72**: 337-347 [PMID: 17457373 DOI: 10.1038/sj.ki.5002302]
20 **Reiser J**, von Gersdorff G, Loos M, Oh J, Asanuma K, Giardino L, Rastaldi MP, Calvaresi N, Watanabe H, Schwarz K, Faul C, Kretzler M, Davidson A, Sugimoto H, Kalluri R, Sharpe AH, Kreidberg JA, Mundel P. Induction of B7-1 in podocytes is associated with nephrotic syndrome. *J Clin Invest* 2004; **113**: 1390-1397 [PMID: 15146236 DOI: 10.1172/JCI200420402]
21 **Odobasic D**, Kitching AR, Tipping PG, Holdsworth SR. CD80 and CD86 costimulatory molecules regulate crescentic glomerulonephritis by different mechanisms. *Kidney Int* 2005; **68**: 584-594 [PMID: 16014035 DOI: 10.1111/j.1523-1755.2005.00436.x]
22 **Tada Y**, Nagasawa K, Ho A, Morito F, Koarada S, Ushiyama O, Suzuki N, Ohta A, Mak TW. Role of the costimulatory molecule CD28 in the development of lupus in MRL/lpr mice. *J Immunol* 1999; **163**: 3153-3159 [PMID: 10477582]
23 **Garin EH**, Mu W, Arthur JM, Rivard CJ, Araya CE, Shimada M, Johnson RJ. Urinary CD80 is elevated in minimal change disease but not in focal segmental glomerulosclerosis. *Kidney Int* 2010; **78**: 296-302 [PMID: 20485332 DOI: 10.1038/ki.2010.143]
24 **Ishimoto T**, Shimada M, Araya CE, Huskey J, Garin EH, Johnson RJ. Minimal change disease: a CD80 podocytopathy? *Semin Nephrol* 2011; **31**: 320-325 [PMID: 21839364 DOI: 10.1016/j.semnephrol.2011.06.002]
25 **Reynolds J**, Khan SB, Allen AR, Benjamin CD, Pusey CD. Blockade of the CD154-CD40 costimulatory pathway prevents the development of experimental autoimmune glomerulonephritis. *Kidney Int* 2004; **66**: 1444-1452 [PMID: 15458437 DOI: 10.1111/j.1523-1755.2004.00907.x]
26 **Yellin MJ**, D'Agati V, Parkinson G, Han AS, Szema A, Baum D, Estes D, Szabolcs M, Chess L. Immunohistologic analysis of renal CD40 and CD40L expression in lupus nephritis and other glomerulonephritides. *Arthritis Rheum* 1997; **40**: 124-134 [PMID: 9008608 DOI: 10.1002/art.1780400117]
27 **Kairaitis L**, Wang Y, Zheng L, Tay YC, Wang Y, Harris DC. Blockade of CD40-CD40 ligand protects against renal injury in chronic proteinuric renal disease. *Kidney Int* 2003; **64**: 1265-1272 [PMID: 12969144 DOI: 10.1046/j.1523-1755.2003.00223.x]
28 **Biancone L**, Andres G, Ahn H, DeMartino C, Stamenkovic I. Inhibition of the CD40-CD40ligand pathway prevents murine membranous glomerulonephritis. *Kidney Int* 1995; **48**: 458-468 [PMID: 7564113 DOI: 10.1038/ki.1995.314]
29 **Ford ML**, Larsen CP. Translating costimulation blockade to the clinic: lessons learned from three pathways. *Immunol Rev* 2009; **229**: 294-306 [PMID: 19426229 DOI: 10.1111/j.1600-065X.2009.00776.x]
30 **Kalled SL**, Cutler AH, Datta SK, Thomas DW. Anti-CD40 ligand antibody treatment of SNF1 mice with established nephritis: preservation of kidney function. *J Immunol* 1998; **160**: 2158-2165 [PMID: 9498753]
31 **Pearson TC**, Trambley J, Odom K, Anderson DC, Cowan S, Bray R, Lin A, Hollenbaugh D, Aruffo A, Siadak AW, Strobert E, Hennigar R, Larsen CP. Anti-CD40 therapy extends renal allograft survival in rhesus macaques. *Transplantation* 2002; **74**: 933-940 [PMID: 12394833 DOI: 10.1097/00007890-200210150-00006]
32 **Kawai T**, Andrews D, Colvin RB, Sachs DH, Cosimi AB. Thromboembolic complications after treatment with monoclonal antibody against CD40 ligand. *Nat Med* 2000; **6**: 114 [PMID: 10655072 DOI: 10.1038/72162]
33 **Suntharalingam G**, Perry MR, Ward S, Brett SJ, Castello-Cortes A, Brunner MD, Panoskaltsis N. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med* 2006; **355**: 1018-1028 [PMID: 16908486 DOI: 10.1056/NEJMoa063842]
34 **Salomon B**, Bluestone JA. Complexities of CD28/B7: CTLA-4 costimulatory pathways in autoimmunity and transplantation. *Annu Rev Immunol* 2001; **19**: 225-252 [PMID: 11244036 DOI: 10.1146/annurev.immunol.19.1.225]
35 **Sayegh MH**, Akalin E, Hancock WW, Russell ME, Carpenter CB, Linsley PS, Turka LA. CD28-B7 blockade after alloantigenic challenge in vivo inhibits Th1 cytokines but spares Th2. *J Exp Med* 1995; **181**: 1869-1874 [PMID: 7536798 DOI: 10.1084/jem.181.5.1869]
36 **Vincenti F**, Charpentier B, Vanrenterghem Y, Rostaing L, Bresnahan B, Darji P, Massari P, Mondragon-Ramirez GA, Agarwal M, Di Russo G, Lin CS, Garg P, Larsen CP. A phase III study of belatacept-based immunosuppression regimens versus cyclosporine in renal transplant recipients (BENEFIT study). *Am J Transplant* 2010; **10**: 535-546 [PMID: 20415897 DOI: 10.1111/j.1600-6143.2009.03005.x]
37 **Pestana JO**, Grinyo JM, Vanrenterghem Y, Becker T, Campistol JM, Florman S, Garcia VD, Kamar N, Lang P, Manfro RC, Massari P, Rial MD, Schnitzler MA, Vitko S, Duan T, Block A, Harler MB, Durrbach A. Three-year outcomes from BENEFIT-EXT: a phase III study of belatacept versus cyclosporine in recipients of extended criteria donor kidneys. *Am J Transplant* 2012; **12**: 630-639 [PMID: 22300431 DOI: 10.1111/j.1600-6143.2011.03914.x]
38 Risk evaluation and mitigation strategy (REMS). Initial REMS Approval: June 2011. Available from: URL: http://www.fda.gov/downloads/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/UCM261934.pdf
39 **Furie R**, Nicholls K, Cheng TT, Houssiau F, Burgos-Vargas R, Chen SL, Hillson JL, Meadows-Shropshire S, Kinaszczuk M, Merrill JT. Efficacy and safety of abatacept in lupus nephritis: a twelve-month, randomized, double-blind study. *Arthritis Rheumatol* 2014; **66**: 379-389 [PMID: 24504810 DOI: 10.1002/art.38260]
40 **Askanase AD**, Byron M, Keyes-Elstein LL, Cagnoli PC, McCune W, Chatham W, Contreras G, Daikh DI, Dall' Era M, Wofsy D, Davidson A, Diamond B, Mackay M, Ding L, Gao W, Dooley MA, Fragoso-Loyo H, Sanchez-Guerrero J, Karp DR, Olsen NJ, Jolly M, Kalunian K, Kamen D, Lee I, Levesque MC, Lim S, Ramos-Remus C, Rovin BH, Sayre PH, Smilek DE, Tosta P, Utset TO, Venuturupalli S, Winchester R, Aranow C. Treatment of lupus nephritis with abatacept: the Abatacept and Cyclophosphamide Combination Efficacy and Safety Study. *Arthritis Rheumatol* 2014; **66**: 3096-3104 [PMID: 25403681 DOI: 10.1002/art.38790]
41 **Yu CC**, Fornoni A, Weins A, Hakroush S, Maiguel D, Sageshima J, Chen L, Ciancio G, Faridi MH, Behr D, Campbell KN, Chang JM, Chen HC, Oh J, Faul C, Arnaout MA, Fiorina P, Gupta V, Greka A, Burke GW, Mundel P. Abatacept in B7-1-positive proteinuric kidney disease. *N Engl J Med* 2013; **369**: 2416-2423 [PMID: 24206430 DOI: 10.1056/NEJMoa1304572]
42 **Benigni A**, Gagliardini E, Remuzzi G. Abatacept in B7-1-positive proteinuric kidney disease. *N Engl J Med* 2014; **370**: 1261-1263 [PMID: 24670179 DOI: 10.1056/NEJMc1400502]
43 **Alachkar N**, Carter-Monroe N, Reiser J. Abatacept in B7-1-positive proteinuric kidney disease. *N Engl J Med* 2014; **370**: 1263-1264 [PMID: 24670180 DOI: 10.1056/NEJMc1400502]
44 **Larsen CP**, Messias NC, Walker PD. B7-1 immunostaining in proteinuric kidney disease. *Am J Kidney Dis* 2014; **64**: 1001-1003 [PMID: 25278092 DOI: 10.1053/j.ajkd.2014.07.023]
45 **Reiser J**, Alachkar N. Proteinuria: abate or applaud abatacept in proteinuric kidney disease? *Nat Rev Nephrol* 2014; **10**: 128-130 [PMID: 24375054 DOI: 10.1038/nrneph.2013.276]
46 **Garin EH**, Reiser J, Cara-Fuentes G, Wei C, Matar D, Wang H, Alachkar N, Johnson RJ. Case series: CTLA4-IgG1 therapy in minimal change disease and focal segmental glomerulosclerosis. *Pediatr Nephrol* 2015; **30**: 469-477 [PMID: 25239302 DOI: 10.1007/s00467-014-2957-6]
47 **Javaid B**, Quigg RJ. Treatment of glomerulonephritis: will we

ever have options other than steroids and cytotoxics? *Kidney Int* 2005; **67**: 1692-1703 [PMID: 15840015 DOI: 10.1111/j.1523-1755.2005.00266.x]

48 **McGrath MM**, Najafian N. The Role of Coinhibitory Signaling Pathways in Transplantation and Tolerance. *Frontiers in Immunology* 2012; **3**: 47 [DOI: 10.3389/fimmu.2012.00047]

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EDITORIAL

Clinical neurological examination *vs* electrophysiological studies: Reflections from experiences in occupational medicine

Jørgen Riis Jepsen

Jørgen Riis Jepsen, Department of Occupational Medicine, Hospital of South-western Jutland, DK-6710 Esbjerg, Denmark

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Correspondence to: Jørgen Riis Jepsen, MD, Associate professor, Consultant, Department of Occupational Medicine, Hospital of South-western Jutland, Østergade 81-83, DK-6710 Esbjerg, Denmark. joergen.riis.jepsen@svs.regionsyddanmark.dk
Telephone: +45-23-285702
Fax: +45-79-182294

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Abstract

Seventy-five percent of upper limb disorders that are related to work are regarded as diagnostically unclassifiable and therefore challenging to the clinician. Therefore it has been generally less successfully to prevent and treat these common and frequently disabling disorders. To reach a diagnosis requires the identification of the responsible pathology and the involved tissues and structures. Consequently, improved diagnostic approaches are needed. This editorial discusses the potentials of using the clinical neurologic examination in patients with upper limb complaints related to work. It is argued that a simple but systematic physical approach permits the examiner to frequently identify patterns of neurological findings that suggest nerve afflictions and their locations, and that electrophysiological studies are less likely to identify pathology. A diagnostic algorithm for the physical assessment is provided to assist the clinician. Failure to include representative neurological items in the physical examination may result in patients being misinterpreted, misdiagnosed and mistreated.

Key words: Neurological examination; Electrophysiology; Work-related disorders; Occupational medicine; Nerve afflictions; Upper limb disorders

Core tip: Patients with work-related upper limb disorders should be subjected to a systematic upper limb examination including neurological items with the main focus on muscle strength testing. A refined version of the classical neurological upper limb examination can be rewarding because it permits the clinician to frequently identify patterns in accordance with nerve afflictions with specific locations. This examination is suitable in any clinical setting because it is simple, inexpensive, noninvasive, and highly reproducible.

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An estimated three quarters of upper limb disorders that are related to work are regarded as diagnostically unclassifiable[1] and consequently these conditions remain a diagnostic challenge to the physician. Therefore evidence-based prevention and treatment have been largely unsuccessful. To reach a diagnosis requires the identification of the tissues and specific structures that are involved, and also of the pathology that causes the condition. To achieve this goal, improved diagnostic approaches to these frequent and disabling conditions should be applied.

Frequently, patients with work-related upper limb disorders have pain with characteristics that suggest that the pain is neuropathic. The pain may be accompanied by sensory abnormalities such as paraesthesia, subjective weakness or heaviness and/or tactile dysfunction. The combination of a neuropathic pain with motor and sensory symptoms suggests that the nervous system is involved and therefore that the upper limb peripheral nerves should be included in the physical examination. Still, clinicians and also researchers tend to ignore this possibility and to rather attribute these conditions to pathology located in muscles, tendons or insertions. Even if involvement of peripheral nerve(s) is considered, the attention tends to be directed - and limited - to carpal tunnel syndrome and afflictions of a cervical root. The intermediate portion of nerve of a length approaching one meter, and other potential locations of focal neuropathies receive less attention.

Focal neuropathies cause rather specific neurological patterns: If muscles are innervated distally to a nerve-lesion they are likely to be weak; the sensibility in supplied cutaneous territories will be altered; there will be abnormal soreness where the nerve trunk is affected. The classical neurological examination is based on these principles and all physicians have been trained in its execution. Still, there seems to be reluctance to perform a detailed neurological examination in upper limb patients with work-related complaints. This is particularly the case with respect to the portions of the upper limb nerves that are located proximally such as in the forearm, arm and shoulder. To the experience of the author this inadequacy applies to physicians in any specialty including neurology. Performing a detailed clinical neurological examination and to interpret its outcome may be regarded as difficult, and this examination may also be assumed to be time consuming. It is true that relevant benefit of the examination requires knowledge of anatomy such as the location of nerves and their innervation patterns. This has been previously learned but might be forgotten. To achieve a neurological diagnosis such as the identification of a focal nerve affliction of the upper limb, the neurological examination may also be perceived as less rewarding than paraclinical studies. Although this view is not justified physical neurological upper limb examinations are rarely more than basic. Rather than performing a detailed examination it may be easier for the clinician to refer the patient to electrophysiological examination (and frequently to MR-imaging - in particular of the cervical spine) and so to leave the diagnostic work to others.

This choice may be justified if electrophysiological studies of the upper limb nerves reflect the truth better than the traditional neurological examination. Clinicians - including many neurologists - tend to view electrophysiological (and imaging) studies as superior to their own physical examination and judgement. Consequently, the outcome of a physical examination based on patterns of neurological abnormalities suggesting focal peripheral neuropathy is likely to be regarded as less valid if electrophysiological studies do not identify abnormalities. However, according to the scientific literature, the superiority of electrophysiology has not been demonstrated and certainly not for disorders of the upper limb studied in an occupational context. While electrophysiological studies tend to be generally viewed as "golden standard" for peripheral neuropathy their sensitivity - in particular with minor nerve afflictions such as may be the case with work-related upper limb complaints - is limited because nerve lesions may be mixed and partial with few myelinated fibres intact and reinnervation taking place. Therefore the electrophysiological findings may be entirely normal[2]. The potentials of a refined electrophysiological assessment are acknowledged but the application of expanded techniques is also very time consuming and consequently expensive, and therefore rarely applied. There is also agreement that a detailed neurological physical examination should precede an electrophysiological study of the peripheral nerves and guide its content. Evidently, it has no sense to study electrophysiologically the median nerve in the carpal tunnel when a nerve affliction is located elsewhere. Some common locations of upper limb nerve entrapment can only rarely be identified by electrophysiological studies, *e.g.*, radial tunnel syndrome[3], pronator syndrome[4] and brachial plexopathy[5].

It is essential that the physical examination focuses on all nerves of relevance and on the entire length of nerves from the roots to their muscular and cutaneous supply. That means that the examiner should include neurological items that are representative to nerve afflictions with any location that one would expect. The physical neurological examination should be reliable and also valid, meaning that it should be capable to identify abnormalities in symptomatic limbs and exclude abnormalities in healthy limbs, respectively.

A semiquantitative detailed upper limb neurological examination, which has been developed for this

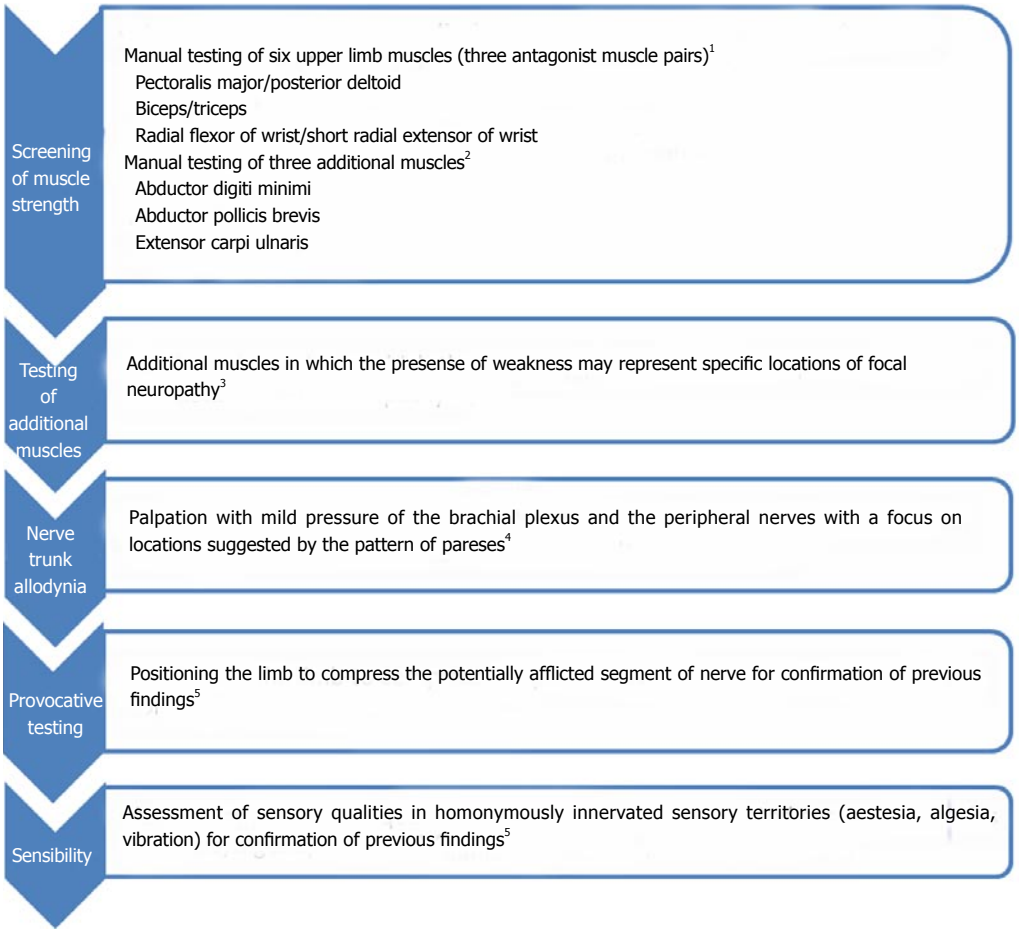

Figure 1 An algorithm for an upper limb neurological examination in patients with work-related upper-limb complaints. [1]Manual testing of six upper limb muscles is a sensitive measure, which can suggest the presence of the majority of upper limb nerve afflictions but confirmation of focal neuropathy requires the demonstration of mechanical nerve trunk allodynia at the location(s) that are suggested by the pattern of pareses[11]. The combination of manual muscle testing and nerve trunk palpation is able to increase the specificity of the neurological examination; [2]Screening of strength in the six muscles applied for the initial screening cannot identify focal neuropathy at three common locations: Ulnar neuropathy will result in weakness in the abductor digiti minimi, carpal tunnel syndrome will be associated with paretic abductor pollicis brevis muscle, and radial tunnel syndrome causes weakness in the extensor carpi ulnaris muscle. Therefore manual testing of these three muscles is also recommended. If all nine muscles are of normal strength, a peripheral nerve affliction is unlikely. If weaknesses are found, additional muscles should be tested[12]; [3]The muscles selected to be recommended for screening are those that according to the author are the more rewarding in the diagnosis of upper limb nerve afflictions. Depending on the clinical situation nerve afflictions of less frequency may be looked for by examining additional muscles[7]; [4]The identification of mechanical allodynia at location(s) suggested by the pattern of pareses will improve the specificity of the examination and contribute to its validity[11]; [5]Provocative testing and assessment of sensory qualities serve to reassure the examiner of the neurological findings. Examples of the former are the well-known Phalen sign with carpal tunnel syndrome and the hyperabduction test with an infraclavicular brachial plexus affliction. Passive compression of the posterior interosseous nerve by passive forearm hyperpronation will provoke the pain associated with a radial tunnel syndrome. While upper limb tension tests have been developed to indicate bias towards the median, radial, and ulnar nerves, respectively[13], they cannot, however, determine the level(s) of afflicted location(s) along these nerves. The same is the case for sensory assessment. Therefore manual muscle testing is the key to the neurological upper limb assessment.

purpose consists of manual strength-testing in selected individual and representative muscles[6]. It also contains of an assessment of sensory deviations from normal in representative territories, which are homonymously innervated. Finally, the presence of allodynia of nerve trunks with mild palpation should be studied at the location(s) where nerves may be affected[7]. This low-tech examination is rapid to perform and requires no equipment beside a needle and a 256 Hz tuning fork.

When this examination was carried out on patients that were referred for assessment in a hospital department of occupational medicine there was a high occurrence of patterns of neurological findings. These patterns were in accordance with the topography of the nerves and their muscular and sensory innervation – and they were also frequently demonstrated in upper limb patients that could not be diagnosed by conventional means. The construct validity of this approach in terms of interrelations of nerve afflictions with various location has previously been demonstrated[8]. There was a high inter-rater reliability of the identification of neurological patterns[7]. The validity of this approach was further indicated by demonstrating that the presence of neurological patterns was related to the presence of symptoms[9]. The most frequent location of nerve afflictions in the upper limb was at the infraclavicular brachial plexus (behind the minor pectoral muscle below the clavicle). This location of nerve affliction was often

found in combination with median neuropathy (just proximally to and medially to the elbow joint) and radial (posterior interosseous) neuropathy (at the Arcade of Frohse) at elbow level. Whether diagnosed by criteria that included a thorough neurological examination or by conventional diagnostic criteria, neuropathic upper limb conditions could also be identified as the most frequent among patients in general practice[10].

It is frequently frustrating to experience when a patient with peripheral upper limb nerve affliction(s) with certain location(s) diagnosed by this examination is subsequently examined by a neurologist. After a mostly limited neurological examination and an electrophysiological study targeting a few selected parameters, the neurologist is likely to conclude the absence of a neurological condition and to interpret the patient's complaints and findings (*e.g.*, "pain induced weakness" or "sensory deviation with a non-dermatomal extension") as either due to a disorder located to muscles or to be functional and without any somatic origin. These patients are likely to be misinterpreted, misdiagnosed and mistreated. Furthermore, the legal authorities seem to trust the basic examination by the neurologist rather than to appreciate a detailed neurological examination.

I regard the assessment of individual muscle strength as the most important part of the clinical neurological tests. I would therefore suggest clinicians who see upper limb patients to routinely integrate in their physical examination a screening approach consisting of manual muscle testing of six to nine representative upper limb muscles (Figure 1). This approach is sensitive and permits the identification of patients that should be physically examined further to determine the location of an upper limb nerve affliction[11]. Any physician can easily learn to manually assess the strength in individual muscles[6,11]. This part of the examination is therefore feasible in any clinical setting whether it be in industrialized countries or in the developing world.

A correct diagnosis is essential for targeted preventive intervention at workplaces as well as for treatment, which may follow the concepts of neuromobilisation[13-15]. There is increasing evidence of an effect of nerve mobilisation in the treatment of upper limb nerve afflictions[14]. Neurolytic surgery for upper limb nerve afflictions has been undertaken for years but its success depends of a precise location of the affliction.

Including a systematic neurologic examination in the diagnostic physical approach to patients with work-related upper limb disorders may eventually constitute a step towards improved prevention but this remains to be demonstrated. The first step would be to demonstrate risk factors in work as has been done for certain neuropathic upper limb conditions such as, *e.g.*, radial tunnel syndrome[16] and brachial plexopathy[17]. Next would be to see whether the removal or reduction of these risk factors would reduce the occurrence.

I would caution against blind faith in diagnostic tools such as electrophysiological studies. As demonstrated by others, it is not automatically the best or safest choice to trust device outputs that have a potential for flawed measurement. Trained judgement should be applied when interpreting results generated from devises[18] such as results from electrophysiological studies. The clinician should know their potentials and limitations, and be able to assess whether they are better or inferior than the clinical examination.

REFERENCES

1 **Palmer K**, Cooper C. Repeated movement and repeated trauma affecting the musculoskeletal disorders of the upper limbs. In: Baxter P, Adams P, Aw T, Cockcroft A, Harrington J, editors. Hunter's Diseases of Occupations. 9th ed. London: Arnold, 2000: 453-475

2 **Krarup C**. Pitfalls in electrodiagnosis. *J Neurol* 1999; **246**: 1115-1126 [PMID: 10653302 DOI: 10.1007/s004150050529]

3 **Werner CO**. Lateral elbow pain and posterior interosseous nerve entrapment. *Acta Orthop Scand Suppl* 1979; **174**: 1-62 [PMID: 286496 DOI: 10.3109/ort.1979.50.suppl-174.01]

4 **Werner CO**, Rosén I, Thorngren KG. Clinical and neurophysiologic characteristics of the pronator syndrome. *Clin Orthop Relat Res* 1985; **(197)**: 231-236 [PMID: 4017339 DOI: 10.1097/00003086-198507000-00029]

5 **Aminoff MJ**, Olney RK, Parry GJ, Raskin NH. Relative utility of different electrophysiologic techniques in the evaluation of brachial plexopathies. *Neurology* 1988; **38**: 546-550 [PMID: 2832784 DOI: 10.1212/WNL.38.4.546]

6 **Jepsen J**, Laursen L, Larsen A, Hagert CG. Manual strength testing in 14 upper limb muscles: a study of inter-rater reliability. *Acta Orthop Scand* 2004; **75**: 442-448 [PMID: 15370589 DOI: 10.1080/00016470410001222-1]

7 **Jepsen JR**, Laursen LH, Hagert CG, Kreiner S, Larsen AI. Diagnostic accuracy of the neurological upper limb examination I: inter-rater reproducibility of selected findings and patterns. *BMC Neurol* 2006; **6**: 8 [PMID: 16483371 DOI: 10.1186/1471-2377-6-8]

8 **Jepsen JR**, Laursen LH, Kreiner S, Larsen AI. Neurological examination of the upper limb: a study of construct validity. *Open Neurol J* 2009; **3**: 54-63 [PMID: 20148172 DOI: 10.2174/1874205X00903010054]

9 **Jepsen JR**, Laursen LH, Hagert CG, Kreiner S, Larsen AI. Diagnostic accuracy of the neurological upper limb examination II: relation to symptoms of patterns of findings. *BMC Neurol* 2006; **6**: 10 [PMID: 16504144 DOI: 10.1186/1471-2377-6-10]

10 **Laursen LH**, Sjøgaard G, Hagert CG, Jepsen JR. Diagnostic distribution of non-traumatic upper limb disorders: vibrotactile sense in the evaluation of structured examination for optimal diagnostic criteria. *Med Lav* 2007; **98**: 127-144 [PMID: 17375606]

11 **Jepsen JR**. Can testing of six individual muscles represent a screening approach to upper limb neuropathic conditions? *BMC Neurol* 2014; **14**: 90 [PMID: 24767511 DOI: 10.1186/1471-2377-14-90]

12 **Jepsen JR**, Hagert CG. Muscle testing in the diagnosis of work-related upper limb complaints. *Europ Neurol J* 2010; **2**: 1-9

13 **Schmid AB**, Nee RJ, Coppieters MW. Reappraising entrapment neuropathies--mechanisms, diagnosis and management. *Man Ther* 2013; **18**: 449-457 [PMID: 24008054 DOI: 10.1016/j.math.2013.07.006]

14 **Butler DS**. Mobilisation of the nervous system. Melbourne: Churchill Livingstone, 1992

15 **Butler DS**. The sensitive nervous system. Adelaide: Noigroup Publications, 2000

16 **Roquelaure Y**, Raimbeau G, Dano C, Martin YH, Pelier-Cady MC, Mechali S, Benetti F, Mariel J, Fanello S, Penneau-Fontbonne D. Occupational risk factors for radial tunnel syndrome in industrial workers. *Scand J Work Environ Health* 2000; **26**: 507-513 [PMID: 11201398 DOI: 10.5271/sjweh.575]

17 **Jepsen JR**. Brachial plexopathy: a case-control study of the relation to physical exposures at work. *J Occup Med Toxicol* 2015; **10**: 14 [PMID: 25897317]

18 **Trohman RG**. Trust in technology: straddling the line between faith and reason. *Crit Care Med* 2010; **38**: 712-713 [PMID: 20083940 DOI: 10.1097/CCM.0b013e3181c0dd5f]

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EDITORIAL

Cross-reactivity between aeroallergens and food allergens

Florin-Dan Popescu

Florin-Dan Popescu, Department of Allergology, "Carol Davila" University of Medicine and Pharmacy, "Nicolae Malaxa" Clinical Hospital, 022441 Bucharest, Romania

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Correspondence to: Florin-Dan Popescu, MD, PhD, Associate Professor, Department of Allergology, "Carol Davila" University of Medicine and Pharmacy, "Nicolae Malaxa" Clinical Hospital, Sector 2, 022441 Bucharest, Romania. florindanpopescu@ymail.com
Telephone: +40-21-2555405
Fax: +40-21-2555275

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Abstract

In patients with respiratory allergy, cross-reactivity between aeroallergens and foods may induce food allergy, symptoms ranging from oral allergy syndrome to severe anaphylaxis. Clinical entities due to IgE sensitization to cross-reactive aeroallergen and food allergen components are described for many sources of plant origin (pollen-food syndromes and associations, such as birch-apple, cypress-peach and celery-mugwort-spice syndromes, and mugwort-peach, mugwort-chamomile, mugwort-mustard, ragweed-melon-banana, goosefoot-melon associations), fungal origin (*Alternaria*-spinach syndrome), and invertebrate, mammalian or avian origin (mite-shrimp, cat-pork, and bird-egg syndromes). Clinical cases of allergic reactions to ingestion of food products containing pollen grains of specific plants, in patients with respiratory allergy to *Asteraceae* pollen, especially mugwort and ragweed, are also mentioned, for honey, royal jelly and bee polen dietary supplements, along with allergic reactions to foods contaminated with mites or fungi in patients with respiratory allergy to these aeroallergens. Medical history and diagnosis approach may be guided by the knowledge about the diverse cross-reacting allergens involved, and by the understanding of these clinical entities which may vary significantly or may be overlapping. The association between primary IgE sensitization with respiratory symptoms to inhaled allergens and food allergy due to cross-reactive allergen components is important to assess in allergy practice. The use of molecular-based diagnosis improves the understanding of clinically relevant IgE sensitization to cross-reactive allergen components from aeroallergen sources and foods.

Key words: Cross-reactivity syndromes and associations; Aeroallergens; Food allergens

Core tip: Many different syndromes and associations due to cross-reactivity between aeroallergens and food allergens of plant, fungal and animal origin have been described. Significant examples are pollen-food syndromes or associations, along with mite-shrimp, cat-pork, and bird-egg syndromes, but rare or more complex clinical entities must also be discussed. It is important to underline the impact of relevant cross-reactivities between aeroallergens and food allergens and of molecular-based allergy diagnosis in clinical practice.

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INTRODUCTION

Respiratory allergies affect 10%-30% of adults and children worldwide[1,2], while food allergy is estimated to affect more than 1%-2% and less than 10% of the population[3]. Allergic rhinitis, asthma and food allergies have significant detrimental effects on health-related quality of life, family economics, social interactions, school and work attendance[4-7]. There is a high co-occurrence of food allergy with other atopic diseases, including allergic rhinitis and asthma. Evidence of respiratory allergy may indicate an increased risk of IgE-mediated food allergy. Moreover, underlying asthma, regardless of severity, has been associated with increased risk of severe reactions and even death caused by food allergy[3].

This editorial underlines the importance of the IgE sensitization *via* the respiratory route to aeroallergens and food allergy due to cross-reactivities between some allergen components. This phenomenom should be distinguished from the common food allergy without sensitization to cross-reactive aeroallergens, in which heat- and enzyme-resistant class 1 food allergens induce allergic sensitization *via* the digestive tract, typically being responsible for systemic allergic reactions. Class 2 food allergens are more heat-labile and susceptible to digestion and therefore do not cause gastrointestinal sensitization, but instead provoke allergic reactions in already sensitized patients to cross-reactive aeroallergens through the respiratory route. Typically, pollen-food syndromes are produced by class 2 food allergens. In contrast to class 1 food allergy which mainly affects young children, class 2 food allergy is observed especially in adults as a consequence of sensitization to cross-reactive aeroallergens[8-10]. This traditional classification has a more modern changed approach from a molecular allergy point of view. Important allergen components families involved in cross-reactivity between aeroallergens and food allergens are presented in Table 1. The clinical expression for IgE sensitization to PR-10 proteins and profilins is mainly oral allergy syndrome.

Cross-reactivity is an immune-mediated phenomenon of an IgE antibody recognizing, binding, and inducing an immune response to similar allergenic molecules (homologues). IgE cross-reactivity often occurs between allergenic molecules in closely related species or well preserved molecules with similar function present in widely different species, belonging to the same protein family[3,11,12].

The clinical relevance of cross-reactivity seems to be influenced by a number of factors including the host immune response against the allergen, exposure and the allergen itself[13]. Cross-reactivity is important for various reasons, such as its immunologic basis, particularly in relation to the regulation of allergic sensitization, the risk of allergic cross-reactivity to novel foods and the identification of the patterns of cross-reactivity, because they often, but not always, may reflect the pattern of clinical sensitivities. Cross-reactivities involve clustering cross-reactive allergens or family-restricted homologous molecules, panallergens and cross-reactive carbohydrate determinants (CCDs)[14]. Panallergens are cross-reactive allergens, belonging to protein families well preserved throughout many widely different species, able to trigger IgE antibody binding[12]. Panallergens are ubiquitous proteins responsible for IgE cross-reactivity to a wide variety of related and unrelated allergenic sources. IgE cross-reactivity is usually approached from an allergen-perspective, meaning that cross-reactivity is a consequence of structural similarity between homologous proteins, which is translated into conserved sequence regions, three-dimensional folding and function[15]. CCDs are carbohydrate moieties of glycoproteins that induce the production of highly cross-reactive IgE. Many plant and invertebrate allergens are glycoproteins containing carbohydrate moieties called N-glycans that interfere with *in vitro* specific IgE determinations. Anti-CCD IgE biomarker indicate the presence in serum of IgE directed against carbohydrate epitopes. Grass pollen sensitization is the most common cause of CCDs sensitization in food allergic patients, anti-CCD IgE antibodies are highly cross-reactive with CCD monovalent peanut allergens, but does not induce clinical symptoms. CCDs rarely cause allergic reactions, but are an important cause of cross-reactivity for *in vitro* specific IgE assays for CCD-containing allergens from pollen, plant foods, insects and venoms. The use of CCD-free recombinant allergen components may be of utility in such cases[16-20].

In general, the term cross-reactivity should be used to describe defined clinical features revealing the reactivity to a source without previous exposure[13,21]. The comprehensive term co-recognition, including by definition cross-reactivity, could be usefully adopted to define the large majority of the IgE reactivity where co-exposure to a number of sources bearing homologous molecules does not allow the identification of the sensitizer. The CCD-IgE co-recognition of similar carbohydrate structures on unrelated sources may lead to *in vitro* false positive results in diagnostic tests[13]. Despite having high sequence homology in some cases, the ability of cross-reactive allergens to mediate clinical allergic reactions is highly variable, and often depends on the specific allergen sources involved. In addition, cross-reactivity between allergens may cause covariation of sensitization, such as a higher frequency of sensitization to two or more allergens than the expected frequency[3,22]. Immunologically, cross-reactivity is distinct from co-sensitization in which

Table 1 Important allergen components families involved in cross-reactivity between aeroallergens and food allergens[9,11,12,20]

Allergen components families (sensitivity to heat and proteases)	Examples of relevant allergen components involved (allergen sources)
PR-10 proteins, Bet v 1 homologues	Bet v 1, Aln g 1 (tree pollen)
(sensitive to heat and digestion)	Mal d 1, Pru p 1, Api g 1, Gly m4 (fruits, vegetables, legumes)
Profilins, Bet v 2 homologues	Bet v 2, Ole e 2 (tree pollen), Che a 2, Art v 4, Amb a 8 (weed pollen)
(sensitive to heat and digestion)	Api g 4, Dau c 4, Pru p 4, Cuc m 2, Mus xp 1, Sin a 4 (vegetables, fruits, seeds)
Lipid transfer proteins	Pla a 3, Ole e 7, Art v 3, Amb a 6 (tree and weed pollen)
(stable to heat and digestion)	Api g 2, Pru p 3, Cuc m LTP, Mus a 3, Sin a 3 (vegetables, fruits, seeds)
Tropomyosins	Der p 10, Bla g 7 (house dust mites, insects)
(stable to heat and digestion)	Pen m 1, Myt e 1 (crustaceans, mollusks)
Serum albumins	Fel d 2, Can f 3, Equ c 3 (cat, dog and horse serum albumins)
(fairly sensitive to heat and digestion)	Bos d 6, Sus s 6 (bovine and porcine serum albumins)

genuine sensitization to more than one allergen sources is not due to cross-reactivity, being not mediated by shared epitope-specific antibodies[3,12].

Accurate epidemiologic data on the prevalence of clinical cross-reactivities between aeroallergens and food allergens are generally limited by the lack of large, controlled population-based studies, incorporating oral food challenges. In adults, up to 80% of all cases of food allergy are preceded by sensitisation (clinical or subclinical) to aeroallergens, food allergic symptoms being caused in these patients by cross-reactions between ingested food and inhaled allergens[23]. Even in children, it is suggested that cross-sensitization may be found in up to 25% of cases[24].

CROSS-REACTIVITY BETWEEN AEROALLERGENS OF PLANT ORIGIN AND FOOD ALLERGENS

Pollen-food syndromes and associations are food allergies affecting pollen-sensitized individuals, that have become the most prevalent types of food allergy in European adolescents and adults, affecting about 5% of the population in central Europe. In United Kingdom, pollen-food syndrome overall prevalence is about 2%, in South-Eastern England urban practice being slightly over 4%. The symptoms of pollen-food allergy syndromes range from oral allergy syndrome to severe anaphylaxis, and the foods involved are of vegetal origin, mostly fruits and vegetables, eaten raw[9,25,26]. Pollinosis patients often display adverse reactions upon ingestion of plant-derived foods as a result of IgE cross-reactive epitopes shared by pollen and food allergen sources[1].

The role of an allergy specialist in recognizing and assessment of pollen-food syndromes and associations is essential. Many purified native and recombinant allergen components have been obtained in order to use them for a detailed molecular component-resolved diagnosis of the genuine sensitization and cross-reactivities profiles, and for a more accurate prescription of allergy immunotherapy[20,27,28].

Specific IgE antibodies to recombinant and native specific allergen components from trees, grasses and weeds pollen are important to differentiate the true sensitization profile in patients with multiple pollen sensitizations, as described below[20,27-32].

Tree pollen-specific allergen components are mentioned for anemophilous trees/shrubs belonging to the *Betulaceae* family: rBet v 1, a 17 kDa pathogenesis-related protein PR-10 with ribonuclease activity from the pollen of birch *Betula verrucosa*, cross-reactive with other *Betulaceae* pollen PR-10 components with about 70% identity to it (alder *Alnus glutinosa* rAln g 1, hazel *Corylus avellana* rCor a 1); *Oleaceae* family: nOle e 1 and rOle e 1, a 19-20 kDa trypsin inhibitor from the pollen of olive *Olea europaea*; *Platanaceae* family: rPla a 1, a 18 kDa invertase inhibitor, and nPla a 2, a 43 kDa polygalacturonase, from the pollen of plane tree *Platanus acerifolia*; *Cupressaceae* family: nCup a 1, a 43 kDa pectate lyase, from the pollen of cypress *Cupressus arizonica*, cross-reactive with other *Cupressaceae* pollen pectate lyase components (cedar *Criptomeria japonica* nCry j).

Grass pollen-specific rPhl p 1 (27 kDa beta-expansin), rPhl p 5b (32 kDa ribonuclease), and natural timothy grass (*Phleum pratense*) extract are used to identify grass pollen allergy. Specific IgE against rPhl p 1 is a *Poaceae* family-specific biomarker for genuine sensitization to grass pollen, and against rPhl p 2, rPhl p5 and rPhl p 6 are *Pooideae* subfamily-specific biomarkers for true sensitization to temperate grass pollen. Specific IgE antibodies to nCyn d 1 (beta-expansin of Bermuda grass *Cynodon dactylon)*, a warm climate grass-specific native pollen allergen component, represent biomarkers of genuine sensitization to *Chloridoideae* subfamily grass pollen.

Weed pollen-specific allergen components are described for herbaceous plants belonging to the *Asteraceae* (*Compositae*) family: nArt v 1, a 28 kDa defensin from the pollen of mugwort *Artemisia vulgaris* and nAmb a 1, a 38 kDa pectate lyase from the pollen of short ragweed *Ambrosia artemisiifolia* var. *elatior*; *Plantaginaceae* family: rPla l 1, a 17 kDa Ole e 1-like trypsin inhibitor from the pollen of plantain *Plantago lanceolata*; *Urticaceae* family: rPar j 2, a 14 kDa lipid transfer protein (LTP) from the pollen of wall pellitory *Parietaria*

Table 2 Significant syndromes and associations due to cross-reactivity between aeroallergens and food allergens of plant origin[9,11]

Syndrome or association (sensitivity to heat and proteases)	Relevant allergen components involved (allergen sources)
Birch-apple syndrome	Bet v 1 homologue Mal d 1
Cypress-peach syndrome	Pru p 3 non-specific lipid transfer protein (nsLTP)
Celery-mugwort-spice syndrome	Art v 4 profilin, Art v 60 kDa homologue to Api g 5
Mugwort-peach association	Art v 4 profilin, Art v 3 LTP
Mugwort-chamomile association	Art v 1 defensin (possible candidate)
Mugwort-mustard syndrome	Art v 3 LTP, Art v 4 profilin, Art v 60 kDa (possible candidates)
Ragweed-melon-banana association	Amb a 6 LTP, Amb a 8 profilin (possible candidates)
Goosefoot-melon association	Che a 2 profilin (possible candidate)

LTP: Lipid transfer protein.

judaica; *Amaranthaceae/Chenopodiaceae* family: rChe a 1, a 24 kDa trypsin inhibitor from the pollen of goosefoot *Chenopodium album* and nSal k 1, a 43 kDa protein belonging to the pectin methylesterase family from the pollen of saltwort *Salsola kali.* Art v 6 (pectate lyase) plays an important role in mugwort allergy and the cross-reactivity between Art v 6 and Amb a 1 is frequent, bidirectional, and clinically relevant.

Many cross-reactive allergen components are involved in pollen-food syndromes and associations, such as plant panallergen profilins (actin-binding proteins with roles in the dynamic turnover and restructuring of the actin cytoskeleton), PR-10 proteins (Bet v 1 homologues), lipid transfer proteins (LTPs)[9,27,33], as presented in Table 2.

Tree/shrub pollen aeroallergens and food allergens of plant origin

Several cross-reactivities between tree, shrubs and lianas pollen and foods are described in patients with respiratory allergy. Trees and shrubs discussed below as a source of pollen cross-reactive with foods belong to different anemophilous plant families: *Betulaceae* (birch family), *Oleaceae* (olive family), *Platanaceae* (plane-tree family), and *Cupressaceae (cypress family).* The temperate liana, vine *Vitis vinifera*, is also mentioned.

Regarding the birch-fruit-vegetable-syndrome, about 70% of birch pollen-allergic patients develop symptoms of allergy to plant foods, most frequently involved being *Rosaceae* fruits (mainly apple), nuts (especially hazelnut), and vegetables from the *Apiaceae* family (mainly celery and carrot). Pollinosis precedes the symptoms of food allergy, which, in the majority of cases, is limited to the oropharynx as oral allergy syndrome, occuring when eating raw food. The main allergen component involved in more than 90% of patients with birch pollinosis-associated food allergies, is Bet v 1, a pathogenesis-related PR-10 protein, which is cross-reactive with its homologous in these foods. Bet v 1 homologues represent major allergens in pollen of trees and shrubs from the order *Fagales* (including the *Betulaceae* and *Fagaceae* families), but can also be found in many allergenic foods belonging to the botanical families *Rosaceae* (50%-60% identity to Bet v 1 for the *Maleae* tribe PR-10 proteins: apple Mal d 1, pear Pyr c 1, and *Amigdaleae* tribe PR-10 proteins: apricot Pru ar 1, plum Pru c 1, peach Pru p 1, cherry Pru av 1), *Betulaceae* (hazelnut Cor a 1.0101 with 50% identity to Bet v 1), and *Apiaceae* (PR-10 proteins with 40%-50% identity to Bet v 1: carrot Dau c 1, celery Api g 1). Less than 25% of patients with this syndrome are sensitized to the panallergen Bet v 2 (birch profilin), its contribution to symptoms being unclear. Bet v 1 homologues and profilins, incriminated in the birch-plant foods syndrome, are denatured by high temperatures and by gastric enzymes[20,25,34-37].

The birch pollen-hazelnut association is a *Betula* pollen-associated food allergy to hazelnuts, with Cor a 1-reactive T cells and specific IgE cross-reactive to Bet v 1[38]. This type of hazelnut (*Corylus avellana*) allergy occurs in adults with pollinosis to *Betulaceae* trees/shrubs, and manifests mainly as an oral allergy syndrome, due to an extensive cross-reactivity between the labile hazelnut Cor a 1.04 and birch pollen Bet v 1 allergen components. In contrast, children predominantly exhibit sensitisation to hazelnut storage proteins, Cor a 9 and Cor a 11, which is unrelated to birch pollen allergy, and had more severe clinical manifestations on consumption on raw and processed hazelnuts. In the absence of a cure, avoidance remains the key measure of effective management, especially in patients with severe symptoms[39].

The most common tree pollen-fruit cross-reactivity is represented by the birch-apple syndrome[11,40]. IgE antibodies formed against either Bet v 1, the birch pollen PR-10 allergen, or Mal d 1, the apple allergen, cross-react and give rise to sensitivity to both birch and apple. Moreover, patients with oral allergy syndrome to apple have a higher Bet v 1-induced T cell proliferation compared with those monosensitized to birch pollen without food allergy[12,41].

Interestingly, soy allergen component Gly m 4 also belongs to the PR-10 protein family, and in birch pollen-allergic patients, the combination of IgE sensitization to Gly m 4 and intake of large amounts of mildly processed soy, like soy drinks, may induce a severe allergic reaction[12].

The birch-apple-carrot association is another possible cross-reaction in patients with birch pollen and food allergy, in which IgE-mediated systemic allergic reaction to both apple and carrot, in both fresh and cooked form, is reported[42]. The birch-*Apiaceae* syndrome is seen

mainly in central Europe, and the typical clinical picture is oral allergy syndrome, which occurs when raw foods are ingested. This food allergy to *Apiaceae* is secondary to pollinosis and is due to the presence in these foods of Bet v 1 homologues (Api g 1, Dau c 1), and less frequently to profilins[34].

IgE sensitization patterns to different cross-reactive allergen components are variable according to climate and eating habits. In the Western Mediterranean region, allergies to *Rosaceae* fruits are caused by monosensitization to profilin, monosensitization to LTP, or co-sensitization to both these allergen molecules. In Northern and Central Europe, monosensitization to PR-10 and, to a lesser degree, co-sensitization to profilin and PR-10, is dominant. LTP sensitization is present both in pollinosis and non-pollinosis patients, and is associated with peach allergy in particular. The disease pattern for patients sensitized to profilin is characterized by several concomitant allergies, including grass pollen, *Rosaceae* and non-*Rosaceae* fruits. Sensitization to PR-10 is primarily associated to concomitant birch pollen and apple allergy[43]. In a birch endemic area in Western Europe, both mild and anaphylactic apple-allergic patients are sensitized to PR-10 proteins, whereas only a few of the mild local and none of the anaphylactic apple-allergic patients is sensitized to LTP. In contrast, anaphylactic hazelnut-allergic patients display no such clear sensitization pattern: few are sensitized to both PR-10 proteins and hazelnut LTP, and others to only LTP or to only PR-10 proteins, or to neither PR-10 proteins, nor LTP[44]. Bet v 1 sensitization is associated to concomitant birch pollen rhinoconjunctivitis and oral allergy syndrome to *Rosaceae* fruits in patients from the Southeastern-Central Europe, in a sylvosteppe area with low density forests[45]. In East-Central Europe, in patients with birch pollen allergy with associated food allergy, IgE sensitization to Bet v 1 is frequently associated with food allergy to fruits from *Rosaceae* family. Bet v 2 profilin may be involved in cross-reactivity with non-*Rosaceae* plants, such as *Apiaceae*/*Umbelliferae* vegetables[46].

Immune tolerance induction in the birch-apple syndrome was evaluated in several studies. In patients with oral allergy to apple, tolerance can be safely induced with slowly, gradually increasing consumption of apple, but relapse after consumption discounting and absence of immunologic changes suggest it is only transient[47]. Allergy immunotherapy is clearly effective for birch pollen allergy, but its efficacy on apple allergy is still controversial. Some patients treated with subcutaneous or sublingual immunotherapy develop complete tolerance to apple. Pre-treatment evaluation of patients using molecular allergy diagnosis tools and choosing the appropriate immunotherapeutical doses of birch pollen allergen extract is important[40,48]. Although most patients became re-sensitized to apple over time, many of them are still able to tolerate eating apple at a 30-mo follow-up visit[49]. Pollen immunotherapy has also a positive impact on oral allergy syndrome to hazelnut in birch pollen-allergic patients, but the amount of hazelnut tolerated is small, the effect remaining limited[50].

In the cross-reactive olive pollen-fruit syndrome the main fruits involved are peach *Prunus persica*, pear *Pyrus communis*, melon *Cucumis melo* and kiwi fruit *Actinidia deliciosa*. Sensitization to the LTP Ole e 7 is associated with more severe clinical symptoms in patients who had anaphylaxis, while to the profilin Ole e 2 in most oral allergy syndrome cases. Cross-reactivities between profilins (Ole e 2, Pru p 4, Pyr c 4, Cuc m 2, Act d 9) and LTPs (Ole e 7, Pru p 3, Pyr c 3, Cuc m LTP, Act d 10) are involved in the olive pollen–fruit syndrome. The glucanase Ole e 9 is an allergen component candidate for an important role in pollen-latex-fruit syndrome in patients allergic to olive pollen. Beta-glucanases are also present in latex *Hevea brasiliensis* (Hev b 2) and banana (Mus xp 5). Other cross-reactive allergens involved in pollen-latex-fruit syndrome are profilins from olive pollen (Ole e 2), latex (Hev b 8), ananas (Ana c 1), banana (Mus xp 1) and kiwi (Act d 9), and superoxide dismutases from olive pollen (Ole e 5) and latex (Hev b 10)[51].

In the ficus-fruit syndrome, allergic reactions to fresh or dried figs (*Ficus carica*) or other tropical fruits, which may be presented as anaphylaxis, are a consequence of primary sensitization to airborne ornamental *Ficus benjamina* allergens, independent of sensitization to rubber latex allergens. Cross-reactive ficus allergen component ficin (Fic c Ficin) belongs to the family of cystein proteases present also in kiwi fruit *Actinidia deliciosa* (Act d 1), pineapple *Ananas comosus* (Ana c 2), papaya *Carica papaya* (Car p 1). Figs may also be involved in the latex-fruit syndrome[52,53].

The *Platanus* pollen-fruit/vegetables association is a cross-reactivity entity observed among plane tree *Platanus acerifolia* pollen and plant-derived food allergic patients. There is an important cross-reactivity between the pollen of plane tree, hazelnut and banana fruit, and an intermediate cross-reactivity with celery and peanut. Other fruits and vegetables may also be mentioned. The cross-reacting LTPs may be involved, being present in *Platanus acerifolia* pollen (Pla a 3), but also in hazelnut *Corylus avellana* (Cor a 8), banana *Musa acuminata* (Mus a 3) fruit, peach (Pru p 3), celery *Apium graveolans* (Api g 2), peanut *Arachis hypogaea* (Ara h 9). It appears that neither profilin Pla a 8, nor the two major allergens invertase inhibitor Pla a 1 and polygalacturonase Pla a 2 can be the cause for the strong cross-reactivity[54-57].

In the cypress-peach syndrome, allergic crossreactions between cypress pollen and peach have been reported, including oral allergy syndrome. Profilins (Cup s 8, Pru p 4) or thaumatins (Cup s 3, Pru p 2) could not explain the observed clinical association between cypress pollen and peach. Pru p 3-like non-specific LTPs are involved in the syndrome[11,58].

Other rarer cross-reactive associations are those between date-palm (*Phoenix dactylifera*) pollen and vegetal foods manifested as oral allergy syndrome[59] and between *Vitis vinifera* vine pollen eliciting seasonal

rhinoconjunctivitis and asthma with subsequent food allergy to grapes[60], in both the cross-reacting proteins are still not well established.

Grass pollen aeroallergens and food allergens of plant origin

Profilins are highly cross-reactive allergen components which bind IgE antibodies of almost 20% of plant-allergic patients. Grass pollen is cross-reactive with some foods in patients with oral allergy syndrome. The Bermuda grass *Cynodon dactylon* pollen profilin (Cyn d 12) has substantial cross-reactivity with profilins from tomato *Solanum lycopersicum* (Sola l 1) and cantaloupe *Cucumis melo* (Cuc m 2)[3,61].

Although several patients with oral allergy syndrome, urticaria, angioedema, gastrointestinal or anaphylaxis symptoms after ingestion of products containing wheat or maize flour were reported in patients suffering from respiratory allergy to grass pollens, cross-reactivity among cereal grains and grass pollen is generally considered clinically insignificant. Beta-expansin 11 (EXPB11), a homologue of the major allergen of timothy grass pollen, Phl p 1, may bear a high cross-reactive potential in patients who suffer from both food allergy and pollinosis[62,63].

Hypersensitivity reactions to Navajo ceremonial use of oral corn pollen in native Americans were previously described[64], and recently a case of corn silk (*Stigma maydis*) infusion (traditional herbal medicinal product) and *Poaceae* pollen allergy was reported[65].

Weed pollen aeroallergens and food allergens of plant origin

Allergenic weeds with pollen involved in respiratory sensitization followed by food allergy due to cross-reactive allergen components can be found in the plant families of *Asteraceae/Compositae* (mugwort *Artemisia vulgaris*, ragweed *Ambrosia artemisiifolia* var. *elatior*), *Urticaceae* (pellitory *Parietaria officinalis*), *Amaranthaceae/Chenopodiaceae* (goosefoot *Chenopodium album),* *Plantaginaceae* (plantain *Plantago lanceolata*), and *Cannabaceae* (hop *Humulus japonicus*)[9,66].

In the celery-birch-mugwort-spices syndrome, patients IgE-sensitized to *Betula* and *Artemisia* spp pollen present food allergy to celery, other vegetables and spices, due to several cross-reactive allergen components, including Bet v 1 homologs, profilins and high molecular weight allergens of 40-60 kDa. The Bet v 1 homologs, Api g 1 (celery) and Dau c 1 (carrot), are responsible for the association between birch pollinosis and *Apiaceae* allergy, as no Bet v 1-homologous proteins are found in mugwort pollen. Thus, the celery-birch association only involves species from the *Apiaceae* family, whereas the celery-birch-mugwort syndrome comprises additional botanical families. Associated allergy to *Amaryllidaceae* family foods and spices is rare[9,34].

The well-known celery-mugwort-spice syndrome consists of respiratory sensitization to mugwort *Artemisia vulgaris* and IgE cross-reactive reactions to foods belonging to *Apiaceae/Umbelliferae* family: celery (*Apium graveolans*), carrot (*Daucus carota*), parsley (*Petroselinum crispum*), caraway seeds (*Carum carvi*), fennel seeds (*Foeniculum vulgare*), coriander seeds (*Corinadrum sativum*), aniseed (*Pimpinella anisum*); *Amaryllidaceae* family: garlic (*Allium sativum*), onion (*Allium cepa*), leek (*Allium porrum*); *Solanaceae* family: paprika (*Capsicum annuum*); and *Piperaceae* family: pepper (*Piper* sp). The number of allergen sources involved, the nature of the allergen components, and influencing factors, such as climate and dietary habits, make the celery-birch-mugwort-spice syndrome a clinical entity of high complexity[9]. Complicated cases may associate curry spice allergy with pollen-food allergy syndrome and latex fruit-syndrome[67]. Cross-reactivity between profilins of mugwort pollen (Art v 4) and *Apiaceae* foods, such as celery (Api g 4), carrot (Dau c 4) and spices, are involved in the pathogenesis of this celery-mugwort-spice syndrome[9]. Moreover, the mugwort-fennel-allergy-syndrome is associated with sensitization to an allergen homologous to Api g 5, a high molecular weight glycoprotein flavoprotein, Foe v 5, with function of *flavin adenine dinucleotide-linked oxidoreductase*. An Api g 5-like protein was also identified in carrot. This 60 kDa fennel allergen, highly homologous to Api g 5, may be involved in the mugwort-celery-spice syndrome, being cross-reactive with Art v 60 kDa allergen component from mugwort pollen[68,69].

The mugwort-mustard allergy syndrome describes the association of mugwort pollinosis with several botanically unrelated plant-derived foods allergy from the *Brassicaceae/Cruciferae* family: white mustard (*Sinapis alba*), Indian mustard (*Brassica juncea*), cabbage (*Brassica oleracea* var. *capitata*), broccoli (*Brassica oleracea* var. *italica*), cauliflower (*Brassica oleracea* var. *botrytis*), and possibly from the *Fabaceae/Leguminosae* family: peanut (*Arachis hypogaea*), and *Rosaceae* family: almond (*Prunus dulcis*). Mustard is sometimes a masked allergen in processed foods, and food allergy symptoms vary from oral allergy syndrome to anaphylaxis. Although the causative cross-reactive allergen components have not yet been clearly identified, the possible candidates are LTPs (Art v 3, Sin a 3), profilins (Art v 4, Sin a 4) and high molecular weight allergens, such as Art v 60 kDa[9].

In the mugwort-peach association, the cross-reactive allergen components involved are LTPs (Art v 3, Pru p 3) and profilins (Art v 4, Pru p 4)[9]. This cross-reactivity association appears in a limited group of patients from Southern Europe, in which Art v 3 behaves as the primary sensitizing allergen, although Pru p 3-associated peach allergy is a food allergy driven by primary sensitization to peach in the Mediterranean region. The *Rosaceae* fruits allergens cross-react with mugwort allergens in the mugwort-peach association[70]. A similar group of patients was reported recently in Northern China, in which the food peach LTP allergy originates

from primary sensitization to cross-reactive pollen allergen component Art v 3. The pattern of geographical distribution of this mugwort-peach association may be explained by the dominant role of mugwort pollen exposure in some regions, which is similar to the importance of birch pollen exposure in Northern Europe[71].

An *Asteraceae*-lychee association was also reported in patients diagnosed with respiratory allergy to *Artemisia vulgaris* pollen and food allergy to sunflower seeds, who have subsequently presented anaphylaxis after the first ingestion of lychee fruit. *Litchi chinensis* is a tropical fruit belonging to the *Sapindaceae* family, in which Lit c 1 profilin, a 16 kDa allergen cross-reactive with Art v 4 profilin, was identified. Another allergen component of 70 kDa identified in lychee, and also present in mugwort pollen, is a possible new candidate for the cross-reactive mechanism in this clinical association[72].

In mugwort-chamomile association, respiratory IgE sensitization to mugwort *Artemisia vulgaris* is a primary risk factor for allergy symptoms up to anaphylaxis to ingestion of chamomile infusions. Some patients present positive conjunctival provocation tests with chamomile extract. German Chamomile (*Matricaria chamomilla* var. *recutita*) is a Southern European plant of the *Asteraceae* family, used frequently as herbal tea medical remedy. There is a high degree cross-reactivity between *Matricaria chamomilla* and *Artemisia vulgaris* pollen[73]. The candidate cross-reactive component proposed in mugwort-chamomile association is the Art v 1 defensin[11]. Bet v 1 homologues (Mat c 1) and high molecular weight allergens may also play a role, but not profilins. Patients sensitised to mugwort pollen sometimes present allergic reactions to chamomile, while most subjects allergic to chamomile are sensitized to mugwort. In clinical practice, the incidence and risks of this mugwort-chamomile association may be underestimated[74,75].

In mugwort-sunflower association, the food allergy was reported either manifested as anaphylaxis to consumption of sunflower-pollen contaminated commercial peeled sunflower seeds in a patient sensitized to *Artemisia* pollen, or as oral allergy syndrome after eating sunflower seeds in a patient with airborne allergy to particles of these seeds used as pet food for small animals, such as birds. Moreover, allergy to sunflower seeds may be associated with respiratory allergy to mugwort pollen[76]. Sunflower (*Helianthus annuus*) belongs to the family of *Asteraceae*. Its pollen allergen component Hel a 4 is an Art v 1-like allergen, while Hel a 3 LTP and Hel a 2S albumin are present in seeds[77-79].

Clinical cases of severe allergic reactions, mostly anaphylaxis, to ingestion of bee products, containing pollen grains of *Compositae* plants, in patients with respiratory allergy to *Asteraceae* pollen, especially mugwort and ragweed pollen, were published, for honey, royal jelly and bee polen dietary supplements[80-83]. Cross-reactivity between the pollen of wind-pollinated weeds and other *Asteraceae* insect-pollinated plants is a major mechanism of bee product-induced allergic reactions, likely attributable to several contained pollen allergen components, including profilins, polcalcins, and LTPs. Bee products may contain not only pollen from insect-pollinated plants, but also from wind-pollinated trees or herbaceous plants that grow in the same area, resulting in systemic allergic reactions after accidental ingestion of these airborne pollen grains[81].

The ragweed-melon-banana association is present in patients with respiratory allergy to ragweed *Ambrosia artemisiifolia* experiencing food allergy, usually oral symptoms, when eating various members of the *Cucurbitaceae* family: watermelon (*Citrullus lanatus* subsp. *vulgaris*), netted muskmelon/cantaloupe (*Cucumis melo* var. *cantalupo*), honeydew melon (*Cucumis melo* var. *inodorus*), zucchini (*Cucurbita pepo*), cucumber (*Cucumis sativus*); and *Musaceae* family: banana (*Musa* x *paradisiaca*) fruit. The possible cross-reactive allergen candidates involved in ragweed-melon-banana association are profilins (Amb a 8, Cit la 2, Cuc m 2, Cuc p 2, Cuc s 2, Mus xp 1) and LTPs (Amb a 6, Cuc m LTP)[9,84]. Another possible cross-reactivity between *Ambrosia* pollen and other plants from the *Asteraceae* family recommends not to administer *Echinacea* botanical supplements in patients allergic to ragweeed[85].

In the pellittory-pistachio association, several cross-reacting proteins were suggested using *in vitro* methods. IgE sensitization to pistachio (*Pistacia vera*) is common in *Parietaria* weed pollen allergy. Pistachio nuts, belonging to the family of *Anacardiaceae*, are widely used to produce ice creams, cakes, and mortadella, or are simply eaten roasted. Oral allergy syndrome to pistachio was reported in adult and child patients with *Parietaria* pollinosis. Minor injuries of the oral mucosa due to pistachio shells may enhance local allergic responses[9,86]. Furthermore, different members of the *Anacardiaceae* family (pistachio, mango, and cashew) have been mentioned to share common allergens[87].

The plantain-melon association represents the clustering of allergy to melon *Cucumis melo* and plantain *Plantago lanceolata*, respiratory sensitization to plantain pollen being important in Australia and Mediterranean countries. Several distinct proteins of 14 and 31 kDa, and a spectrum of proteins migrating between 40 and 70 kDa were discussed as cross-reactive allergens[9]. Moreover, the seeds of *Plantago ovata* (psyllium, ispaghula) used in the manufacture of bulk laxatives may cause occupational respiratory allergy in health care and pharmaceutical workers. Cases of anaphylaxis were reported after ingestion of laxatives or breakfast cereals containing *Plantago ovata* seeds, in most of those subjects sensitization occurring previously by inhalation of seed dust. In addition, immunologic cross-reactivity between *Plantago ovata* seed and *Plantago lanceolata* pollen is possible[88].

The goosefoot-melon association or the goosefoot-fruit association was revealed in several cases of patients with pollen allergy to *Chenopodium album*

Table 3 Examples of syndromes due to cross-reactivity between aeroallergens of animal and fungal origin and food allergens[9,11]

Syndrome or association	Relevant allergen components involved
Alternaria-spinach syndrome	Al t a 1
Mite-shrimp syndrome	Der p 10 tropomyosin
Cat-pork syndrome	Fel d 2 cat serum albumin
Bird-egg syndrome	Gal d 5 alpha-livetin (chicken serum albumin)

(allergenic weed of the *Amaranthaceae/Chenopodiaceae* family), who displayed oral allergy syndrome after eating fresh fruits, such as melon, banana and peach. The panallergen profilins (Che a 2, Mus xp 1, Pru p 4) might play a role in goosefoot IgE cross-reactivity[9]. The risk for Russian thistle-saffron association is due to the possible cross-reactivity between *Salsola* and saffron, keeping in mind the use of saffron as a *Crocus sativus* flower-derived spice and the reported flower sensitization in saffron workers[9].

Finally, a hop-celery association is mentioned for Japanese hop (*Humulus japonicus*) pollinosis with allergy to another representative of the *Cannabaceae* family, the common hop (*Humulus lupulus*), and to the unrelated celery (*Apium graveolans*), with no significant associations with ragweed or mugwort pollen[9,89].

CROSS-REACTIVITY BETWEEN AEROALLERGENS OF FUNGAL ORIGIN AND FOOD ALLERGENS

Respiratory allergy to environmental molds is relatively common, fungi representing a prominent source of aeroallergens[90]. *Alternaria alternata* is one of the most common molds associated with allergic diseases, and 80% of *Alternaria*-sensitive patients produce IgE antibodies to Alt a 1, a major allergen with an unique, dimeric beta-barrel structure. Alt a 1 and homologous proteins are characteristic for the *Dothideomycetes* class of ascomycetes[91].

The *Alternaria*-spinach syndrome, in which Alt a 1 is mentioned as an involved allergen component (Table 3), was recently recognized[11]. This cross-reactivity between aeroallergens from fungi imperfecti and allergens from spinach and mushroom *Agaricus bisporus* as foods is mentioned. In the first report of anaphylaxis to spinach and concomitant oral allergy syndrome to mushrooms, cross-reactivity was suggested to be due to common epitopes[92]. A further study identified the cross-reactive 30 kDa protein, probably the Alt a 1 allergen component, present both in spinach and mushroom extracts[93]. This syndrome is different from another possible association between IgE sensitization to mannitol, naturally present in cultivated mushrooms and pomegranate, and anaphylaxis to this sugar alcohol as a drug excipient[94].

In addition, non-fatal anaphylaxis was reported after mycoprotein burger eating, in a young female patient with respiratory IgE-allergy to *Alternaria alternata*, the edible mycoprotein being produced from the fungus *Fusarium venenatum*. Cross-reactivity studies revealed that it shares allergenic determinants with *Alternaria alternata* and *Cladosporium herbarum*[95]. Sensitization to mold allergens *via* the respiratory tract and subsequent oral ingestion of cross-reactive fungal proteins may lead to severe food-allergic reactions, such as those caused by *Fusarium venenatum* acidic ribosomal protein P2 allergen[96]. An unusual case of fatal anaphylaxis was also reported due to heavy mold contamination of a pancake mix with *Fusarium, Penicillium*, *Mucor*, and *Aspergillus* spp, in a teenager allergic to molds[97]. It is also possible that bee products, such as bee pollen supplements, to be contaminated with fungi such as *Aspergillus* and *Cladosporium* spp, and may cause severe allergic reactions in patients sensitized to these molds[81].

Moreover, yeasts should be considered as possible ingestive allergens in mold-allergic patients. A patient with a clustered respiratory IgE sensitization to fungi (*Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus fumigatus*, and *Penicillium notatum*) and baker's yeast (*Saccharomyces cerevisiae*), developed multiple anaphylactic reactions after ingesting pasta yeast sauces containing cross-reacting fungal allergens[98].

CROSS-REACTIVITY BETWEEN AEROALLERGENS OF ANIMAL ORIGIN AND FOOD ALLERGENS

Aeroallergens of animal origin, such as those from domestic arthropods (house dust mites, cockroaches) and pets are usual indoor allergens involved in allergic rhinitis and asthma, along with indoor fungal aeroallergens from certain molds. Spending more time in areas inside buildings (homes, workplaces, schools, and indoor public spaces) creates conditions for more exposure to multiple indoor aeroallergens[99]. Examples of syndromes due to cross-reactivity between inhaled allergens of animal origin and food allergens are presented in Table 3. Other data from case reports defining rare or distinct associations are also discussed below, but are not simplistic put in the table as general rule for clinical practice.

Aeroallergens and food allergens of invertebrate animal origin

Dust mites are the most common indoor environmental cause of respiratory allergies. The main sources of allergens in house dust worldwide are the mite species belonging to the phylum of *Arthropoda*, class *Arachnida*, subclass *Acari*), order *Astigmata*. The most important house dust mites are those from the *Pyroglyphidae* family, especially *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*. Pyroglyphid mites are highly cross-reactive, but have limited cross-reactivity with *Blomia tropicalis*, mite species from *Echimyopodidae*

family significant for tropical/subtropical regions, or with other storage mites from *Acaridae* and *Glycyphagidae* families[100,101].

Patients sensitized to house dust mites can be classified according to their pattern of sensitization into those sensitized only to the major allergens from group 1 and group 2 allergens of pyroglyphid mites, and those with a broader profile of sensitization, including highly cross-reactive allergens, the most important being tropomyosin, belonging to the group 10 allergens[102].

Allergic reactions to edible invertebrates can generate a variety of clinical manifestations ranging from mild oral allergy syndrome, urticaria and/or angioedema, to severe anaphylaxis. Although cross-reactivity between dust mites and invertebrates consumed as food is demonstrated, sometimes there is a poor correlation of IgE reactivity and clinical symptoms[103].

Edible invertebrates are represented mostly by shellfish (culinary term for exoskeleton-bearing aquatic invertebrates, such as crustaceans and mollusks) and less by edible insects (in some parts of the world). Although most kinds of shellfish are aquatic invertebrate animals from saltwater environments (seafood), some are harvested from freshwater, and, in addition, few species of land snails and crabs are also edible invertebrates. Crustaceans are classified among arthropods, together with arachnids (including house dust mites) and insects, whereas mollusks include bivalves, cephalopods and gastropods. Shellfish is one of the leading causes of food allergy in adults and is a common cause of food-induced anaphylaxis. Most frequent causative types of shellfish are shrimp, crab, lobster, clam, oyster and mussel. Specific invertebrate seafood allergy can reflect regional consumption of particular species. It is also important to differentiate shellfish toxic syndromes frequently masquerading as an allergic reaction[104].

The house dust mites-crustaceans-mollusks syndrome is a relatively rare variant of food allergy in which the house dust mites are the primary IgE sensitising agents, while shellfish can induce food allergy, up to anaphylaxis, even at first ingestion[23]. In the more usual mite-shrimp syndrome, the typical allergen component mentioned is tropomyosin Der p 10[11]. Allergenic molecules involved in shellfish allergy are cross-reactive with allergen components from house dust mites, especially certain proteins with a role in muscular contraction. Cross-reactivity with species that are not closely related is common in shellfish-allergic patients, some seafood allergens being widely distributed invertebrate panallergens[105]. Tropomyosin was the first identified allergen involved in cross-reactivity between *Dermatophagoides pteronyssinus* mite, shrimp and insects[106] and it is still considered a major shellfish allergen frequently responsible for clinical cross-reactivity with inhaled house dust mites[107]. Besides been assumed to be a major cause of cross-reactivity between astigmatid mites and other invertebrates, tropomyosin may be a major cause of covariation of sensitization between house dust mites, crustaceans, and some species of insects and mollusks[22]. Allergenic tropomyosins are highly conserved muscle proteins found in invertebrates, such as arachnids (house dust mites), insects (cockroaches), crustaceans (shrimp, prawn, lobster, crawfish, crab), and mollusks (mussel, oyster, squid, cuttlefish, octopus, abalone, limpet, snail), therefore being considered panallergens. In contrast to invertebrate tropomyosin, vertebrate tropomyosins, such as those from beef, pork, rabbit or chicken, are not allergenic[104,108].

Tropomyosins are a large family of alpha-helical proteins that form a coiled-coil structure of two parallel helices containing two sets of seven alternating actin-binding sites, playing a critical role in regulating the function of actin filaments. Tropomyosins are present in all eukaryotic cells, associated with the thin filament in muscle and microfilament in many nonmuscle cells, being involved in the contractile activity of these cells, and also in helping regulation of cell morphology and motility. As panallergens, these are resistant to heat, low gastric pH and gastroenteric peptidase, therefore their allergenicity is maintained in cooked and digested foods, causing allergic systemic reactions up to anaphylaxis. Natural tropomyosin has an average molecular weight of 37 kDa. House dust mite group 10 allergens are composed of 284 amino acids. Sequence identity within the house dust mite tropomyosins is higher than any other mite allergen. The Der p 10 tropomyosin shares more than 65% identical residues with other invertebrate tropomyosins[22,109-111]. Regions adjacent to the positions 133-135 and 201 of the invertebrate tropomyosins present lower probability of alpha helix folding than those of vertebrates, and are candidates responsible for allergenicity[112]. There is a high cross-reactivity between house dust mite tropomyosins: *Dermatophagoides pteronyssinus* (Der p 10), *Dermatophagoides farinae* (Der f 10) and *Blomia tropicalis* (Blo t 10)[22].

The prevalence of sensitization to tropomyosin among house dust mite-allergic patients, assessed using recombinant group 10 mite allergen components, varies with geographical area[22,113]. In many European countries, this sensitization prevalence to rDer p 10 varies between 9%-18%[114]. Other studies revealed that sensitization rates to tropomyosin are found in higher rates (30%-55%) in subtropical or tropical regions, such as Australia or Central Africa. This variability can be explained by various exposure to sensitizing invertebrate allergens in different parts of the world[113]. The resulting IgE antibodies are able to cross-react with different tropomyosins, even with those which did not induce their production. For example, IgE antibody reactivity to a major food allergen, the cross-reactive brown shrimp tropomyosin, can occur in unexposed subjects (Orthodox Jews, with Kosher dietary laws that prohibit eating shellfish) with clinically significant allergy to house dust mites and/or cockroaches[21]. Another study revealed that half of the house dust mite-allergic

European patients with IgE sensitization to tropomyosin (Der p 10) have a history of clinically relevant cross-reactivity reactions to eating seafood, the other half having no allergic reactions when consuming such edible invertebrates[107].

Cross-reactivity of tropomyosin allergens from house dust mites with crustaceans (subphylum *Crustacea*) is significant, mentioning decapods (order *Decapoda*) from the *Penaeidae* family: brown shrimp *Farfantepenaeus/Penaeus aztecus* (Pen a 1), black tiger shrimp *Penaeus monodon* (Pen m 1), Indian prawn *Fenneropenaeus/Penaeus indicus* (Pen i 1), whiteleg shrimp *Litopenaeus vannamei* (Lit v 1), sand shrimp *Metapenaeus ensis* (Met e 1); *Crangonidae* family: common shrimp *Crangon crangon* (Cra c 1); *Pandalidae* family: Northern shrimp *Pandalus borealis* (Pan b 1); *Nephropidae* family: European lobster *Homarus gammarus* (Hom g 1), American lobster *Homarus americanus* (Hom a 1); *Palinuridae* family: Chinese spiny lobster *Panulirus stimpsoni* (Pan s 1); *Portunidae* family: coral crab *Charybdis feriata* (Cha f 1), and *Cambaridae* family: red swamp crayfish *Procambarus clarkii (*Pro cl 1)[22,104,115]. Cross-reactivity of tropomyosin allergens from house dust mites with mollusks (phylum *Mollusca*) is also reported, mentioning *Bivalvia* class mussels (*Mytilidae* family): blue mussel *Mytilus edulis* (Myt e 1), Mediterranean mussel Mytilus galloprovincialis (Myt g 1), Asian green mussel Perna viridis (Per v 1), oysters (Osteridae family): Pacific oyster *Crassostrea gigas* (Cra g 1), scallops (*Pectinidae* family): scallop *Mimachlamys nobilis* (Mim n 1), razor clams (*Solecurtidae* family): constricted tagelus *Sinonovacula constricta* (Sin c 1); *Gastropoda* class abalones (*Haliotidae* family): disk abalone *Haliotis discus hannai* (Hal di 1), Japanese abalone *Haliotis diversicolor* (Hal d 1), turban snails (*Turbinidae* family): horned turban *Turbo cornutus* (Tur c 1), land snails (*Helicidae* family): brown garden snail *Helix aspersa* (Hel as 1); *Cephalopoda* class decapod arrow squids (*Ommastrephidae* family): Japanese flying squid *Todarodes pacificus* (Tod p 1), cuttlefish (*Sepiidae* family): golden cuttlefish *Sepia esculenta* (Sep e 1), and octopods (*Octopodidae* family): common octopus *Octopus vulgaris* (Oct v 1)[22,104,115,116]. In addition to tropomyosin, other muscle protein crustacean allergens involved in shellfish allergy and cross-reactivity with other invertebrates are arginine kinase and myosin light chain[117].

Arginine kinase, a 40-kDa enzyme involved in the storage of excess energy as arginine phosphate, is a potential new class of invertebrate panallergens, identified mainly in crustaceans, such as black tiger shrimp *Penaeus monodon* (Pen m 2), common shrimp *Crangon crangon* (Cra c 2), whiteleg shrimp *Litopenaeus vannamei* (Lit v 2), Chinese shrimp *Fenneropenaeus chinensis* (Fen c 2), snow crab *Chionoecetes opilio* (Chi o 2), mangrove mud crab *Scylla serrata* (Scy s 2), Atlantic Horseshoe Crab *Limulus polyphemus* (Lim p 2), but also in mollusks: common octopus *Octopus vulgaris* (Oct v 2), ocellated octopus *Octopus fangsiao* (Oct f 2). These are cross-reactive with arginin kinase allergens from house dust mites (Der p 20, Der f 20, Blo t 20), cockroaches (Bla g 9, Per a 9) and moths, such as Indian-meal moth *Plodia interpunctella* (Plo i 1) and silk moth/silkworm larvae *Bombyx mori* (Bomb m 1)[104,118-121].

Myosin light chain is a 20 kDa crustacean allergen identified in common shrimp *Crangon crangon* (Cra c 5), brine shrimp *Artemia franciscana* (Art fr 5), black tiger shrimp *Penaeus monodon* (Pen m 3), whiteleg shrimp *Litopenaeus vannamei* (Lit v 3), American lobster *Homarus americanus* (Hom a 3), with potential cross-reactivity with aeroallergens from dust mite *Dermatophagoides farinae* (Der f 26) and German cockroach (Bla g 8)[104,120,122,123].

Paramyosin is a 103 kDa cross-rective muscle protein described in diverse invertebrates. Molluskan paramyosin is responsible for the "catch" mechanism that enables sustained contraction of muscles with very little energy expenditure. House dust mite allergen Der p 11 (paramyosin with 89% identity with Der f 11) presents significant homology with the paramyosin of mollusks: Mediterranean black Mussel *Mytilus galloprovincialis* (Myt g PM), Japanese Abalone *Haliotis discus* (Hal di PM), Horned Turban *Turbo cornutus* (Tur c PM), common Octopus *Octopus vulgaris* (Oct v PM)[116,120,124,125].

Amylase and haemocyanin are other allergens found in mollusks and are possibly involved in cross-reactivity with house-dust mite aeroallergens. Alpha-amylase Der p 4 may be involved in *Dermatophagoides pteronyssinus* cross-reactivity in gastropod allergy[22,116,126].

It is important to differentiate primary sensitization to pyroglyphid mite aeroallergens, with subsequent food allergy to edible invertebrates, from allergy to shellfish in patients not allergic to house dust mites. There is a profile of sensitization to shellfish in which tropomyosin is involved as a panallergen, with patients not tolerating several crustaceans and/or mollusks, selective sensitization to only one type of seafood being uncommon. Another profile was has been also described in cases with house dust mites as primary sensitizing agents and selective allergy to mollusks or crustaceans, described for common European limpet (*Patella vulgata*), terrestrial green garden snail (*Helix* spp) and Mediterranean spiny lobster (*Palinurus elephas*)[126,127]. The role of tropomyosin as a clear cause of cross-reactivity is a matter of debate in some circumstances. Clinical cases of shrimp allergy without snail allergy in relation to house dust mites sensitization, and of allergy to snails without shrimp allergy in context of respiratory mite allergies, were reported, with the observation that in shrimp allergy the symptoms are mainly urticaria or angioedema, while in snail allergy the clinical picture is usually dominated by severe asthma[128].

Moreover, there seems to exist differences in mite-shellfish cross-reactivity depending on climate. A recent study designed to identify which of the shrimp allergen molecules (tropomyosin, arginine kinase, sarcoplasmic

calcium-binding protein, actinins, aldolase, ubiquitin) are involved in mite-seafood cross-reactivity in two different climate populations, revealed that tropomyosin and ubiquitin are responsible for mite-seafood cross-reactivity from both continental dry and humid climates, while alpha-actinin and arginine kinase are involved in dry- and humid-climate groups, respectively. Mites are the primary sensitizer in humid-climate, while shrimps in the continental dry-climate population[129]. Patients sensitized to tropomyosin Der p 10 usually are sensitized to several other house dust mite allergen components besides the major allergens (Der p 1, Der p 2), whereas Der p 10-negative patients are primarily sensitized to Der p 1 and/or Der p 2. Therefore, Der p 10 may be a diagnostic biomarker for mite-allergic patients with additional sensitization to allergens other than Der p 1 and Der p 2, such patients requiring more attention when immunotherapy with allergen extracts is considered[130].

Because allergy immunotherapy with house dust mites extracts is an effective method of treating respiratory allergy, and most of the currently available extracts for subcutaneous or sublingual route of administration contain high concentrations of group 1 and 2 allergens, but may also contain lower concentrations of other sensitizing molecules, including group 10 allergens, it is still unclear whether this type of treatment may induce clinically relevant sensitization to tropomyosin[113,131]. Some studies suggested that food allergy to shrimp or snail can worsen in some patients treated with subcutaneous immunotherapy. However, many patients already had mild allergic reactions to tropomyosin-containing foods before starting immunotherapy, and a new sensitization was confirmed in only one patient[132,133]. Other studies revealed a lack of induction of new sensitization to tropomyosin during house dust mite injection or sublingual immunotherapy[131,134]. Additional studies suggested that, apart from not inducing new sensitization to tropomyosin, house mite subcutaneous immunotherapy could have possible beneficial effects in patients with snail, squid or shrimp allergy[113,135]. Moreover, a recently published report presented a shrimp allergy case improved from anaphylactic symptoms observed before, to mild oral allergy syndrome after one year of sublingual mite immunotherapy with a known and relatively high dosage of tropomyosin[136]. Therefore, induction of clinically relevant sensitization to tropomyosin is an unlikely consequence of house dust mite immunotherapy, but since the risk of adverse allergic reactions to seafood needs to be closely monitored, levels of serum specific IgE to tropomyosins Der p 10 and Pen a 1 may be useful biomarkers[113].

From another point of view, there is a significant prevalence of occupational asthma in shellfish-processing workers, and airborne shellfish exposure not only can cause symptoms in highly allergic subjects, but can also cause *de novo* sensitization. Symptoms may be limited to respiratory tract or may systemic, as in anaphylaxis[104,137]. Moreover, respiratory allergy to aquarium fish food (aquarium syndrome) is due to exposure to aeroallergens (either at work or as a hobby) from a variety of dried arthropod species, including common water fleas (*Daphnia* spp, crustaceans of the *Daphniidae* family), freshwater shrimps (*Gammarus* spp, crustaceans of the *Gammaridae* family), red midge larvae (*Chironomus thummi*, insect of the *Chironomidae* family), mosquito black larvae (*Culex* spp, insect of the *Culicidae* family), and segmented earthworms (*Tubifex tubifex*, annelid of the *Naididae* family). Variable degree of cross-reactivity between these arthropods with house dust mites, cockroaches and edible shrimps, was reported[137,138].

Oral mite anaphylaxis is a new syndrome characterized by severe allergic symptoms occurring immediately after eating mite-contaminated foods, in patients with a previous history of house dust mite-allergic rhinitis and/or asthma. This type of food allergy to mite ingestion was reported in various countries, including the United States, Japan, Taiwan, Venezuela, Brazil and in Southern Europe, being more prevalent in tropical/subtropical environments. Different mite species involved are house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), and storage mites (*Tyrophagus putrescentiae*, *Lepidoglyphus destructor*, *Blomia tropicalis etc.*). Allergenic cross-reactivity between different domestic and storage mite species could explain why patients sensitized to house dust mites may present systemic reactions when exposed to storage mites by oral route. Mite-contaminated foods are usually prepared with wheat and/or corn flour, including pancakes, sponge cakes, pizza, pasta, bread, white sauce, beignets, cornmeal cakes, and polenta. Because exposure to low temperatures inhibits mite proliferation, it is recommended to store the flour in sealed containers in the refrigerator. Other foods that can be contaminated with mites when stored for long periods at ambient temperature are cheese, ham, chorizo, and salami[139]. In Japan, cases involving ingested okonomiyaki or takoyaki prepared at home were reported, due to mite-contaminated flavored flour, the okonomiyaki-mix or takoyaki-mix being previously opened and stored for months at room temperature[140]. It is suggested that thermoresistant mite allergens are involved in pathogenesis, because many cooked mite-contaminated foodstuffs are able to induce symptoms. A variety of this syndrome is the mite ingestion-associated exercise-induced anaphylaxis[141]. Patients with oral mite anaphylaxis present also an increased prevalence of nonsteroidal anti-inflammatory drugs (NSAIDs) hypersensitivity. Even no salicylates were detected in mite-contaminated wheat flour, the opisthonotal gland secretion from *pyroglyphid* mites contains salicylaldehyde analog 2-formyl-3-hydrobenzyl formate[142]. Moreover, intake of NSAIDs sometimes enhance immediate reactions in food-dependent exercise-induced anaphylaxis[143], and salicylate hypersensitivity with reactions to salicylate food additives may occur in patients with cross-reactive NSAIDs hypersensitivity[144].

Domestic cockroaches, especially *Blattella germanica* (German cockroach), are the most important urban indoor inhalant insect allergen sources. Major German cockroach allergens, Bla g 1 and Bla g 2, are cross-reactive with similar American cockroach *Periplaneta americana* allergen components Per a 1 and Per a 2, respectively[145]. Molecular mimicry between cockroach Bla g 5 and helminth glutathione S-transferases promotes cross-reactivity and cross-sensitization[146]. Cockroaches also contain cross-reactive tropomyosin (Bla g 7), which indicates a risk for allergic reactions to shellfish or snail, which can be severe[12,103]. German cockroach allergen molecule Bla g 7 has cross-reactivity with tropomyosins from other cockroaches, such as American cockroach *Periplaneta americana* (Per a 7), but also from dust mites *Dermatophagoides pteronyssinus* (Der p 10), *Dermatophagoides farinae* (Der f 10), and ascarid nematodes *Anisakis simplex* (Ani s 3), *Ascaris lumbricoides* (Asc l 3)[22,147]. Recombinant Bla g 7 sensitization rate in German cockroach-allergic Korean patients is 16.2%[148]. Besides tropomyosin (Bla g 7), myosin light chain (Bla g 8) and arginine kinase (Bla g 9), hemocyanin is another cockroach aeroallergen (Bla g 3) cross-reactive with shellfish allergens, such as the one identified in giant keyhole limpet *Megathura crenulata* (Meg c Hemocyanin)[120,149,150].

An association between sensitization to arthropod aeroallergens and food allergy to edible insects is also possibile. The silkworm *Bombyx mori* is an important insect in the textile industry and its pupa are used in Chinese cuisine, being the most commonly eaten insect in China. The silk, urine and dander of silkworms are often the cause of allergies in sericulture workers, and silkworm pupa is known to be allergenic. Silkworm moth-sensitized patients with rhinoconjunctivitis and asthma from Southern China are frequently concomitantly sensitized to other aeroallergens from relevant mites and cockroaches (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Blomia tropicalis*, *Blattella germanica*, *Periplaneta americana*). The silk moth allergen component Bomb m 1 is the significant cross-reactive arginine kinase, similar to house dust mites and cockroaches allergenic molecules, the tropomyosin Bomb b 7 being probable less important[151-153].

Caterpillars are commonly eaten insects in Africa, ingested Mopane worm *Imbrasia belina* being reported as a cause of allergic anaphylaxis in a Zimbabwean adolescent with IgE sensitization to house dust mites and cockroaches (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Blattella germanica*), suggesting cross-reactivity due to glutathione transferases (Der p 8, Der f 8, Bla g 5) or tropomyosin[154].

Infestation of food with insects is a different type of allergy described in Spain, lentil pest *Bruchus lentis* proteins being a cause of IgE-mediated rhinoconjunctivitis and asthma in patients eating or inhaling infested legume particles[155].

Cochineal red/carmine is a natural red color, used as food additive (E120, FDA 73.100) or pharmaceutical excipient, and obtained from the dried bodies of the female scale insect *Dactylopius coccus*, which contain dye protein residues attributed to IgE-mediated sensitization, food allergy, including anaphylaxis, and occupational asthma and rhinoconjunctivitis. Insect-derived proteins possibly complexed with carminic acid may be responsible for carmine allergy, and a 38 kDa major allergen in cochineal extract was described as an insect phospholipase or related enzyme. Carmine insect allergens can act both *via* inhalation and digestion, inducing both respiratory allergy and alimentary allergy[156-159].

Aeroallergens and food allergens of vertebrate animal origin

Syndromes and associations related to clinical cross-reactivity bewteen aeroallergens and food allergens of mammalian and avian origin are described below.

Domestic mammals with fur kept as pets induce respiratory symptoms in allergic patients. The popularity of cats and dogs as pets put them among the most important sources of indoor allergens. In Europe and United States of America, at least one person in four is exposed every day to aeroallergens of mammalian origin, and almost everyone is occasionally exposed to inhalant allergens from pets or domesticated animals[160]. Specific *Carnivora* order pet allergen components are described for cat (*Felis catus* syn. *Felis domesticus*): Fel d 1, Fel d 4, and dog (*Canis lupus familiaris* syn. *Canis familiaris*): Can f 1, Can f 2, Can f 5[18,161,162].

Cross-reactive serum albumins (66-69 kDa) from mammals kept as pets or domestic animals are described as allergen components: cat *Felis domesticus* Fel d 2, dog *Canis domesticus* Can f 3, horse *Equus caballus* Equ c 3, cattle *Bos domesticus* Bos d 6, pork *Sus scrofa domestica* Sus s 6, rabbit *Oryctolagus cuniculus* Ory c 6. Allergic sensitization to serum albumins can occur by inhalation as well as ingestion. These proteins are a major component in the circulatory system of mammals, contributing to colloid osmotic blood pressure and the transport of many ligands. As important allergen components, they are present in body fluids, including saliva, in meat, and on dander. IgE cross-reactivity between inhaled (aeroallergens from pets or occupational settings) and ingested or systemically administered serum albumins must be considered in clinical practice[160,163-165].

The cat-pork syndrome consists primarily of IgE-mediated respiratory symptoms following exposure to cat dander, and secondarily of food allergy symptoms after the ingestion of pork meat[166]. The first report on cat-allergic patients experiencing anaphylaxis to pork meat suggested cross-reactivity due to a 67 kDa protein[167], later on the sensitization to cat serum albumin being considered an useful biomarker of possible cross-sensitization not only to porcine serum albumin, but also to other mammalian serum albumins[168]. Despite being a dominant protein in dander, Fel d 2 is a 67-kDa serum albumin regarded as a minor cat

allergen, about 15%-35% of cat allergic patients being sensitized to it. Only 1%-3% of cat-allergic patients seem to be at risk for food allergy to pork meat, under the circumstances that about a third of the subjects sensitized to porcine serum albumin are likely to present allergic reactions to pork consumption[160,165,168].

Although the term cat-pork syndrome seems to be appropriate because the sensitization to cat serum albumin represents the primary event in this cross-reactivity entity, it is also frequently named pork-cat syndrome. Clinical picture varies from oral itching to anaphylaxis. Because albumin is a heat-labile protein, fresh meat or dried and smoked pork are more consistent triggers than well-cooked meat. Pork grilled meat, sausages, ham and pork ribs, hamburger or barbecue were mentioned as causative factors. Small amount of pork meat in a strip of bacon or cooked pork meat may be tolerated without severe reactions, as well as seasoned pork products, such as salami. Fatal anaphylaxis after eating wild boar meat was reported in a patient with pork-cat syndrome. Symptoms usually occur within 30-45 min after eating pork meat, and does not appear to be related to tick bites. In general, patients with pork-cat syndrome, neither react to beef, nor have serum evidence of sensitization. These aspects are helpful in differentiating from delayed food allergy to red meat, due to IgE antibodies to alpha-gal (galactose-alpha-1,3-galactose, a nonprimate mammalian oligosaccharide epitope), in patients with recent tick bite/bites (1-4 wk). These alpha-gal allergic patients present delayed anaphylaxis, angioedema or urticaria, 3-6 h after eating red meat (beef, pork, lamb), but not chicken, turkey, or fish. Both cross-reactivity syndromes do not appear early in life, most reported patients are older than age of five years, with the majority being adults or adolescents[168-171].

Pig allergy was reported from the point of view of food sensitization, but also as occupational allergy. An unusual case of occupational asthma resulting from pork-cat syndrome was also recently described in a female patient having respiratory allergy with sensitization to cat dander, working at a grocery store selling cured meats (having the duty to cut pork bones), and presenting symptoms caused by inhalation. In this case cat dander was the primary sensitizer and sensitization to galactose-α-1,3-galactose, a source of cross-reactivity between meat and dander was ruled out[172]. Pig hair and dander are also important inducers of occupational allergies in farmers exposed in swine production. Moreover, popular uncommon pets include small pigs, mini-pigs, or teapot pigs[163].

Several other cross-reactivity associations between mammalian inhalant allergies with subsequent food allergy were also reported. A case of confirmed occupational respiratory allergy due to pork was followed by food allergy to pork, and later by food allergy to chicken. Porcine and chicken hemoglobin were found to be cross-reactive allergens. Although IgE cross-reactivity is most frequent between mammalian albumins, cross-reactions may also occur between cat and chicken albumins, which share 46% identical amino acids. Cross-reactivity between porcine and chicken serum albumins was possibly linked to a prior sensitization to cat serum albumin[173]. A case of occupational asthma induced by the inhalation of bovine serum albumin powder in a laboratory researcher, was followed by symptoms of food allergy after drinking milk, without symptoms on ingesting beef, pork, or chicken meat[174]. It is important to mention that milk allergic children sensitized to the allergen component Bos d 6 (bovine serum albumin) may also have concomitant beef allergy[175]. Another patient diagnosed with initial sensitization to inhaled rabbit products (such as epithelium, urine, serum) in childhood presented anaphylaxis with severe bronchospasm secondary to ingestion of rabbit meat in adolescence, the allergen involved being the 60-kDa albumin, responsible for cross-reactivity between rabbit epithelium and rabbit meat[176].

Airborne exposure to pet birds antigens may cause allergic rhinitis and/or asthma in IgE sensitized patients or hypersensitivity pneumonitis/extrinsic allergic alveolitis in others.

The bird-egg syndrome consists of primary IgE-mediated sensitization with respiratory symptoms to exposure to bird aeroallergens, and secondarily of allergy symptoms after the ingestion of eggs. This syndrome is due to cross-reactivity between egg yolk and bird allergens (feathers, serum, droppings, and meat). Its pathomechanism is different from hypersensitivity pneumonitis induced by bird antigens, such as pigeon fancier's lung. There are also differences between the bird-egg syndrome and the common allergy without sensitization to bird proteins. Patients with bird-egg syndrome are typical adults, with allergic rhinoconjunctivitis and/or asthma due to repeated exposure to household pet birds, such as budgerigars, canaries, parrots or lovebirds, and the symptoms associated with egg ingestion are usually gastrointestinal, but also cutaneous or respiratory. Food-dependent, exercise-induced anaphylaxis to egg has also been reported. While ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin (Gal d 3) and lysozyme (Gal d 4) are involved in common hen's egg white allergy, alpha-livetin found in egg yolk also known as chicken serum albumin (Gal d 5) is the allergen component involved in both respiratory and food-allergy symptoms in the bird-egg syndrome. Gal d 5 is a water-soluble, partially heat-labile, 70 kDa allergen present in egg yolk and avian meat and serum, and induce cross-reactivity to bird allergens, egg yolk, and chicken meat. A minority of patients with egg allergy are reactive to chicken meat. The role in food allergy of several other allergens identified in egg yolk, including apovitellenin I (Gal d Apo I) and apovitellenin VI (Gal d Apo VI), is still unclear[163,177-180]. Two similar cross-reactivity syndromes must also be mentioned. While the bird-egg syndrome is described in patients primarily sensitized to bird antigens, the egg-bird syndrome was reported

in patients in which egg allergy started in infancy and the primary sensitization was to egg yolk. The egg-egg syndrome is an occupational respiratory allergy to airborne egg proteins with subsequent nutritive egg allergy, in bakery and confectionery industry workers[181].

CONCLUSION

The knowledge of significant syndromes and associations related to cross-reactive allergen components and the impact of relevant cross-reactivities between aeroallergens and food allergens are of great importance for the allergy specialist. Allergen cross-reactivity may be an underestimated problem in clinical practice[13,182]. Moreover, molecular-based allergy diagnosis is essential for an accurate allergy evaluation of cross-reactions, sometimes with impact on the therapeutic strategy[12]. Patients allergic to certain food allergens and inhaled allergens should be carefully instructed about cross-reactions to other food allergens[183]. Dietary avoidance of foods that are related and have potentially cross-reactive proteins should be individualized according to the risk of clinical cross-reactivity[3].

Very recently, a trustable expert system was developed to support the interpretation of molecular tests for allergy based on microarrays. Allergen microarrays facilitate the simultaneous testing of more than 100 allergen components, represent the state-of-the-art technology for allergy diagnosis in poly-sensitized patients, and have an important role in the accurate diagnosis of syndromes and associations related to the IgE sensitization to cross-reactive allergens components. A section termed "post molecular anamnesis" suggests any clinical supplemental questions that should arise from the microarray interpretation[11,184-186].

Because the era of the characterization of molecular features of food allergens has begun, new data started to bring useful information about cross-reactivity between different sources of food allergens and aeroallergens in order to help the clinicians to provide appropriate prophylaxis approach, and to estimate the types and severity of allergic reactions[187].

Component-resolved diagnosis is a research method that explains on molecular level allergen cross-reactivity, and allows to distinguish cross-reactions occurring after ingestion of food in patients with IgE sensitization primarily to aeroallergens from the coexistence of inhaled and food allergies. Due to the geographic diversity resulting in different exposure to airborne allergens and dietary factors, studies on allergen components in populations living in different climatic zones give different results. This suggests that the diagnostic and prognostic assessment based on the component-resolved diagnosis results is limited and should always be considered in clinical context[188].

Medical history and diagnosis approach may be guided by the knowledge about the diverse cross-reacting allergens involved, and by the understanding of these clinical entities which may vary significantly or may be overlapping. The use of molecular-based allergy diagnosis improves the understanding of clinically relevant cross-reactive allergen components from aero-allergen sources and foods.

REFERENCES

1 **Bousquet J**, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, Zuberbier T, Baena-Cagnani CE, Canonica GW, van Weel C, Agache I, Aït-Khaled N, Bachert C, Blaiss MS, Bonini S, Boulet LP, Bousquet PJ, Camargos P, Carlsen KH, Chen Y, Custovic A, Dahl R, Demoly P, Douagui H, Durham SR, van Wijk RG, Kalayci O, Kaliner MA, Kim YY, Kowalski ML, Kuna P, Le LT, Lemiere C, Li J, Lockey RF, Mavale-Manuel S, Meltzer EO, Mohammad Y, Mullol J, Naclerio R, O'Hehir RE, Ohta K, Ouedraogo S, Palkonen S, Papadopoulos N, Passalacqua G, Pawankar R, Popov TA, Rabe KF, Rosado-Pinto J, Scadding GK, Simons FE, Toskala E, Valovirta E, van Cauwenberge P, Wang DY, Wickman M, Yawn BP, Yorgancioglu A, Yusuf OM, Zar H, Annesi-Maesano I, Bateman ED, Ben Kheder A, Boakye DA, Bouchard J, Burney P, Busse WW, Chan-Yeung M, Chavannes NH, Chuchalin A, Dolen WK, Emuzyte R, Grouse L, Humbert M, Jackson C, Johnston SL, Keith PK, Kemp JP, Klossek JM, Larenas-Linnemann D, Lipworth B, Malo JL, Marshall GD, Naspitz C, Nekam K, Niggemann B, Nizankowska-Mogilnicka E, Okamoto Y, Orru MP, Potter P, Price D, Stoloff SW, Vandenplas O, Viegi G, Williams D. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 2008; **63** Suppl 86: 8-160 [PMID: 18331513 DOI: 10.1111/j.1398-9995.2007.01620.x]

2 **Papadopoulos NG**, Agache I, Bavbek S, Bilo BM, Braido F, Cardona V, Custovic A, Demonchy J, Demoly P, Eigenmann P, Gayraud J, Grattan C, Heffler E, Hellings PW, Jutel M, Knol E, Lötvall J, Muraro A, Poulsen LK, Roberts G, Schmid-Grendelmeier P, Skevaki C, Triggiani M, Vanree R, Werfel T, Flood B, Palkonen S, Savli R, Allegri P, Annesi-Maesano I, Annunziato F, Antolin-Amerigo D, Apfelbacher C, Blanca M, Bogacka E, Bonadonna P, Bonini M, Boyman O, Brockow K, Burney P, Buters J, Butiene I, Calderon M, Cardell LO, Caubet JC, Celenk S, Cichocka-Jarosz E, Cingi C, Couto M, Dejong N, Del Giacco S, Douladiris N, Fassio F, Fauquert JL, Fernandez J, Rivas MF, Ferrer M, Flohr C, Gardner J, Genuneit J, Gevaert P, Groblewska A, Hamelmann E, Hoffmann HJ, Hoffmann-Sommergruber K, Hovhannisyan L, Hox V, Jahnsen FL, Kalayci O, Kalpaklioglu AF, Kleine-Tebbe J, Konstantinou G, Kurowski M, Lau S, Lauener R, Lauerma A, Logan K, Magnan A, Makowska J, Makrinioti H, Mangina P, Manole F, Mari A, Mazon A, Mills C, Mingomataj E, Niggemann B, Nilsson G, Ollert M, O'Mahony L, O'Neil S, Pala G, Papi A, Passalacqua G, Perkin M, Pfaar O, Pitsios C, Quirce S, Raap U, Raulf-Heimsoth M, Rhyner C, Robson-Ansley P, Alves RR, Roje Z, Rondon C, Rudzeviciene O, Ruëff F, Rukhadze M, Rumi G, Sackesen C, Santos AF, Santucci A, Scharf C, Schmidt-Weber C, Schnyder B, Schwarze J, Senna G, Sergejeva S, Seys S, Siracusa A, Skypala I, Sokolowska M, Spertini F, Spiewak R, Sprikkelman A, Sturm G, Swoboda I, Terreehorst I, Toskala E, Traidl-Hoffmann C, Venter C, Vlieg-Boerstra B, Whitacker P, Worm M, Xepapadaki P, Akdis CA. Research needs in allergy: an EAACI position paper, in collaboration with EFA. *Clin Transl Allergy* 2012; **2**: 21 [PMID: 23121771 DOI: 10.1186/2045-7022-2-21]

3 **Sampson HA**, Aceves S, Bock SA, James J, Jones S, Lang D, Nadeau K, Nowak-Wegrzyn A, Oppenheimer J, Perry TT, Randolph C, Sicherer SH, Simon RA, Vickery BP, Wood R, Bernstein D, Blessing-Moore J, Khan D, Lang D, Nicklas R, Oppenheimer J, Portnoy J, Randolph C, Schuller D, Spector S, Tilles SA, Wallace D, Sampson HA, Aceves S, Bock SA, James J, Jones S, Lang D, Nadeau K, Nowak-Wegrzyn A, Oppenheimer J, Perry TT, Randolph C, Sicherer SH, Simon RA, Vickery BP, Wood R. Food allergy: a

practice parameter update-2014. *J Allergy Clin Immunol* 2014; **134**: 1016-25.e43 [PMID: 25174862 DOI: 10.1016/j.jaci.2014.05.013]
4 **Chafen JJ**, Newberry SJ, Riedl MA, Bravata DM, Maglione M, Suttorp MJ, Sundaram V, Paige NM, Towfigh A, Hulley BJ, Shekelle PG. Diagnosing and managing common food allergies: a systematic review. *JAMA* 2010; **303**: 1848-1856 [PMID: 20460624 DOI: 10.1001/jama.2010.582]
5 **Small M**, Vickers A, Anderson P, Kay S. The patient-physician partnership in asthma: real-world observations associated with clinical and patient-reported outcomes. *Adv Ther* 2010; **27**: 591-599 [PMID: 20680534 DOI: 10.1007/s12325-010-0054-1]
6 **Jonsson M**, Egmar AC, Hallner E, Kull I. Experiences of living with asthma - a focus group study with adolescents and parents of children with asthma. *J Asthma* 2014; **51**: 185-192 [PMID: 24192017 DOI: 10.3109/02770903.2013.853080]
7 **Meltzer EO**. Allergic rhinitis: the impact of discordant perspectives of patient and physician on treatment decisions. *Clin Ther* 2007; **29**: 1428-1440 [PMID: 17825694]
8 **Breiteneder H**, Ebner C. Molecular and biochemical classification of plant-derived food allergens. *J Allergy Clin Immunol* 2000; **106**: 27-36 [PMID: 10887301]
9 **Egger M**, Mutschlechner S, Wopfner N, Gadermaier G, Briza P, Ferreira F. Pollen-food syndromes associated with weed pollinosis: an update from the molecular point of view. *Allergy* 2006; **61**: 461-476 [PMID: 16512809]
10 **Han Y**, Kim J, Ahn K. Food allergy. *Korean J Pediatr* 2012; **55**: 153-158 [PMID: 22670149 DOI: 10.3345/kjp.2012.55.5.153]
11 **Melioli G**, Spenser C, Reggiardo G, Passalacqua G, Compalati E, Rogkakou A, Riccio AM, Di Leo E, Nettis E, Canonica GW. Allergenius, an expert system for the interpretation of allergen microarray results. *World Allergy Organ J* 2014; **7**: 15 [PMID: 24995073 DOI: 10.1186/1939-4551-7-15]
12 **Canonica GW**, Ansotegui IJ, Pawankar R, Schmid-Grendelmeier P, van Hage M, Baena-Cagnani CE, Melioli G, Nunes C, Passalacqua G, Rosenwasser L, Sampson H, Sastre J, Bousquet J, Zuberbier T. A WAO - ARIA - GA²LEN consensus document on molecular-based allergy diagnostics. *World Allergy Organ J* 2013; **6**: 17 [PMID: 24090398 DOI: 10.1186/1939-4551-6-17]
13 **Ferreira F**, Hawranek T, Gruber P, Wopfner N, Mari A. Allergic cross-reactivity: from gene to the clinic. *Allergy* 2004; **59**: 243-267 [PMID: 14982506]
14 **Aalberse RC**, Akkerdaas J, van Ree R. Cross-reactivity of IgE antibodies to allergens. *Allergy* 2001; **56**: 478-490 [PMID: 11421891]
15 **Hauser M**, Roulias A, Ferreira F, Egger M. Panallergens and their impact on the allergic patient. *Allergy Asthma Clin Immunol* 2010; **6**: 1 [PMID: 20298513 DOI: 10.1186/1710-1492-6-1]
16 **Ebo DG**, Hagendorens MM, Bridts CH, De Clerck LS, Stevens WJ. Sensitization to cross-reactive carbohydrate determinants and the ubiquitous protein profilin: mimickers of allergy. *Clin Exp Allergy* 2004; **34**: 137-144 [PMID: 14720274]
17 **Guilloux L**, Morisset M, Codreanu F, Parisot L, Moneret-Vautrin DA. Peanut allergy diagnosis in the context of grass pollen sensitization for 125 patients: roles of peanut and cross-reactive carbohydrate determinants specific IgE. *Int Arch Allergy Immunol* 2009; **149**: 91-97 [PMID: 19127064 DOI: 10.1159/000189190]
18 **Sastre J**. Molecular diagnosis in allergy. *Clin Exp Allergy* 2010; **40**: 1442-1460 [PMID: 20682003 DOI: 10.1111/j.1365-2222.2010.03585.x]
19 **Manduzio H**, Fitchette AC, Hrabina M, Chabre H, Batard T, Nony E, Faye L, Moingeon P, Gomord V. Glycoproteins are species-specific markers and major IgE reactants in grass pollens. *Plant Biotechnol J* 2012; **10**: 184-194 [PMID: 21951299 DOI: 10.1111/j.1467-7652.2011.00654.x]
20 **Popescu FD**. Molecular biomarkers for grass pollen immunotherapy. *World J Methodol* 2014; **4**: 26-45 [PMID: 25237628 DOI: 10.5662/wjm.v4.i1.26]
21 **Fernandes J**, Reshef A, Patton L, Ayuso R, Reese G, Lehrer SB. Immunoglobulin E antibody reactivity to the major shrimp allergen, tropomyosin, in unexposed Orthodox Jews. *Clin Exp Allergy* 2003; **33**: 956-961 [PMID: 12859453]
22 **Shafique RH**, Inam M, Ismail M, Chaudhary FR. Group 10 allergens (tropomyosins) from house-dust mites may cause covariation of sensitization to allergens from other invertebrates. *Allergy Rhinol* (Providence) 2012; **3**: e74-e90 [PMID: 23342293 DOI: 10.2500/ar.2012.3.0036]
23 **Kütting B**, Brehler R. House dust mite-crustaceans-molluscs syndrome. A rare variant of food allergy in primary sensitization to inhaled allergens. *Hautarzt* 2001; **52**: 708-711 [PMID: 11544942]
24 **Cudowska B**, Kaczmarski M, Wasilewska J. Cross allergic reactions in infants and toddlers with atopic dermatitis. *Adv Med Sci* 2013; **58**: 401-407 [PMID: 24176963 DOI: 10.2478/ams-2013-0022]
25 **Bohle B**. The impact of pollen-related food allergens on pollen allergy. *Allergy* 2007; **62**: 3-10 [PMID: 17156335]
26 **Skypala IJ**, Bull S, Deegan K, Gruffydd-Jones K, Holmes S, Small I, Emery PW, Durham SR. The prevalence of PFS and prevalence and characteristics of reported food allergy; a survey of UK adults aged 18-75 incorporating a validated PFS diagnostic questionnaire. *Clin Exp Allergy* 2013; **43**: 928-940 [PMID: 23889246 DOI: 10.1111/cea.12104]
27 **Passali D**, Bellusi LM, Kern EB, Sarafoleanu C, Popescu FD. Genes, allergens and inflammation in allergic rhinitis. In: Rhinosinusal inflammation and infections: modern thinking and current treatment. Bucharest: Editura Academiei Romane, 2013: 101-167
28 **Douladiris N**, Savvatianos S, Roumpedaki I, Skevaki C, Mitsias D, Papadopoulos NG. A molecular diagnostic algorithm to guide pollen immunotherapy in southern Europe: towards component-resolved management of allergic diseases. *Int Arch Allergy Immunol* 2013; **162**: 163-172 [PMID: 23921568]
29 **Popescu FD**, Vieru M, Tudose AM. Evaluation of birch pollen sensitization profile in allergic rhinoconjunctivitis patients from Bucharest region using component-resolved diagnosis. *Clin Transl Allergy* 2013; **3** Suppl 2: P3 [DOI: 10.1186/2045-7022-3-S2-P3]
30 **Rossi RE**, Melioli G, Monasterolo G, Harwanegg C, Rossi L, Canonica GW, Passalacqua G. Sensitization profiles in polysensitized patients from a restricted geographical area: further lessons from multiplexed component resolved diagnosis. *Eur Ann Allergy Clin Immunol* 2011; **43**: 171-175 [PMID: 22360133]
31 **Scaparrotta A**, Verini M, Consilvio NP, Cingolani A, Rapino D, Attanasi M, Cerasa M, Di Pillo S, Chiarelli F. Sensitization to timothy grass pollen allergenic molecules in children. *Multidiscip Respir Med* 2013; **8**: 17 [PMID: 23448255 DOI: 10.1186/2049-6958-8-17]
32 **Asero R**, Bellotto E, Ghiani A, Aina R, Villalta D, Citterio S. Concomitant sensitization to ragweed and mugwort pollen: who is who in clinical allergy? *Ann Allergy Asthma Immunol* 2014; **113**: 307-313 [PMID: 25053399 DOI: 10.1016/j.anai.2014.06.009]
33 **Rashid RS**, Smith KA, Nambiar KZ, Frew AJ, Tarzi MD. Pollen-food syndrome is related to Bet v 1/PR-10 protein sensitisation, but not all patients have spring rhinitis. *Allergy* 2011; **66**: 1391-1392 [PMID: 21569050 DOI: 10.1111/j.1398-9995.2011.02618.x]
34 **García BE**, Lizaso MT. Cross-reactivity syndromes in food allergy. *J Investig Allergol Clin Immunol* 2011; **21**: 162-170; quiz 2 p following 170 [PMID: 21548443]
35 **Asero R**, Villalta D. Are anti-Phl p 12 IgE levels predictive of oral allergy syndrome in profilin hypersensitive patients? *Eur Ann Allergy Clin Immunol* 2011; **43**: 184-187 [PMID: 22360135]
36 **Pauli G**, Metz-Favre C. Cross reactions between pollens and vegetable food allergens. *Rev Mal Respir* 2013; **30**: 328-337 [PMID: 23664291 DOI: 10.1016/j.rmr.2012.10.633]
37 **Lavaud F**, Fore M, Fontaine JF, Pérotin JM, de Blay F. Birch pollen allergy. *Rev Mal Respir* 2014; **31**: 150-161 [PMID: 24602682 DOI: 10.1016/j.rmr.2013.08.006]
38 **Hofmann C**, Scheurer S, Rost K, Graulich E, Jamin A, Foetisch K, Saloga J, Vieths S, Steinbrink K, Adler HS. Cor a 1-reactive T cells and IgE are predominantly cross-reactive to Bet v 1 in patients with birch pollen-associated food allergy to hazelnut. *J Allergy Clin Immunol* 2013; **131**: 1384-1392.e6 [PMID: 23246018 DOI: 10.1016/j.jaci.2012.10.037]
39 **Ebo DG**, Verweij MM, Sabato V, Hagendorens MM, Bridts CH, De Clerck LS. Hazelnut allergy: a multi-faced condition with

demographic and geographic characteristics. *Acta Clin Belg* 2012; **67**: 317-321 [PMID: 23189537]
40 **Mauro M**, Russello M, Incorvaia C, Gazzola G, Frati F, Moingeon P, Passalacqua G. Birch-apple syndrome treated with birch pollen immunotherapy. *Int Arch Allergy Immunol* 2011; **156**: 416-422 [PMID: 21832831 DOI: 10.1159/000323909]
41 **Ciprandi G**, Fenoglio G, Kalli F, De Amici M, Leonardi S, Miraglia Del Giudice M, Salpietro C, La Rosa M, Caimmi S, Marseglia GL. Patients with oral allergic syndrome to apple have intense proliferative response to BET V 1. *J Biol Regul Homeost Agents* 2012; **26**: S113-S117 [PMID: 22691258]
42 **Minov J**, Karadzinska-Bislimovska J, Stoleski S, Mijakoski D, Marsenic M, Risteska-Kuc S, Milkovska S. Pollen-fruit syndromes: a case with birch-apple-carrot association. *Am J Immunol* 2014; **10**: 88-92 [DOI: 10.3844/ajisp.2014.88.92]
43 **Andersen MB**, Hall S, Dragsted LO. Identification of european allergy patterns to the allergen families PR-10, LTP, and profilin from Rosaceae fruits. *Clin Rev Allergy Immunol* 2011; **41**: 4-19 [PMID: 19851893 DOI: 10.1007/s12016-009-8177-3]
44 **Le TM**, van Hoffen E, Lebens AF, Bruijnzeel-Koomen CA, Knulst AC. Anaphylactic versus mild reactions to hazelnut and apple in a birch-endemic area: different sensitization profiles? *Int Arch Allergy Immunol* 2013; **160**: 56-62 [PMID: 22948203 DOI: 10.1159/000339244]
45 **Vieru M**, Popescu FD, Tudose AM. Sensitisation pattern to birch pollen allergen components in oral allergy syndrome to Rosaceae fruits in patients with spring pollinosis from an East European Sylvosteppe area with low density forests. *Clin Transl Allergy* 2014; **4** Suppl 2: P47 [DOI: 10.1186/2045-7022-4-S2-P47]
46 **Cudowska B**, Kaczmarski M. Diagnostic value of birch recombinant allergens (rBet v 1, profilin rBet v 2) in children with pollen-related food allergy. *Rocz Akad Med Bialymst* 2004; **49**: 111-115 [PMID: 15631325]
47 **Kopac P**, Rudin M, Gentinetta T, Gerber R, Pichler Ch, Hausmann O, Schnyder B, Pichler WJ. Continuous apple consumption induces oral tolerance in birch-pollen-associated apple allergy. *Allergy* 2012; **67**: 280-285 [PMID: 22070352 DOI: 10.1111/j.1398-9995.2011.02744.x]
48 **Okamoto Y**, Kurihara K. [A case of oral allergy syndrome whose symptoms were dramatically improved after rush subcutaneous injection immunotherapy with pollen extracts of birch]. *Arerugi* 2012; **61**: 652-658 [PMID: 22705787]
49 **Asero R**. How long does the effect of birch pollen injection SIT on apple allergy last? *Allergy* 2003; **58**: 435-438 [PMID: 12752332]
50 **Bucher X**, Pichler WJ, Dahinden CA, Helbling A. Effect of tree pollen specific, subcutaneous immunotherapy on the oral allergy syndrome to apple and hazelnut. *Allergy* 2004; **59**: 1272-1276 [PMID: 15507095]
51 **Quiralte J**, Palacios L, Rodríguez R, Cárdaba B, Arias de Saavedra JM, Villalba M, Florido JF, Lahoz C. Modelling diseases: the allergens of Olea europaea pollen. *J Investig Allergol Clin Immunol* 2007; **17** Suppl 1: 24-30 [PMID: 18050568]
52 **Focke M**, Hemmer W, Wöhrl S, Götz M, Jarisch R. Cross-reactivity between Ficus benjamina latex and fig fruit in patients with clinical fig allergy. *Clin Exp Allergy* 2003; **33**: 971-977 [PMID: 12859455]
53 **Hemmer W**, Focke M, Götz M, Jarisch R. Sensitization to Ficus benjamina: relationship to natural rubber latex allergy and identification of foods implicated in the Ficus-fruit syndrome. *Clin Exp Allergy* 2004; **34**: 1251-1258 [PMID: 15298566]
54 **Miralles JC**, Caravaca F, Guillén F, Lombardero M, Negro JM. Cross-reactivity between Platanus pollen and vegetables. *Allergy* 2002; **57**: 146-149 [PMID: 11929418]
55 **Enrique E**, Cisteró-Bahíma A, Bartolomé B, Alonso R, San Miguel-Moncín MM, Bartra J, Martínez A. Platanus acerifolia pollinosis and food allergy. *Allergy* 2002; **57**: 351-356 [PMID: 11906368]
56 **Enrique E**, Alonso R, Bartolomé B, San Miguel-Moncín M, Bartra J, Fernández-Parra B, Tella R, Asturias JA, Ibarrola I, Martínez A, Cisteró-Bahíma A. IgE reactivity to profilin in Platanus acerifolia pollen-sensitized subjects with plant-derived food allergy. *J Investig Allergol Clin Immunol* 2004; **14**: 335-342 [PMID: 15736720]
57 **Salari F**, Vahedi F, Chamani J, Varasteh A, Ketabdar H, Sankian M. Efficient expression of a soluble lipid transfer protein (LTP) of Platanus orientalis using short peptide tags and structural comparison with the natural form. *Biotechnol Appl Biochem* 2015; **62**: 218-225 [PMID: 24750344 DOI: 10.1002/bab.1235]
58 **Caimmi D**, Barber D, Hoffmann-Sommergruber K, Amrane H, Bousquet PJ, Dhivert-Donnadieu H, Demoly P. Understanding the molecular sensitization for Cypress pollen and peach in the Languedoc-Roussillon area. *Allergy* 2013; **68**: 249-251 [PMID: 23205629 DOI: 10.1111/all.12073]
59 **Kwaasi AA**, Harfi HA, Parhar RS, Saleh S, Collison KS, Panzani RC, Al-Sedairy ST, Al-Mohanna FA. Cross-reactivities between date palm (Phoenix dactylifera L.) polypeptides and foods implicated in the oral allergy syndrome. *Allergy* 2002; **57**: 508-518 [PMID: 12028116]
60 **Mur P**, Feo Brito F, Bartolomé B, Galindo PA, Gómez E, Borja J, Alonso A. Simultaneous allergy to vine pollen and grape. *J Investig Allergol Clin Immunol* 2006; **16**: 271-273 [PMID: 16889288]
61 **Sankian M**, Varasteh A, Pazouki N, Mahmoudi M. Sequence homology: a poor predictive value for profilins cross-reactivity. *Clin Mol Allergy* 2005; **3**: 13 [PMID: 16153305]
62 **Pasini G**, Simonato B, Curioni A, Vincenzi S, Cristaudo A, Santucci B, Peruffo AD, Giannattasio M. IgE-mediated allergy to corn: a 50 kDa protein, belonging to the Reduced Soluble Proteins, is a major allergen. *Allergy* 2002; **57**: 98-106 [PMID: 11929411]
63 **Weichel M**, Vergoossen NJ, Bonomi S, Scibilia J, Ortolani C, Ballmer-Weber BK, Pastorello EA, Crameri R. Screening the allergenic repertoires of wheat and maize with sera from double-blind, placebo-controlled food challenge positive patients. *Allergy* 2006; **61**: 128-135 [PMID: 16364168]
64 **Freeman GL**. Oral corn pollen hypersensitivity in Arizona Native Americans: some sociologic aspects of allergy practice. *Ann Allergy* 1994; **72**: 415-417 [PMID: 8179227]
65 **Popescu FD**, Vieru M. Molecular allergology approach in a patient with corn silk (Stigma maydis) infusion and pollen allergy. *Allergy* 2014; **69** (Suppl 99): 462 [DOI: 10.1111/all.12478]
66 **Gadermaier G**, Hauser M, Ferreira F. Allergens of weed pollen: an overview on recombinant and natural molecules. *Methods* 2014; **66**: 55-66 [PMID: 23806644 DOI: 10.1016/j.ymeth.2013.06.014]
67 **Yagami A**, Nakazawa Y, Suzuki K, Matsunaga K. Curry spice allergy associated with pollen-food allergy syndrome and latex fruit-syndrome. *J Dermatol* 2009; **36**: 45-49 [PMID: 19207436 DOI: 10.1111/j.1346-8138.2008.00584.x]
68 **Borghesan F**, Mistrello G, Amato S, Giuffrida MG, Villalta D, Asero R. Mugwort-fennel-allergy-syndrome associated with sensitization to an allergen homologous to Api g 5. *Eur Ann Allergy Clin Immunol* 2013; **45**: 130-137 [PMID: 24067338]
69 **Faeste CK**, Jonscher KR, Sit L, Klawitter J, Løvberg KE, Moen LH. Differentiating cross-reacting allergens in the immunological analysis of celery (Apium graveolens) by mass spectrometry. *J AOAC Int* 2010; **93**: 451-461 [PMID: 20480890]
70 **Lombardero M**, García-Sellés FJ, Polo F, Jimeno L, Chamorro MJ, García-Casado G, Sánchez-Monge R, Díaz-Perales A, Salcedo G, Barber D. Prevalence of sensitization to Artemisia allergens Art v 1, Art v 3 and Art v 60 kDa. Cross-reactivity among Art v 3 and other relevant lipid-transfer protein allergens. *Clin Exp Allergy* 2004; **34**: 1415-1421 [PMID: 15347375]
71 **Gao ZS**, Yang ZW, Wu SD, Wang HY, Liu ML, Mao WL, Wang J, Gadermaier G, Ferreira F, Zheng M, van Ree R. Peach allergy in China: a dominant role for mugwort pollen lipid transfer protein as a primary sensitizer. *J Allergy Clin Immunol* 2013; **131**: 224-226. e1-e3 [PMID: 22939759 DOI: 10.1016/j.jaci.2012.07.015]
72 **Garrido S**, García BE, Echechipía S, Sanz ML, Ariz S, Tabar AI. Anaphylaxis following the first ingestion of lychee fruit: clinical features and immunological cross-reactivity implications. *Allergy* 2007; **62**: 962-963 [PMID: 17484730]
73 **de la Torre Morín F**, Sánchez Machín I, García Robaina JC, Fernández-Caldas E, Sánchez Triviño M. Clinical cross-reactivity between Artemisia vulgaris and Matricaria chamomilla (chamomile). *J Investig Allergol Clin Immunol* 2001; **11**: 118-122 [PMID:

11642570]
74 **Reider N**, Sepp N, Fritsch P, Weinlich G, Jensen-Jarolim E. Anaphylaxis to camomile: clinical features and allergen cross-reactivity. *Clin Exp Allergy* 2000; **30**: 1436-1443 [PMID: 10998021]
75 **Andres C**, Chen WC, Ollert M, Mempel M, Darsow U, Ring J. Anaphylactic reaction to camomile tea. *Allergol Int* 2009; **58**: 135-136 [PMID: 19050375 DOI: 10.2332/allergolint.C-08-63]
76 **Rottem M**, Waisel Y. Food allergy to concealed sunflower pollen. *Allergy* 1998; **53**: 719-720 [PMID: 9700046]
77 **Palma-Carlos AG**, Palma-Carlos ML, Tengarrinha F. Allergy to sunflower seeds. *Eur Ann Allergy Clin Immunol* 2005; **37**: 183-186 [PMID: 15984317]
78 **Gruber P**, Gadermaier G, Bauer R, Weiss R, Wagner S, Leonard R, Breiteneder H, Ebner C, Ferreira F, Egger M. Role of the polypeptide backbone and post-translational modifications in cross-reactivity of Art v 1, the major mugwort pollen allergen. *Biol Chem* 2009; **390**: 445-451 [PMID: 19361284 DOI: 10.1515/BC.2009.063]
79 **Berecz B**, Clare Mills EN, Parádi I, Láng F, Tamás L, Shewry PR, Mackie AR. Stability of sunflower 2S albumins and LTP to physiologically relevant in vitro gastrointestinal digestion. *Food Chem* 2013; **138**: 2374-2381 [PMID: 23497898 DOI: 10.1016/j.foodchem.2012.12.034]
80 **Lombardi C**, Senna GE, Gatti B, Feligioni M, Riva G, Bonadonna P, Dama AR, Canonica GW, Passalacqua G. Allergic reactions to honey and royal jelly and their relationship with sensitization to compositae. *Allergol Immunopathol* (Madr) 1998; **26**: 288-290 [PMID: 9934408]
81 **Choi JH**, Jang YS, Oh JW, Kim CH, Hyun IG. Bee pollen-induced anaphylaxis: a case report and literature review. *Allergy Asthma Immunol Res* 2014; **6**: e295. Available from: URL: http://e-aair.org/Synapse/Data/PDFData/9999AAIR/aair-6-e295.pdf
82 **Fuiano N**, Incorvaia C, Riario-Sforza GG, Casino G. Anaphylaxis to honey in pollinosis to mugwort: a case report. *Eur Ann Allergy Clin Immunol* 2006; **38**: 364-365 [PMID: 17274522]
83 **Paola F**, Pantalea DD, Gianfranco C, Antonio F, Angelo V, Eustachio N, Elisabetta DL. Oral allergy syndrome in a child provoked by royal jelly. *Case Rep Med* 2014; **2014**: 941248 [PMID: 24799914 DOI: 10.1155/2014/941248]
84 **Asero R**, Mistrello G, Amato S. The nature of melon allergy in ragweed-allergic subjects: A study of 1000 patients. *Allergy Asthma Proc* 2011; **32**: 64-67 [PMID: 21262100 DOI: 10.2500/aap.2011.32.3416]
85 Don't take echinacea if you're allergic to ragweed. *Consum Rep* 2012; **77**: 12 [PMID: 22368799]
86 **Liccardi G**, Russo M, Mistrello G, Falagiani P, D'Amato M, D' Amato G. Sensitization to pistachio is common in Parietaria allergy. *Allergy* 1999; **54**: 643-645 [PMID: 10435484]
87 **Fernández C**, Fiandor A, Martinez-Garate A, Martinez Quesada J. Allergy to pistachio: crossreactivity between pistachio nut and other Anacardiaceae. *Clin Exp Allergy* 1995; **25**: 1254-1259 [PMID: 8821307]
88 **Bernedo N**, García M, Gastaminza G, Fernández E, Bartolomé B, Algorta J, Muñoz D. Allergy to laxative compound (Plantago ovata seed) among health care professionals. *J Investig Allergol Clin Immunol* 2008; **18**: 181-189 [PMID: 18564629]
89 **Park HS**, Jung KS, Jee SY, Hong SH, Kim HY, Nahm DH. Are there any links between Hop Japanese pollen and other weed pollens or food allergens on skin prick tests? *Allergy Asthma Proc* 2001; **22**: 43-46 [PMID: 11227917]
90 **Crameri R**, Garbani M, Rhyner C, Huitema C. Fungi: the neglected allergenic sources. *Allergy* 2014; **69**: 176-185 [PMID: 24286281 DOI: 10.1111/all.12325]
91 **Chruszcz M**, Chapman MD, Osinski T, Solberg R, Demas M, Porebski PJ, Majorek KA, Pomés A, Minor W. Alternaria alternata allergen Alt a 1: a unique β-barrel protein dimer found exclusively in fungi. *J Allergy Clin Immunol* 2012; **130**: 241-7.e9 [PMID: 22664167 DOI: 10.1016/j.jaci.2012.03.047]
92 **Herrera I**, Moneo I, Caballero ML, de Paz S, Perez Pimiento A, Rebollo S. Food allergy to spinach and mushroom. *Allergy* 2002; **57**: 261-262 [PMID: 11906344]
93 **Herrera-Mozo I**, Ferrer B, Luís Rodriguez-Sanchez J, Juarez C. Description of a novel panallergen of cross-reactivity between moulds and foods. *Immunol Invest* 2006; **35**: 181-197 [PMID: 16698676]
94 **Hegde VL**, Venkatesh YP. Anaphylaxis to excipient mannitol: evidence for an immunoglobulin E-mediated mechanism. *Clin Exp Allergy* 2004; **34**: 1602-1609 [PMID: 15479277]
95 **Katona SJ**, Kaminski ER. Sensitivity to Quorn mycoprotein (Fusarium venenatum) in a mould allergic patient. *J Clin Pathol* 2002; **55**: 876-877 [PMID: 12401831]
96 **Hoff M**, Trüeb RM, Ballmer-Weber BK, Vieths S, Wuethrich B. Immediate-type hypersensitivity reaction to ingestion of mycoprotein (Quorn) in a patient allergic to molds caused by acidic ribosomal protein P2. *J Allergy Clin Immunol* 2003; **111**: 1106-1110 [PMID: 12743577]
97 **Bennett AT**, Collins KA. An unusual case of anaphylaxis. Mold in pancake mix. *Am J Forensic Med Pathol* 2001; **22**: 292-295 [PMID: 11563743]
98 **Airola K**, Petman L, Mäkinen-Kiljunen S. Clustered sensitivity to fungi: anaphylactic reactions caused by ingestive allergy to yeasts. *Ann Allergy Asthma Immunol* 2006; **97**: 294-297 [PMID: 17042133]
99 **Dales R**, Liu L, Wheeler AJ, Gilbert NL. Quality of indoor residential air and health. *CMAJ* 2008; **179**: 147-152 [PMID: 18625986 DOI: 10.1503/cmaj.070359]
100 **Mary CL**, López-Malpica F, Díaz AM. Analysis of cross-reactivity between group 1 allergens from mites. *P R Health Sci J* 2008; **27**: 163-170 [PMID: 18616045]
101 **Fernández-Caldas E**, Puerta L, Caraballo L. Mites and allergy. *Chem Immunol Allergy* 2014; **100**: 234-242 [PMID: 24925403 DOI: 10.1159/000358860]
102 **Iancovici-Kidon M**, Tim CF. Component-specific immunoglobulin E in the diagnosis of allergic disease in childhood: more of the same or something more? *Isr Med Assoc J* 2007; **9**: 476-478 [PMID: 17642400]
103 **Lopata AL**, Lehrer SB. New insights into seafood allergy. *Curr Opin Allergy Clin Immunol* 2009; **9**: 270-277 [PMID: 19398906 DOI: 10.1097/ACI.0b013e32832b3e6f]
104 **Woo CK**, Bahna SL. Not all shellfish "allergy" is allergy! *Clin Transl Allergy* 2011; **1**: 3 [PMID: 22410209 DOI: 10.1186/2045-7022-1-3]
105 **Pedrosa M**, Boyano-Martínez T, García-Ara C, Quirce S. Shellfish Allergy: a Comprehensive Review. *Clin Rev Allergy Immunol* 2014 May 29; Epub ahead of print [PMID: 24870065]
106 **Witteman AM**, Akkerdaas JH, van Leeuwen J, van der Zee JS, Aalberse RC. Identification of a cross-reactive allergen (presumably tropomyosin) in shrimp, mite and insects. *Int Arch Allergy Immunol* 1994; **105**: 56-61 [PMID: 8086829]
107 **Becker S**, Gröger M, Canis M, Pfrogner E, Kramer MF. Tropomyosin sensitization in house dust mite allergic patients. *Eur Arch Otorhinolaryngol* 2012; **269**: 1291-1296 [PMID: 22081096 DOI: 10.1007/s00405-011-1826-1]
108 **Reese G**, Ayuso R, Lehrer SB. Tropomyosin: an invertebrate pan-allergen. *Int Arch Allergy Immunol* 1999; **119**: 247-258 [PMID: 10474029]
109 **Asturias JA**, Arilla MC, Gómez-Bayón N, Martínez A, Martínez J, Palacios R. Sequencing and high level expression in Escherichia coli of the tropomyosin allergen (Der p 10) from Dermatophagoides pteronyssinus. *Biochim Biophys Acta* 1998; **1397**: 27-30 [PMID: 9545522]
110 **Ivanciuc O**, Garcia T, Torres M, Schein CH, Braun W. Characteristic motifs for families of allergenic proteins. *Mol Immunol* 2009; **46**: 559-568 [PMID: 18951633 DOI: 10.1016/j.molimm.2008.07.034]
111 **Liu GM**, Huang YY, Cai QF, Weng WY, Su WJ, Cao MJ. Comparative study of in vitro digestibility of major allergen, tropomyosin and other proteins between Grass prawn (Penaeus monodon) and Pacific white shrimp (Litopenaeus vannamei). *J Sci Food Agric* 2011; **91**: 163-170 [PMID: 20853272 DOI: 10.1002/jsfa.4167]
112 **González-Fernández J**, Rodero M, Daschner A, Cuéllar C. New insights into the allergenicity of tropomyosin: a bioinformatics approach. *Mol Biol Rep* 2014; **41**: 6509-6517 [PMID: 24985979

DOI: 10.1007/s11033-014-3534-6]
113 **Pevec B**, Pevec MR, Markovic AS, Batista I. House dust mite subcutaneous immunotherapy does not induce new sensitization to tropomyosin: does it do the opposite? *J Investig Allergol Clin Immunol* 2014; **24**: 29-34 [PMID: 24765878]
114 **Weghofer M**, Thomas WR, Kronqvist M, Mari A, Purohit A, Pauli G, Horak F, Grönlund H, van Hage M, Valenta R, Vrtala S. Variability of IgE reactivity profiles among European mite allergic patients. *Eur J Clin Invest* 2008; **38**: 959-965 [PMID: 19021722 DOI: 10.1111/j.1365-2362.2008.02048.x]
115 **Shiomi K**. Current knowledge on molecular features of seafood allergens. *Shokuhin Eiseigaku Zasshi* 2010; **51**: 139-152 [PMID: 20827050]
116 **Taylor SL**. Molluscan shellfish allergy. *Adv Food Nutr Res* 2008; **54**: 139-177 [PMID: 18291306 DOI: 10.1016/S1043-4526(07)00004-6]
117 **Bauermeister K**, Wangorsch A, Garoffo LP, Reuter A, Conti A, Taylor SL, Lidholm J, Dewitt AM, Enrique E, Vieths S, Holzhauser T, Ballmer-Weber B, Reese G. Generation of a comprehensive panel of crustacean allergens from the North Sea Shrimp Crangon crangon. *Mol Immunol* 2011; **48**: 1983-1992 [PMID: 21784530 DOI: 10.1016/j.molimm.2011.06.216]
118 **Binder M**, Mahler V, Hayek B, Sperr WR, Schöller M, Prozell S, Wiedermann G, Valent P, Valenta R, Duchêne M. Molecular and immunological characterization of arginine kinase from the Indianmeal moth, Plodia interpunctella, a novel cross-reactive invertebrate pan-allergen. *J Immunol* 2001; **167**: 5470-5477 [PMID: 11673567]
119 **Uda K**, Fujimoto N, Akiyama Y, Mizuta K, Tanaka K, Ellington WR, Suzuki T. Evolution of the arginine kinase gene family. *Comp Biochem Physiol Part D Genomics Proteomics* 2006; **1**: 209-218 [PMID: 20483252 DOI: 10.1016/j.cbd.2005.10.007]
120 **Anas M**, Rahman A, Helleur RJ, Jeebhay MF, Lopata AL. Characterization of seafood proteins causing allergic diseases, allergic diseases - Highlights in the Clinic, Mechanisms and Treatment. Pereira C, editor. 2012 [DOI: 10.5772/25316]
121 **Shen HW**, Cao MJ, Cai QF, Ruan MM, Mao HY, Su WJ, Liu GM. Purification, cloning, and immunological characterization of arginine kinase, a novel allergen of Octopus fangsiao. *J Agric Food Chem* 2012; **60**: 2190-2199 [PMID: 22303807 DOI: 10.1021/jf203779w]
122 **Ayuso R**, Grishina G, Bardina L, Carrillo T, Blanco C, Ibáñez MD, Sampson HA, Beyer K. Myosin light chain is a novel shrimp allergen, Lit v 3. *J Allergy Clin Immunol* 2008; **122**: 795-802 [PMID: 18760458 DOI: 10.1016/j.jaci.2008.07.023]
123 **An S**, Chen L, Long C, Liu X, Xu X, Lu X, Rong M, Liu Z, Lai R. Dermatophagoides farinae allergens diversity identification by proteomics. *Mol Cell Proteomics* 2013; **12**: 1818-1828 [PMID: 23481662 DOI: 10.1074/mcp.M112.027136]
124 **Tsai LC**, Peng HJ, Lee CS, Chao PL, Tang RB, Tsai JJ, Shen HD, Hung MW, Han SH. Molecular cloning and characterization of full-length cDNAs encoding a novel high-molecular-weight Dermatophagoides pteronyssinus mite allergen, Der p 11. *Allergy* 2005; **60**: 927-937 [PMID: 15932384]
125 **Galler S**. Molecular basis of the catch state in molluscan smooth muscles: a catchy challenge. *J Muscle Res Cell Motil* 2008; **29**: 73-99 [PMID: 19039672 DOI: 10.1007/s10974-008-9149-6]
126 **Azofra J**, Lombardero M. Limpet anaphylaxis: cross-reactivity between limpet and house-dust mite Dermatophagoides pteronyssinus. *Allergy* 2003; **58**: 146-149 [PMID: 12622746]
127 **Iparraguirre A**, Rodríguez-Pérez R, Juste S, Ledesma A, Moneo I, Caballero ML. Selective allergy to lobster in a case of primary sensitization to house dust mites. *J Investig Allergol Clin Immunol* 2009; **19**: 409-413 [PMID: 19862942]
128 **Bessot JC**, Metz-Favre C, Rame JM, De Blay F, Pauli G. Tropomyosin or not tropomyosin, what is the relevant allergen in house dust mite and snail cross allergies? *Eur Ann Allergy Clin Immunol* 2010; **42**: 3-10 [PMID: 20355359]
129 **Gámez C**, Zafra M, Boquete M, Sanz V, Mazzeo C, Ibáñez MD, Sánchez-García S, Sastre J, del Pozo V. New shrimp IgE-binding proteins involved in mite-seafood cross-reactivity. *Mol Nutr Food Res* 2014; **58**: 1915-1925 [PMID: 24978201 DOI: 10.1002/mnfr.201400122]
130 **Resch Y**, Weghofer M, Seiberler S, Horak F, Scheiblhofer S, Linhart B, Swoboda I, Thomas WR, Thalhamer J, Valenta R, Vrtala S. Molecular characterization of Der p 10: a diagnostic marker for broad sensitization in house dust mite allergy. *Clin Exp Allergy* 2011; **41**: 1468-1477 [PMID: 21711470 DOI: 10.1111/j.1365-2222.2011.03798.x]
131 **Rossi RE**, Monasterolo G, Incorvaia C, Moingeon P, Frati F, Passalacqua G, Rossi L, Canonica GW. Lack of neo-sensitization to Pen a 1 in patients treated with mite sublingual immunotherapy. *Clin Mol Allergy* 2010; **8**: 4 [PMID: 20230633 DOI: 10.1186/1476-7961-8-4]
132 **van Ree R**, Antonicelli L, Akkerdaas JH, Garritani MS, Aalberse RC, Bonifazi F. Possible induction of food allergy during mite immunotherapy. *Allergy* 1996; **51**: 108-113 [PMID: 8738516]
133 **Pajno GB**, La Grutta S, Barberio G, Canonica GW, Passalacqua G. Harmful effect of immunotherapy in children with combined snail and mite allergy. *J Allergy Clin Immunol* 2002; **109**: 627-629 [PMID: 11941311]
134 **Asero R**. Lack of de novo sensitization to tropomyosin in a group of mite-allergic patients treated by house dust mite-specific immunotherapy. *Int Arch Allergy Immunol* 2005; **137**: 62-65 [PMID: 15832051]
135 **Meglio P**, Plantamura M, Arabito E, Falagiani P, Torre A, Rossi P. Does SIT to Der p protect from snail sensitization? *Allergy* 2002; **57**: 868-869 [PMID: 12169197]
136 **Cortellini G**, Spadolini I, Santucci A, Cova V, Conti C, Corvetta A, Passalacqua G. Improvement of shrimp allergy after sublingual immunotherapy for house dust mites: a case report. *Eur Ann Allergy Clin Immunol* 2011; **43**: 162-164 [PMID: 22145252]
137 **Panzani RC**, Ariano R. Arthropods and invertebrates allergy (with the exclusion of mites): the concept of panallergy. *Allergy* 2001; **56** Suppl 69: 1-22 [PMID: 11990954]
138 **Meseguer Arce J**, Villajos IM, Iraola V, Carnés J, Fernández Caldas E. Occupational allergy to aquarium fish food: red midge larva, freshwater shrimp, and earthworm. A clinical and immunological study. *J Investig Allergol Clin Immunol* 2013; **23**: 462-470 [PMID: 24654310]
139 **Sánchez-Borges M**, Suárez-Chacon R, Capriles-Hulett A, Caballero-Fonseca F, Iraola V, Fernández-Caldas E. Pancake syndrome (oral mite anaphylaxis). *World Allergy Organ J* 2009; **2**: 91-96 [PMID: 23283016 DOI: 10.1097/WOX.0b013e3181a0db50]
140 **Takahashi K**, Taniguchi M, Fukutomi Y, Sekiya K, Watai K, Mitsui C, Tanimoto H, Oshikata C, Tsuburai T, Tsurikisawa N, Minoguchi K, Nakajima H, Akiyama K. Oral Mite Anaphylaxis Caused by Mite-Contaminated Okonomiyaki/Pancake-Mix in Japan: 8 Case Reports and a Review of 28 Reported Cases. *Allergol Int* 2013 Dec 25; Epub ahead of print [PMID: 24368585]
141 **Sánchez-Borges M**, Suárez Chacón R, Capriles-Hulett A, Caballero-Fonseca F, Fernández-Caldas E. Anaphylaxis from ingestion of mites: pancake anaphylaxis. *J Allergy Clin Immunol* 2013; **131**: 31-35 [PMID: 23154081 DOI: 10.1016/j.jaci.2012.09.026]
142 **Sánchez-Borges M**, Fernández-Caldas E, Capriles-Hulett A, Caballero-Fonseca F. Mite-induced inflammation: More than allergy. *Allergy Rhinol* (Providence) 2012; **3**: e25-e29 [PMID: 22852126 DOI: 10.2500/ar.2012.3.0025]
143 **Izumi K**, Aihara M, Ikezawa Z. Effects of non steroidal antiinflammatory drugs (NSAIDs) on immediate-type food allergy analysis of Japanese cases from 1998 to 2009. *Arerugi* 2009; **58**: 1629-1639 [PMID: 20220304]
144 **Fernando SL**, Clarke LR. Salicylate intolerance: a masquerader of multiple adverse drug reactions. *BMJ Case Rep* 2009; **2009**: pii: bcr02.2009.1602 [PMID: 21918670 DOI: 10.1136/bcr.02.2009.1602]
145 **Portnoy J**, Chew GL, Phipatanakul W, Williams PB, Grimes C, Kennedy K, Matsui EC, Miller JD, Bernstein D, Blessing-Moore J, Cox L, Khan D, Lang D, Nicklas R, Oppenheimer J, Randolph C, Schuller D, Spector S, Tilles SA, Wallace D, Seltzer J, Sublett J. Environmental assessment and exposure reduction of cockroaches: a practice parameter. *J Allergy Clin Immunol* 2013; **132**: 802-808.

e1-8.25 [PMID: 23938214 DOI: 10.1016/j.jaci.2013.04.061]
146 **Santiago HC**, LeeVan E, Bennuru S, Ribeiro-Gomes F, Mueller E, Wilson M, Wynn T, Garboczi D, Urban J, Mitre E, Nutman TB. Molecular mimicry between cockroach and helminth glutathione S-transferases promotes cross-reactivity and cross-sensitization. *J Allergy Clin Immunol* 2012; **130**: 248-56.e9 [PMID: 22541242 DOI: 10.1016/j.jaci.2012.02.045]
147 **Mohamad Yadzir ZH**, Misnan R, Abdullah N, Bakhtiar F, Leecyous B, Murad S. Component-resolved diagnosis (CRD): Is it worth it? frequency and differentiation in rhinitis patients with mite reactivity. *Iran J Allergy Asthma Immunol* 2014; **13**: 240-246 [PMID: 24659159]
148 **Jeong KY**, Lee J, Lee IY, Ree HI, Hong CS, Yong TS. Allergenicity of recombinant Bla g 7, German cockroach tropomyosin. *Allergy* 2003; **58**: 1059-1063 [PMID: 14510726]
149 **Giuffrida MG**, Villalta D, Mistrello G, Amato S, Asero R. Shrimp allergy beyond Tropomyosin in Italy: clinical relevance of Arginine Kinase, Sarcoplasmic calcium binding protein and Hemocyanin. *Eur Ann Allergy Clin Immunol* 2014; **46**: 172-177 [PMID: 25224947]
150 **Pomés A**, Arruda LK. Investigating cockroach allergens: aiming to improve diagnosis and treatment of cockroach allergic patients. *Methods* 2014; **66**: 75-85 [PMID: 23916425 DOI: 10.1016/j.ymeth.2013.07.036]
151 **Liu Z**, Xia L, Wu Y, Xia Q, Chen J, Roux KH. Identification and characterization of an arginine kinase as a major allergen from silkworm (Bombyx mori) larvae. *Int Arch Allergy Immunol* 2009; **150**: 8-14 [PMID: 19339797 DOI: 10.1159/000210375]
152 **Belluco S**, Losasso C, Maggioletti M, Alonzi CC, Paoletti MG, Ricci A. Edible insects in a food safety and nutritional perspective: a critical review. *CRFSFS* 2013; **12**: 296-313 [DOI: 10.1111/1541-4337.12014]
153 **Sun B**, Zheng P, Wei N, Huang H, Zeng G. Co-sensitization to silkworm moth (Bombyx mori) and 9 inhalant allergens among allergic patients in Guangzhou, Southern China. *PLoS One* 2014; **9**: e94776 [PMID: 24787549 DOI: 10.1371/journal.pone.0094776]
154 **Kung SJ**, Fenemore B, Potter PC. Anaphylaxis to Mopane worms (Imbrasia belina). *Ann Allergy Asthma Immunol* 2011; **106**: 538-540 [PMID: 21624756 DOI: 10.1016/j.anai.2011.02.003]
155 **Armentia A**, Lombardero M, Blanco C, Fernández S, Fernández A, Sánchez-Monge R. Allergic hypersensitivity to the lentil pest Bruchus lentis. *Allergy* 2006; **61**: 1112-1116 [PMID: 16918515]
156 **Tabar AI**, Acero S, Arregui C, Urdánoz M, Quirce S. [Asthma and allergy due to carmine dye]. *An Sist Sanit Navar* 2003; **26** Suppl 2: 65-73 [PMID: 13679965]
157 **Ohgiya Y**, Arakawa F, Akiyama H, Yoshioka Y, Hayashi Y, Sakai S, Ito S, Yamakawa Y, Ohgiya S, Ikezawa Z, Teshima R. Molecular cloning, expression, and characterization of a major 38-kd cochineal allergen. *J Allergy Clin Immunol* 2009; **123**: 1157-1162, 1157-1162e1-e4 [PMID: 19249084 DOI: 10.1016/j.jaci.2008.12.1111]
158 **Gultekin F**, Doguc DK. Allergic and immunologic reactions to food additives. *Clin Rev Allergy Immunol* 2013; **45**: 6-29 [PMID: 22278172 DOI: 10.1007/s12016-012-8300-8]
159 **Voltolini S**, Pellegrini S, Contatore M, Bignardi D, Minale P. New risks from ancient food dyes: cochineal red allergy. *Eur Ann Allergy Clin Immunol* 2014; **46**: 232-233 [PMID: 25398168]
160 **Hentges F**, Léonard C, Arumugam K, Hilger C. Immune responses to inhalant Mammalian allergens. *Front Immunol* 2014; **5**: 234 [PMID: 24904583 DOI: 10.3389/fimmu.2014.00234]
161 **Borres MP**, Ebisawa M, Eigenmann PA. Use of allergen components begins a new era in pediatric allergology. *Pediatr Allergy Immunol* 2011; **22**: 454-461 [PMID: 21771081 DOI: 10.1111/j.1399-3038.2011.01197.x]
162 **Nordlund B**, Konradsen JR, Kull I, Borres MP, Önell A, Hedlin G, Grönlund H. IgE antibodies to animal-derived lipocalin, kallikrein and secretoglobin are markers of bronchial inflammation in severe childhood asthma. *Allergy* 2012; **67**: 661-669 [PMID: 22339365 DOI: 10.1111/j.1398-9995.2012.02797.x]
163 **Díaz-Perales A**, González-de-Olano D, Pérez-Gordo M, Pastor-Vargas C. Allergy to uncommon pets: new allergies but the same allergens. *Front Immunol* 2013; **4**: 492 [PMID: 24416032 DOI: 10.3389/fimmu.2013.00492]
164 **Zahradnik E**, Raulf M. Animal allergens and their presence in the environment. *Front Immunol* 2014; **5**: 76 [PMID: 24624129 DOI: 10.3389/fimmu.2014.00076]
165 **Grönlund H**, Saarne T, Gafvelin G, van Hage M. The major cat allergen, Fel d 1, in diagnosis and therapy. *Int Arch Allergy Immunol* 2010; **151**: 265-274 [PMID: 19844127 DOI: 10.1159/000250435]
166 **Savi E**, Rossi A, Incorvaia C. Cat-pork syndrome: a case report with a thee years follow-up. *Eur Ann Allergy Clin Immunol* 2006; **38**: 366-368 [PMID: 17274523]
167 **Sabbah A**, Lauret MG, Chène J, Boutet S, Drouet M. [The pork-cat syndrome or crossed allergy between pork meat and cat epithelia (2)]. *Allerg Immunol* (Paris) 1994; **26**: 173-714, 177-180 [PMID: 7522011]
168 **Hilger C**, Kohnen M, Grigioni F, Lehners C, Hentges F. Allergic cross-reactions between cat and pig serum albumin. Study at the protein and DNA levels. *Allergy* 1997; **52**: 179-187 [PMID: 9105522]
169 **Drouet M**, Sabbah A, Le Sellin J, Bonneau JC, Gay G, Dubois-Gosnet C. [Fatal anaphylaxis after eating wild boar meat in a patient with pork-cat syndrome]. *Allerg Immunol* (Paris) 2001; **33**: 163-165 [PMID: 11434195]
170 **Posthumus J**, James HR, Lane CJ, Matos LA, Platts-Mills TA, Commins SP. Initial description of pork-cat syndrome in the United States. *J Allergy Clin Immunol* 2013; **131**: 923-925 [PMID: 23352634 DOI: 10.1016/j.jaci.2012.12.665]
171 **Commins SP**, Platts-Mills TA. Delayed anaphylaxis to red meat in patients with IgE specific for galactose alpha-1,3-galactose (alpha-gal). *Curr Allergy Asthma Rep* 2013; **13**: 72-77 [PMID: 23054628 DOI: 10.1007/s11882-012-0315-y]
172 **Alvarez-Perea A**, Caralli ME, Zubeldia JM, Baeza ML. Pork-cat syndrome as a cause of occupational asthma. *J Investig Allergol Clin Immunol* 2014; **24**: 209-211 [PMID: 25011365]
173 **Hilger C**, Swiontek K, Hentges F, Donnay C, de Blay F, Pauli G. Occupational inhalant allergy to pork followed by food allergy to pork and chicken: sensitization to hemoglobin and serum albumin. *Int Arch Allergy Immunol* 2010; **151**: 173-178 [PMID: 19752572 DOI: 10.1159/000236008]
174 **Choi GS**, Kim JH, Lee HN, Sung JM, Lee JW, Park HS. Occupational asthma caused by inhalation of bovine serum albumin powder. *Allergy Asthma Immunol Res* 2009; **1**: 45-47 [PMID: 20224670 DOI: 10.4168/aair.2009.1.1.45]
175 **Martelli A**, De Chiara A, Corvo M, Restani P, Fiocchi A. Beef allergy in children with cow's milk allergy; cow's milk allergy in children with beef allergy. *Ann Allergy Asthma Immunol* 2002; **89**: 38-43 [PMID: 12487203]
176 **Gómez Galán C**, Ferré Ybarz L, Sansosti Viltes A, de la Borbolla Morán JM, Peña Peloche MA, Duocastella Selvas P, Llusá Serra A, Torredemer Palau A, Ledesma A, Nevot Falcó S. Rabbit meat anaphylaxis. *J Investig Allergol Clin Immunol* 2014; **24**: 136-138 [PMID: 24834782]
177 **Szépfalusi Z**, Ebner C, Pandjaitan R, Orlicek F, Scheiner O, Boltz-Nitulescu G, Kraft D, Ebner H. Egg yolk alpha-livetin (chicken serum albumin) is a cross-reactive allergen in the bird-egg syndrome. *J Allergy Clin Immunol* 1994; **93**: 932-942 [PMID: 8182236]
178 **Quirce S**, Marañón F, Umpiérrez A, de las Heras M, Fernández-Caldas E, Sastre J. Chicken serum albumin (Gal d 5*) is a partially heat-labile inhalant and food allergen implicated in the bird-egg syndrome. *Allergy* 2001; **56**: 754-762 [PMID: 11488669]
179 **Villas F**, Compes E, Fernández-Nieto M, Muñoz MP, Bartolome B, de las Heras M. Bird-egg syndrome caused by Agapornis species (lovebird). *J Investig Allergol Clin Immunol* 2009; **19**: 71-72 [PMID: 19274938]
180 **Caubet JC**, Wang J. Current understanding of egg allergy. *Pediatr Clin North Am* 2011; **58**: 427-443, xi [PMID: 21453811 DOI: 10.1016/j.pcl.2011.02.014]
181 **Leser C**, Hartmann AL, Praml G, Wüthrich B. The "egg-egg" syndrome: occupational respiratory allergy to airborne egg proteins with consecutive ingestive egg allergy in the bakery and confectionery industry. *J Investig Allergol Clin Immunol* 2001; **11**: 89-93 [PMID: 11642578]
182 **Pfiffner P**, Truffer R, Matsson P, Rasi C, Mari A, Stadler BM.

Allergen cross reactions: a problem greater than ever thought? *Allergy* 2010; **65**: 1536-1544 [PMID: 20584004 DOI: 10.1111/j.1398-9995.2010.02420.x]

183 **Muraro A**, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, Cardona V, Dubois A, duToit G, Eigenmann P, Fernandez Rivas M, Halken S, Hickstein L, Høst A, Knol E, Lack G, Marchisotto MJ, Niggemann B, Nwaru BI, Papadopoulos NG, Poulsen LK, Santos AF, Skypala I, Schoepfer A, Van Ree R, Venter C, Worm M, Vlieg-Boerstra B, Panesar S, de Silva D, Soares-Weiser K, Sheikh A, Ballmer-Weber BK, Nilsson C, de Jong NW, Akdis CA. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. *Allergy* 2014; **69**: 1008-1025 [PMID: 24909706 DOI: 10.1111/all.12429]

184 **Canonica GW**, Passalacqua G, Melioli G. Allergenius®: an expert system for the interpretation of allergen microarrays. Poster 1001. *World Allergy Organ J* 2014; **7** (Suppl 1): P2 [DOI: 10.1186/1939-4551-7-S1-P2]

185 **Melioli G**, Passalacqua G, Canonica GW. Novel in silico technology in combination with microarrays: a state-of-the-art technology for allergy diagnosis and management? *Expert Rev Clin Immunol* 2014; **10**: 1559-1561 [PMID: 25370475 DOI: 10.1586/1744666X.2014.978761]

186 **Barber D**, Díaz-Perales A, Villalba M, Chivato T. Challenges for allergy diagnosis in regions with complex pollen exposures. *Curr Allergy Asthma Rep* 2015; **15**: 496 [PMID: 25504260 DOI: 10.1007/s11882-014-0496-7]

187 **Valenta R**, Hochwallner H, Linhart B, Pahr S. Food allergies: the basics. *Gastroenterology* 2015; **148**: 1120-1131.e4 [PMID: 25680669 DOI: 10.1053/j.gastro.2015.02.006]

188 **Balińska-Miśkiewicz W**. [Molecular diagnosis of food allergy--do we know more?]. *Postepy Hig Med Dosw* (Online) 2014; **68**: 754-767 [PMID: 24934534]

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EDITORIAL

Exercise for tendinopathy

Stasinopoulos Dimitrios

Stasinopoulos Dimitrios, Physiotherapy Program, Department Health Sciences, School of Sciences, European University of Cyprus, 1516 Nicosia, Cyprus

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Correspondence to: Dr. Stasinopoulos Dimitrios, Physiotherapy Program, Department Health Sciences, School of Sciences, European University of Cyprus, 6, Diogenes Str. Engomi, 1516 Nicosia, Cyprus. d.stassinopoulos@euc.ac.cy
Telephone: +357-22-713044

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Abstract

Tendinopathies are one of the most common sports/musculoskeletal injury in modern western societies. Many physiotherapy approaches have been recommended in the literature for the management of tendinopathy. The most effective treatment in the management of tendinopathy is the eccentric training. Load, speed and frequency of contractions are the three principles of eccentric exercises, discussed in this report. However, eccentric training is not effective for all patients with tendinopathy and the effectiveness of this approach when applied as monotherapy is lower than it is applied as part of the rehabilitation process. For this reason, clinicians combine eccentric training with other physiotherapy techniques such as stretching, isometric and lumbar stability exercises, electrotherapy, manual therapy, soft tissue manipulation techniques, taping and acupuncture in the management of tendinopathies. Further research is needed to find out which treatment strategy combined with eccentric training will provide the best results in the rehabilitation of tendinopathy.

Key words: Tendinopathy; Exercise; Physiotherapy; Electrotherapy; Eccentric exercises; Stretching exercises; Electrotherapy; Manual therapy

Core tip: Eccentric exercises are effective in the management of tendinopathy. Eccentric training improves patients' symptoms and reverses tendinopathys' pathology. The ideal eccentric protocol is unknown in the literature. Eccentric training alone does not respond positively in many patients. Therefore, clinicians combine eccentric training with other forms of therapy such as stretching exercises, isometric contraction, electrotherapy, manual therapy, deep transverse friction, taping, acupuncture and improvement of lumbo - pelvic control. More research is needed to find out which treatment strategy combined with eccentric training will provide the best results in the rehabilitation of tendinopathy.

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Tendinopathies are one of the most common sports/musculoskeletal injury in modern western societies.

Table 1 Recommended therapies for the management of tendinopathy

Exercise	Electrotherapy	Manual therapy	Other therapies
Eccentric	Therapeutic ultrasound	MWMT for LET	DTFM
Stretching	Laser	Cyriax for LET	Taping
Isometric	ESWT		Acupuncture
Lumbo - pelvic control for lower limb tendinopathies	Iontophoresis		

ESWT: Extracorporeal shockwave therapy; LET: Lateral elbow tendinopathy; DTFM: Deep transverse friction massage; MWMT: Mobilization with manual therapy.

The most common tendinopathies of the upper limb are Rotator cuff (mainly supraspinatus) and lateral elbow tendinopathy (LET) usually seen in sports such as volley - ball, tennis, basketball, swimming and so on whereas Achilles and patellar tendinopathy are the most common tendinopathies of the lower limb commonly seen in sports such as volley-ball, soccer, running, jumping and so on[1]. Many physical therapy strategies have been proposed for the rehabilitation of tendon disorders. These strategies are electrotherapeutic such as therapeutic ultrasound, extracorporeal shockwave therapy, low level laser, iontophoresis and non-electro-therapeutic modalities such as eccentric training, soft tissue techniques, taping and needle therapy (Table 1). These treatments intend to reduce pain and improve function in tendinopathy but act in a totally different mechanism of action. Generally speaking, the efficacy of a treatment is based on reversing the pathology of the tendinopathy and not only improving the symptoms. Nowadays, eccentric exercise program is the most effective conservative approach in the treatment of tendinopathy[2,3].

Load, speed and frequency of contractions are the three principles of eccentric exercises. The results are poor when the load of eccentric exercises should not be increased according to the patient's symptoms[4]. It is impossible to standardize the rate of increase of the load during the treatment period[5] but if the eccentric loading exercise can be performed without experiencing any minor pain or discomfort, it will be increased by adding weight.

The speed of eccentric training should be increased in every treatment session[6,7], Stanish *et al*[8] (2000) state to simulate the mechanism of injury, which usually occurs at relatively high velocities the load on the tendon should be increased. However, to allow tissue healing and to avoid the possibility of re-injury, eccentric exercises should be performed at a slow velocity[9]. Low velocity eccentric loading generates less injurious heat within the tendon and does not exceed the elastic limit of the tendon[10]. It is not possible to define the "slowness" of eccentric contractions. This lack of definition is based on the therapists' claim that patients perform the eccentric exercises slowly anyway in order to avoid pain[11]. However, the slowness of eccentric training should be defined when researchers develop an exercise programme treatment protocol. It is difficult for therapists to replicate the exercise training and put it into practice when the slowness is not defined.

Repetitions and sets can vary in the literature. Three sets of 15 repetitions are usually recommended. The sets are performed once or twice per day. The performance of sets based on home or supervised eccentric training. An exercise programme that can be performed any time during the day without requiring supervision by a physiotherapist called home exercise programme. The pain in patellar tendinopathy[2]. Achilles tendinopathy[2] and LET[11,12] was reduced when a home exercise program was performed for about three months. Patients fail to comply with this regimen[13,14]. The solution in the above problem is to be performed an exercise program in a clinical setting under the supervision of a physiotherapist. The supervised exercise programme may give good long-term results in one month[15-20]. This occurred because a higher degree of patient compliance can be achieved by the supervised exercise programme.

Eccentric programme reduces the pain and improves the function in all sites of tendinopathy? For example, patients with mid-portion Achilles tendinopathy respond positively in eccentric training with dorsiflexion[21-26], but patients with insertional Achilles tendinopathy respond positively in eccentric training without dorsiflexion[27]. Therefore, the two sites of Achilles tendinopathy respond positively in two different protocols of eccentric training. Patients with patellar tendinopathy at the inferior pole of the patella respond positively in squats[2]; however, the effectiveness of eccentric loading training programme on other sites of patellar tendinopathy has not been investigated. Thus, research is needed to determine the effectiveness of eccentric training at all sites of tendinopathies.

Eccentric training alone is not effective for many patients with tendinopathies[9]. Therefore, eccentric training is combined with static stretching exercises in the treatment of tendinopathies with positive results[15-20]. The way that eccentric and stretching exercises reverse the pathology of tendinopathy is unknown because evidenced - based studies to confirm that physiological effects translate into clinically meaningful outcomes and vice versa are lacking. In addition, research supports that the combination of eccentric training, with a physical therapy modality, such as therapeutic ultrasound[28,29], low level laser[30], extracorporeal shockwave therapy[31] and iontophoresis[32], is more effective therapeutic approach than the eccentric training alone

in the rehabilitation of tendinopathy. Furthermore, clinicians thought that patients with patellar and Achilles tendinopathy have lack of lumbopelvic control (lumbopelvic control defines as the reestablishment of the impairment or deficit in motor control around the neutral zone of the spinal motion segment) and this loss has the potential to alter load distribution on the lower limb kinetic chain[33]. My colleagues and I think that the improvement of lumbo-pelvic control can be achieved by performing simple exercises such as single leg bridging in supine and four point prone bridging exercises. Future research is needed to confirm the above relief. Furthermore, a plethora of manual therapies have been advocated for the management of tendinopathy, but there is minimal experimental evidence to support the efficacy of the use of manual therapy for the management of tendinopathy[34]. Mulligan Mobilization with Movement and Cyriax physiotherapy are the most common manipulative techniques for the management of LET. It is unknown whether an analogous manipulation procedure may be found for the rehabilitation of other tendinopathies comparable to that used in management of LET or may be difficult in practice of attempting such a technique at other joints[35,36]. It is believed that even if a similar technique is found for the rehabilitation of all tendinopathies, this technique will be combined with an exercise training in the treatment of tendinopathy. Finally, a recently published case trial showed that isometric contractions of the wrist extensors as a supplement to eccentric and static exercises of wrist extensors is an effective treatment approach in a patient with LET[37]. Future trials to confirm the results of the present case report in all tendinopathies are needed.

Finally, deep transverse friction massage (DTFM), taping and acupuncture have also recommended in the management of tendinopathy. DTFM is a specific type of massage applied precisely to the tendons[35]. Details about the application and mechanism of action of DTFM can be found in the article by Stasinopoulos and Johnson[35] (2007). The conducted trials do not recommend the use of DTFM in the management of tendinopathy[15,17,38]. Taping and acupuncture improve the signs of tendinopathy but it does not reverse the pathology of tendinopathy[39,40].

In conclusion, eccentric training is the most promising treatment approach in the management of tendinopathy. The optimal protocol of eccentric training is needed to investigate. The effectiveness of this approach when applied as monotherapy is lower than it is applied as part of the rehabilitation process. Further research is needed to find out which treatment strategy combined with eccentric training will provide the best results in the rehabilitation of tendinopathy.

REFERENCES

1 **Bass E**. Tendinopathy: why the difference between tendinitis and tendinosis matters. *Int J Ther Massage Bodywork* 2012; **5**: 14-17 [PMID: 22553479]

2 **Malliaras P**, Barton CJ, Reeves ND, Langberg H. Achilles and patellar tendinopathy loading programmes : a systematic review comparing clinical outcomes and identifying potential mechanisms for effectiveness. *Sports Med* 2013; **43**: 267-286 [PMID: 23494258]

3 **Murtaugh B**, Ihm JM. Eccentric training for the treatment of tendinopathies. *Curr Sports Med Rep* 2013; **12**: 175-182 [PMID: 23669088]

4 **Jensen K**, Di Fabio RP. Evaluation of eccentric exercise in treatment of patellar tendinitis. *Phys Ther* 1989; **69**: 211-216 [PMID: 2919192]

5 **Croisier JL**, Foidart-Dessalle M, Tinant F, Crielaard JM, Forthomme B. An isokinetic eccentric programme for the management of chronic lateral epicondylar tendinopathy. *Br J Sports Med* 2007; **41**: 269-275 [PMID: 17224433]

6 **Stanish WD**, Rubinovich RM, Curwin S. Eccentric exercise in chronic tendinitis. *Clin Orthop Relat Res* 1986; **(208)**: 65-68 [PMID: 3720143]

7 **Fyfe I**, Stanish WD. The use of eccentric training and stretching in the treatment and prevention of tendon injuries. *Clin Sports Med* 1992; **11**: 601-624 [PMID: 1638642]

8 **Stanish W**, Curwin S, Mandell S. Tendinitis: its etiology and treatment. Oxford: Oxford University Press, 2000

9 **Cannell LJ**, Taunton JE, Clement DB, Smith C, Khan KM. A randomised clinical trial of the efficacy of drop squats or leg extension/leg curl exercises to treat clinically diagnosed jumper's knee in athletes: pilot study. *Br J Sports Med* 2001; **35**: 60-64 [PMID: 11157465]

10 **Kraushaar BS**, Nirschl RP. Tendinosis of the elbow (tennis elbow). Clinical features and findings of histological, immunohistochemical, and electron microscopy studies. *J Bone Joint Surg Am* 1999; **81**: 259-278 [PMID: 10073590]

11 **Pienimaki TT**, Tarvainen TK, Siira PY, Vanharanta H. Progressive strengthening and stretching exercises and ultrasound for chronic lateral epicondylitis. *Physiotherapy* 1996; **82**: 522-530 [DOI: 10.1016/S0031-9406(05)66275-X]

12 **Malliaras P**, Maffulli N, Garau G. Eccentric training programmes in the management of lateral elbow tendinopathy. *Disabil Rehabil* 2008; **30**: 1590-1596 [PMID: 18608381]

13 **Stasinopoulos D**, Stasinopoulou K, Johnson MI. An exercise programme for the management of lateral elbow tendinopathy. *Br J Sports Med* 2005; **39**: 944-947 [PMID: 16306504]

14 **Stasinopoulos D**, Johnson MI. Treatment/management for tendinopathy. Rapid response to Khan et al (2002) article Time to abandon the 'tendinitis' myth. *BMJ* 2002; **324**: 626 [DOI: 10.1136/bmj.324.7338.626]

15 **Stasinopoulos D**, Stasinopoulos I. Comparison of effects of exercise programme, pulsed ultrasound and transverse friction in the treatment of chronic patellar tendinopathy. *Clin Rehabil* 2004; **18**: 347-352 [PMID: 15180116]

16 **Manias P**, Stasinopoulos D. A controlled clinical pilot trial to study the effectiveness of ice as a supplement to the exercise programme for the management of lateral elbow tendinopathy. *Br J Sports Med* 2006; **40**: 81-85 [PMID: 16371498]

17 **Stasinopoulos D**, Stasinopoulos I. Comparison of effects of Cyriax physiotherapy, a supervised exercise programme and polarized polychromatic non-coherent light (Bioptron light) for the treatment of lateral epicondylitis. *Clin Rehabil* 2006; **20**: 12-23 [PMID: 16502745]

18 **Dimitrios S**, Pantelis M, Kalliopi S. Comparing the effects of eccentric training with eccentric training and static stretching exercises in the treatment of patellar tendinopathy. A controlled clinical trial. *Clin Rehabil* 2012; **26**: 423-430 [PMID: 21856721]

19 **Stasinopoulos D**, Manias P. Comparing Two Exercise Programmes for the Management of Lateral Elbow Tendinopathy (Tennis Elbow/Lateral Epicondylitis) - A Controlled Clinical Trial. *OAJoST* 2013; **1**: 8 [DOI: 10.11131/2013/100013]

20 **Stasinopoulos D**, Stasinopoulos I, Pantelis M, Stasinopoulou K. Comparison of effects of a home exercise programme and a supervised exercise programme for the management of lateral elbow tendinopathy. *Br J Sports Med* 2010; **44**: 579-583 [PMID: 19887440]

21 **Bahr R**, Fossan B, Løken S, Engebretsen L. Surgical treatment compared with eccentric training for patellar tendinopathy (Jumper' s Knee). A randomized, controlled trial. *J Bone Joint Surg Am* 2006; **88**: 1689-1698 [PMID: 16882889]

22 **Alfredson H**, Pietilä T, Jonsson P, Lorentzon R. Heavy-load eccentric calf muscle training for the treatment of chronic Achilles tendinosis. *Am J Sports Med* 1998; **26**: 360-366 [PMID: 9617396]

23 **Mafi N**, Lorentzon R, Alfredson H. Superior short-term results with eccentric calf muscle training compared to concentric training in a randomized prospective multicenter study on patients with chronic Achilles tendinosis. *Knee Surg Sports Traumatol Arthrosc* 2001; **9**: 42-47 [PMID: 11269583]

24 **Ohberg L**, Lorentzon R, Alfredson H. Eccentric training in patients with chronic Achilles tendinosis: normalised tendon structure and decreased thickness at follow up. *Br J Sports Med* 2004; **38**: 8-11; discussion 11 [PMID: 14751936]

25 **Roos EM**, Engström M, Lagerquist A, Söderberg B. Clinical improvement after 6 weeks of eccentric exercise in patients with mid-portion Achilles tendinopathy -- a randomized trial with 1-year follow-up. *Scand J Med Sci Sports* 2004; **14**: 286-295 [PMID: 15387802]

26 **Silbernagel KG**, Thomeé R, Thomeé P, Karlsson J. Eccentric overload training for patients with chronic Achilles tendon pain--a randomised controlled study with reliability testing of the evaluation methods. *Scand J Med Sci Sports* 2001; **11**: 197-206 [PMID: 11476424]

27 **Jonsson P**, Alfredson H, Sunding K, Fahlström M, Cook J. New regimen for eccentric calf-muscle training in patients with chronic insertional Achilles tendinopathy: results of a pilot study. *Br J Sports Med* 2008; **42**: 746-749 [PMID: 18184750]

28 **Chester R**, Costa ML, Shepstone L, Cooper A, Donell ST. Eccentric calf muscle training compared with therapeutic ultrasound for chronic Achilles tendon pain--a pilot study. *Man Ther* 2008; **13**: 484-491 [PMID: 17662639]

29 **Stasinopoulos D**, Cheimonidou AZ, Chatzidamianos T. Are there Effective Ultrasound Parameters in the Management of Lateral Elbow Tendinopathy? A Systematic Review of the Literature. *Int J Phys Med Rehabil* 2013; **1**: 117 [DOI: 10.4172/jpmr.1000117]

30 **Stergioulas A**. Effects of low-level laser and plyometric exercises in the treatment of lateral epicondylitis. *Photomed Laser Surg* 2007; **25**: 205-213 [PMID: 17603862]

31 **Rompe JD**, Furia J, Maffulli N. Eccentric loading versus eccentric loading plus shock-wave treatment for midportion achilles tendinopathy: a randomized controlled trial. *Am J Sports Med* 2009; **37**: 463-470 [PMID: 19088057]

32 **Stergioulas A**, Sotiropoulos D, Dimitrios S. The Influence of Dexamethasone with Lidocaine Hydrochloride Iontophoresis in Recreational Tennis Players Suffering from Lateral Elbow Tendinopathy. *J Nov Physiother Phys Rehabil* 2014; **1**: 114 [DOI: 10.1016/j.jht.2014.12.005]

33 **Kountouris A**, Cook J. Rehabilitation of Achilles and patellar tendinopathies. *Best Pract Res Clin Rheumatol* 2007; **21**: 295-316 [PMID: 17512484]

34 **Bennett A**, Watson T, Simmonds J. The Efficacy of The Use Of Manual Therapy In The Management Of Tendinopathy: A Systematic Review. *BJSM* 2014; **48** (Suppl 2): A11-A12 [DOI: 10.1136/bjsports-2014-094114.17]

35 **Stasinopoulos D**, Johnson MI. 'Lateral elbow tendinopathy' is the most appropriate diagnostic term for the condition commonly referred-to as lateral epicondylitis. *Med Hypotheses* 2006; **67**: 1400-1402 [PMID: 16843614]

36 **Stasinopoulos D**. Mulligan Mobilization with Movement: Can it be used for the management of any tendinopathy? *Trauma Mon* 2015

37 **Stasinopoulos D**. The Effectiveness of Isometric Contractions Combined with Eccentric Contractions and Stretching Exercises on Pain and Disability in Lateral Elbow Tendinopathy. A Case Report. *J Nov Physiother* 2015; **5**: 238 [DOI: 10.4172/2165-7025.1000238]

38 **Brosseau L**, Casimiro L, Milne S, Robinson V, Shea B, Tugwell P, Wells G. Deep transverse friction massage for treating tendonitis (Cochrane review). *In the Cochrane Library* 2003; **4**: CD003528

39 **Shamsoddini A**, Hollisaz MT. Effects of taping on pain, grip strength and wrist extension force in patients with tennis elbow. *Trauma Mon* 2013; **18**: 71-74 [PMID: 24350156]

40 **Speed C**. Acupuncture's role in tendinopathy: new possibilities. *Acupunct Med* 2015; **33**: 7-8 [PMID: 25576594]

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EDITORIAL

Bartter and Gitelman syndromes: Spectrum of clinical manifestations caused by different mutations

Amar Al Shibli, Hassib Narchi

Amar Al Shibli, Department of Pediatrics, Tawam Hospital, Al Ain, United Arab Emirates
Hassib Narchi, Department of Pediatrics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates

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Correspondence to: Hassib Narchi, MD, FRCPCH, Department of Pediatrics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, PO Box 17666, United Arab Emirates. hassib.narchi@uaeu.ac.ae
Telephone: +971-3-7137414
Fax: +971-3-7672022

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Abstract

Bartter and Gitelman syndromes (BS and GS) are inherited disorders resulting in defects in renal tubular handling of sodium, potassium and chloride. Previously considered as genotypic and phenotypic heterogeneous diseases, recent evidence suggests that they constitute a spectrum of disease caused by different genetic mutations with the molecular defects of chloride reabsorption originating at different sites of the nephron in each condition. Although they share some characteristic metabolic abnormalities such as hypokalemia, metabolic alkalosis, hyperplasia of the juxtaglomerular apparatus with hyperreninemia, hyperaldosteronism, the clinical and laboratory manifestations may not always allow distinction between them. Diuretics tests, measuring the changes in urinary fractional excretion of chloride from baseline after administration of either hydrochlorothiazide or furosemide show very little change (< 2.3%) in the fractional excretion of chloride from baseline in GS when compared with BS, except when BS is associated with *KCNJ1* mutations where a good response to both diuretics exists. The diuretic test is not recommended for infants or young children with suspected BS because of a higher risk of volume depletion in such children. Clinical symptoms and biochemical markers of GS and classic form of BS (type III) may overlap and thus genetic analysis may specify the real cause of symptoms. However, although genetic analysis is available, its use remains limited because of limited availability, large gene dimensions, lack of hot-spot mutations, heavy workup time and costs involved. Furthermore, considerable overlap exists between the different genotypes and phenotypes. Although BS and GS usually have distinct presentations and are associated with specific gene mutations, there remains considerable overlap between their phenotypes and genotypes. Thus, they are better described as a spectrum of clinical manifestations caused by different gene mutations.

Key words: Gitelman syndrome; Bartter syndrome; Potassium; Chloride; Magnesium; Metabolic alkalosis; Genetics

Core tip: As inherited disorders of renal tubular excretion and reabsorption of electrolytes, Bartter and Gitelman syndromes were previously considered as genotypic and phenotypic heterogeneous diseases. Although they share some characteristic features, the clinical and laboratory manifestations may not always allow distinction between them. Different genetic mutations inducing impairment of electrolytes transport across different sites of the nephron have been reported in each condition. However, considerable overlap exists between the different genotypes and phenotypes of these two conditions that are now better described as a spectrum of clinical manifestations caused by different gene mutations.

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INTRODUCTION

Bartter syndrome (BS) and Gitelman syndrome (GS) are inherited autosomal recessive conditions resulting in defects of renal tubular excretion and reabsorption of electrolytes. A brief reminder of the physiology of renal handling of water and electrolytes homeostasis is helpful to understand these two conditions.

PHYSIOLOGY[1]

Water and electrolyte homeostasis is maintained by the kidney. To avoid significant losses of electrolytes in the urine following glomerular filtration, their reabsorption in the renal tubule is required. The distal nephron reabsorbs approximately 30% of the filtered sodium: while one quarter is reabsorbed in the thick ascending limb (TAL) of Henle's loop; the distal convoluted tubule (DCT) and the cortical collecting duct (CCD) reabsorb 10%. Dysfunction of distal tubular functions, due to either genetic or acquired causes, will result in a clinical presentation specific to the affected part of the distal nephron.

The TAL is not permeable to water and reabsorbs a large proportion of the filtered sodium chloride, which leads to interstitial hypertonicity that powers the countercurrent exchange and urinary concentration mechanisms. In case of impairment of this function, a major loss of water and sodium occur, as seen with loop diuretics.

The DCT, composed of an early segment (DCT1), a late portion (DCT2), and the connecting tubule leading to the CCD, finely regulates renal excretion of sodium chloride, calcium and magnesium. These segments express the different transport proteins involved in the reabsorption sodium, calcium and magnesium and their functional impairment leads to extracellular volume depletion, initially compensated for by hyperaldosteronism and resulting in increased potassium urinary losses.

The total distal nephron has a finely regulated reabsorption capacity to cope with the intake and/or extrarenal losses of salt and water. The macula densa (MD) in the distal nephron modulates renal hemodynamics and tubular reabsorption with the modulation of the renin-angiotensin Ⅱ system and intrarenal cyclooxygenase 2 activity regulating the glomerular arterial resistance.

GENERALITIES

Although BS and GS share some characteristic metabolic abnormalities such as hypokalemia, metabolic alkalosis, hyperplasia of the juxtaglomerular apparatus with hyperreninemia, hyperaldosteronism, and, sometimes hypomagnesemia[2-4], the clinical and laboratory manifestations may not always allow distinction between them[5]. They were previously considered as genotypic and phenotypic heterogeneous diseases, with urinary calcium and prostaglandin E excretion as well as serum magnesium levels enabling distinction between them. However recent evidence suggests that they constitute, instead, a spectrum of disease characterized by defective chloride reabsorption caused by different genetic mutations at different sites of the nephron in each condition, resulting in three major types of tubulopathy[1,6]: (1) abnormality in the sodium-potassium-chloride cotransporter NKCC2 or the renal outer medullary potassium (ROMK) channel will lead to an impairment in the thick ascending limb of Henle which has a greater salt reabsorption capacity, resulting in severe polyuric loop dysfunction with major urinary salt and water losses. This condition is also known as antenatal BS or hyperprostaglandin E syndrome; (2) defects in the sodium-chloride cotransporter NCCT or the chloride channel ClC-Kb in the DCT which modulates urinary calcium and magnesium excretion, will induce hypokalemia (in BS) with hypomagnesemia (in GS); and (3) abnormality in the chloride channels ClC-Ka and ClC-Kb or their beta-subunit Barttin in combined loop and distal convoluted tubule will lead to more manifestations (antenatal BS or hyperprostaglandin E syndrome with sensorineural deafness).

CHARACTERISTICS SHARED BY BOTH CONDITIONS

Both conditions are inherited in an autosomal recessive mode. Chronic hypokalemia results in fatigue, dizziness, constipation, muscle cramps and weakness. Although usually mildly symptomatic, hypokalemia can be ex-

Table 1 Genetics and presentation of Bartter and Gitelman syndromes

Disorder	Gene affected	Gene product	Clinical presentation
Bartter syndrome type I	SLC12A1	NKCC2	Antenatal Bartter syndrome (hyperprostaglandin E syndrome)
Bartter syndrome type II	KCNJ1	ROMK	Antenatal Bartter syndrome
Bartter syndrome type III	ClC-Kb	CLC-Kb	Hypochloremia, mild hypomagnesemia, failure to thrive in infancy
Bartter syndrome type IVA	BSND	Barttin (B-subunit of CLC-Ka and CLC-Kb)	Antenatal Bartter syndrome (hyperprostaglandin E syndrome) and sensorineural deafness
Bartter syndrome type IVB	ClC-Ka and ClC-Kb	CLC-Ka and CLC-Kb	Antenatal Bartter syndrome (hyperprostaglandin E syndrome) and sensorineural deafness
Bartter syndrome type V	*CaSR* gene	CaSR	Bartter syndrome with hypocalcemia
Gitelman syndrome	SLC12A3	NCC	Hypomagnesemia, hypocalcuria, growth retardation

There are six Bartter syndrome subtypes (I, II, III, IV, IVB, and V) corresponding to six genetic defects. Modified from Seyberth *et al*[6]. NKCC2: Furosemide-sensitive sodium-potassium-2 chloride cotransporter; ROMK: Renal outer medullary potassium channel; CLC-Kb: Chloride channel Kb; CLC-Ka: Chloride channel Ka; CaSR: Calcium sensing receptor; NCC: Thiazide-sensitive sodium-chloride cotransporter.

acerbated by fluid and electrolytes losses caused by diarrhea or vomiting, or by abuse of alcohol, cocaine or other drugs, and can lead to rhabdomyolysis, prolonged QT interval, life-threatening arrhythmia, syncope and sudden death[7,8].

Biochemical findings common to both conditions include hypokalemia, hypochloremia and metabolic alkalosis associated with hyperreninemia and hyperaldosteronism. Hypomagnesemia used to be considered a feature of GS; however, many reports have also described it in patients with BS[9,10].

DISTINCTIVE CHARACTERISTICS OF BS

The defect of NaCl reabsorption in the thick ascending limb of Henle's loop is central to the pathophysiology of BS[3]. The condition has a prevalence of approximately 1.2 per million[11]. Severe failure to thrive commonly presents in early childhood. Blood pressure is usually normal. BS is classified into five subtypes corresponding to specific defective transport proteins in the renal tubules secondary to different gene mutations[12] as shown in Table 1.

At the luminal (urinary) side of the terminal ascending loop

BS type Ⅰ or antenatal Bartter syndrome or hyperprostaglandin E syndrome. This autosomal recessive condition is caused by mutations of the *SLC12A1* gene coding the Na-K-Cl co-transporter protein in the renal tubule. Antenatal manifestations such as polyhydramnios secondary to fetal polyuria may occur. As transepithelial voltage gradient cannot be maintained to absorb calcium and magnesium, hypercalciuria and hypermagnesiuria occur and may result in nephrocalcinosis. There is no associated sensorineural deafness.

BS type Ⅱ or neonatal Bartter syndrome with transient hyperkalemic metabolic acidosis or antenatal Bartter syndrome. This autosomal recessive condition is caused by mutations of the *KCNJ1* gene that codes the inward rectifying ROMK channel. The initial neonatal presentation is hyperkalemic, metabolic acidosis that may mimic pseudohypoaldosteronism. Antenatal manifestations such as polyhydramnios secondary to fetal polyuria may occur. There is no associated sensorineural deafness.

On the basal lateral (blood) side of the terminal ascending loop

The autosomal recessive BS type Ⅲ or classic BS is caused by a defect in the chloride channel Kb (ClC-Kb) secondary to mutations encoding the basolateral chloride channel. As ClC-Ka chloride permeability is preserved, the symptoms are usually very mild but might overlap with those of GS because the ClC-Ka is also present in the DCT. Sensorineural deafness, nephrocalcinosis and nephrolithiasis do not occur.

The autosomal recessive BS type Ⅳ or antenatal BS with sensorineural deafness is due to *BSND* gene mutations leading to an altered Barttin β-subunit of both chloride channel Ka (ClC-Ka) and ClC-Kb needed for potassium chloride membrane localization. As both ClC-Ka and ClC-Kb channels are affected, the symptoms are usually severe and may initially mimic pseudohypoaldosteronism. However, when the ROMK channel and other potassium channels or transporters start to compensate, the neonates develop hypokalemia and metabolic alkalosis. The resulting disturbances in potassium transport cause the sensorineural deafness, because cochlear hearing function relies on several processes governing potassium flow such as its inflow into cochlear hair cells, its entry into hair cells by electro-chemical forces and its recycling either *via* KCNQ4 channels or by entering Deiter's cells (*via* KCC3, KCC4)[13-15].

BS type V is an autosomal dominant condition caused by *L125P* mutations of the extracellular basolateral calcium sensing receptor located on chromosome 16q13. This results in hypocalcemia hypercalciuria and suppression of parathyroid hormone function, asso-

Table 2 Features differentiating Bartter and Gitelman syndromes

Features	Classic Bartter syndrome	Gitelman syndrome
Age at onset	Childhood (early)	Childhood or later
Maternal hydramnios	Rare	Absent
Polyuria, polydipsia	Present	Rare
Dehydration	Often present	Absent
Tetany	Rare	Present
Growth retardation	Present	Absent
Urinary calcium	Normal or high	Low
Nephrocalcinosis	Rare	Absent
Serum magnesium	Occasionally low	Low
Urine prostaglandins (PGE2)	High or normal	Normal

Modified from Urbanová *et al*[20].

ciated with Bartter-like syndrome[16,17].

DISTINCTIVE CHARACTERISTICS OF GS

The autosomal recessive GS, or familial hypokalemic metabolic alkalosis with hypomagnesemia and low urinary calcium excretion has a prevalence of approximately 1 in 40000[18]. It results from transport defects located in the DCT caused by mutations in the solute carrier family 12, member 3 gene (*SLC12A3*) that encodes the thiazide-sensitive NaCl cotransporter (NCC). Mutations in the gene encoding the chloride channel ClC-Kb have also been identified in some individuals[19].

GS is very often asymptomatic. If symptoms occur, this is usually after the age of six years but the condition is often diagnosed in adolescents or adults. The initial presentation is usually the incidental discovery of an asymptomatic and isolated hypokalemia. Some patients present with fatigue, dizziness, muscle weakness, cramps, vomiting, abdominal pain, fever, nocturia and polyuria. Facial Paresthesias may also occur and, occasionally, hypotension. Failure to thrive is not usually severe unless severe hypokalemia and hypomagnesemia are present. Hypocalciuria is a distinct feature and interstitial nephritis may develop because of the persistent hypokalemia. Adults can present with chondrocalcinosis with swollen and warm joints with overlying tenderness. Sudden cardiac arrest has been reported occasionally[8]. Hearing defect is absent. The most important differential diagnosis is BS (especially type Ⅲ). Antenatal diagnosis is available but not usually required because most patients have a good prognosis[18].

DISTINGUISHING BETWEEN THE TWO CONDITIONS

Clinical and biochemical findings

The main differences in the clinical presentation of BS and GS are explained in Table 2[20]. Although the symptoms of BS type Ⅲ (classical BS) often occur before the age of two, patients can present at any age until adolescence, with an initial history of polyuria and polydipsia, followed by growth retardation if the diagnosis and treatment were delayed[21]. Patients usually have high urinary prostaglandins E2 (PGE2) production and hypercalciuria[1,22]. However, the distinction between BS and GS is not always that simple because phenotypic variances. Although genetic diagnosis is possible, its use remains limited because it is costly and not always readily available. In addition, its usefulness remains limited because the "hot spot" mutations along the gene are not always present.

Diuretics test: Response to thiazide and furosemide

The diuretic test involves measuring the change in the urinary fractional excretion of chloride after administration of a diuretic. This consists of either oral hydrochlorothiazide (1 mg/kg up to 50 mg) or furosemide (a single dose of 2 mg/kg). The diuretic is administered after a 7-d "washout" period, during which therapies other than potassium and magnesium supplements are withheld. In GS caused by a defect is in the thiazide-sensitive NCCT, the thiazide test results in only a minimal change (< 2.3%) in the fractional excretion of chloride from baseline. In BS with ClC-Kb mutations, this blunted response does not occur but a normal response to furosemide exists. In BS with KCNJ1 mutations, there is a good response to both diuretics[23]. Because of a higher risk of volume depletion in infants or young children, diuretic tests are not recommended for them when they are suspected to suffer from BS. In patients with the normotensive hypokalemic alkalosis phenotype, an abnormal hydrochlorothiazide test allows to predict with a very high sensitivity and specificity the GS genotype and thus avoid the need for genotyping[24].

Genetic investigations

Clinical symptoms and biochemical markers of GS and classic form of BS (type Ⅲ) may overlap and thus genetic analysis is required to make an accurate diagnosis[1]. Although genetic tests are available, they still face technical difficulties caused by large gene dimensions and the absence of hot-spot mutations. They are also lengthy and costly. Furthermore, considerable overlap exists between the different genotypes and phenotypes.

In most patients with GS, DNA variants are found in the thiazide-sensitive NaCl co-transporter (NCC) encoding *SLC12A3* gene. In others, variants in the chloride channel ClC-Kb encoding *ClC-Kb* gene are identified, causing not only classical BS (type Ⅲ), but also other phenotypes that overlap with antenatal BS (Types Ⅰ-Ⅱ) or with GS[1,3,5,12,20,25-41]. Other genetic and/or environmental factors also act as effect modifiers in other cases of ClC-Kb mutation[29,30] and in polycystic kidney disease[31]. Furthermore, in one family sharing *ClC-Kb* variant some relatives presented clinical characteristics specific for GS, on the one side of the

spectrum, to classic BS on the other[33]. As a result, screening for the *ClC-Kb* gene in patients with the GS phenotype who do not have variants in the *SLC12A3* gene is therefore required[10].

TREATMENT

Bartter syndrome

Hypokalemia, often in the range of 2-3 mmol/L, is caused by increasing urinary potassium losses due to the activation of the renin-angiotensin-aldosterone system and hyperaldosteronism secondary to salt and water depletion caused by the inability to reabsorb sodium in the TAL of the loop of Henle or the DCT. Correcting it is the mainstay of treatment.

Potassium chloride supplements are preferred salt because of the coexisting chloride deficiencies in these patients. Several hundred mmol of potassium per day may be required to correct the hypokalemia.

Spironolactone, a specific aldosterone antagonist, binds competitively binding to the receptors present at the aldosterone-dependent sodium-potassium exchange site in the DCT. It increases water excretion while retaining potassium.

By inhibiting sodium reabsorption at the DCT, cortical collecting tubule, and collecting duct, Amiloride reduces potassium and hydrogen excretion.

By interfering with the active transport exchange of potassium and sodium in the distal tubule, cortical collecting tubule, and collecting duct Triamterene decreases calcium excretion and increases magnesium loss.

Angiotensin-converting enzyme (ACE) inhibitors, such as captopril, enalapril and lisinopril, block the conversion of angiotensin Ⅰ (ANG Ⅰ) to ANG Ⅱ and prevent the secretion of aldosterone from the adrenal cortex.

Nonsteroidal drug anti-inflammatory drugs (NSAID) decrease prostaglandin PGE2 synthesis, which causes the pressor resistance to ANGⅡ and norepinephrine, hyperreninemia, and increased sympathoadrenal activity. The resulting hyporeninemic hypoaldosteronism leads to potassium retention. Medications include indomethacin and naproxen which decrease the activity of the enzyme cyclo-oxygenase (COX) which increases prostaglandin synthesis.

Administration of growth hormone (GH) is required for the treatment of short stature and growth failure, which are common.

In the presence of muscle spasms or tetany, calcium or magnesium supplements may be required.

GS

Asymptomatic patients often require no treatment but need outpatient monitoring once or twice yearly. A high-sodium and potassium diet is recommended.

Lifelong magnesium supplementation is required. As high doses of magnesium cause diarrhea, normalization of serum magnesium level is difficult to achieve. Oral magnesium-chloride supplementation is initially started with a daily dose of 3 mmol/m^2 or 4-5 mg/kg, divided in 3-4 administrations to avoid diarrhea. The dose will subsequently be adjusted according to serum magnesium levels. It has also to be increased during periods of intercurrent illness, especially in the presence of vomiting and diarrhea. If tetany develops, intravenous administration of 20% $MgCl_2$ (0.1 mmol mg/kg per dose) should be administered and can be repeated every 6 h if needed.

Hypokalemia may require large amounts of potassium chloride supplements, up to 10 mmol/kg in children and 500 mmol/d in adults, but poor gastric tolerance frequently occurs. If symptomatic, hypokalemia is treated by a combination therapy of amiloride (5–10 mg/1.73 m^2 per day) and spironolactone (200-300 mg/d), in addition to KCl supplementation (1-3 mmol/kg per day divided in 3-4 doses). Amiloride therapy should be started with a lower dose initially to avoid the development of hypotension.

Symptomatic chondrocalcinosis (pseudo-gout attacks) requires NSAID.

PROGNOSIS

Bartter syndrome

The prognosis depends on the degree of the receptor dysfunction. Without treatment, there is significant morbidity and mortality. Once treated, most patients lead fairly normal lives. Nearly all patients have growth retardation and/or short stature, which improve with potassium, indomethacin, and GH therapy. A small proportion of patients develops slow progression to chronic renal failure, due to interstitial fibrosis, and may require renal replacement therapy. Nephrocalcinosis may occur and is often associated with hypercalciuria. Cardiac arrhythmias, sometimes leading to sudden death, may occur when significant electrolyte imbalances are present. Sensorineural deafness, associated with Bartter syndrome IV, requires appropriate treatment.

GS

The long-term prognosis is generally excellent. The musculoskeletal and constitutional symptoms, the nocturia and polydipsia, may seriously hamper the daily activities and negatively affect the patients' quality of life. There is a risk of developing sudden cardiac arrhythmias, sometimes life-threatening, especially in the presence of severe hypokalemia, hypomagnesemia and alkalosis. These episodes are sometimes precipitated by non-adherence to therapy, the presence of concomitant diarrhea or vomiting or competitive sports that induce potassium and magnesium loss by sweating.

CONCLUSION

Although BS and GS usually have distinct presentations and are associated with specific gene mutations, there remains considerable overlap between their phenotypes

and genotypes. Thus, they are better described as a spectrum of clinical manifestations caused by different gene mutations.

REFERENCES

1 **Seyberth HW**, Schlingmann KP. Bartter- and Gitelman-like syndromes: salt-losing tubulopathies with loop or DCT defects. *Pediatr Nephrol* 2011; **26**: 1789-1802 [PMID: 21503667 DOI: 10.1007/s00467-011-1871-4]

2 **Amirlak I**, Dawson KP. Bartter syndrome: an overview. *QJM* 2000; **93**: 207-215 [PMID: 10787448 DOI: 10.1093/qjmed/93.4.207]

3 **Konrad M**, Vollmer M, Lemmink HH, van den Heuvel LP, Jeck N, Vargas-Poussou R, Lakings A, Ruf R, Deschênes G, Antignac C, Guay-Woodford L, Knoers NV, Seyberth HW, Feldmann D, Hildebrandt F. Mutations in the chloride channel gene CLCNKB as a cause of classic Bartter syndrome. *J Am Soc Nephrol* 2000; **11**: 1449-1459 [PMID: 10906158]

4 **Naesens M**, Steels P, Verberckmoes R, Vanrenterghem Y, Kuypers D. Bartter's and Gitelman's syndromes: from gene to clinic. *Nephron Physiol* 2004; **96**: p65-p78 [PMID: 15056980 DOI: 10.1159/000076752]

5 **Jeck N**, Konrad M, Peters M, Weber S, Bonzel KE, Seyberth HW. Mutations in the chloride channel gene, CLCNKB, leading to a mixed Bartter-Gitelman phenotype. *Pediatr Res* 2000; **48**: 754-758 [PMID: 11102542 DOI: 10.1203/00006450-200012000-00009]

6 **Seyberth HW**. An improved terminology and classification of Bartter-like syndromes. *Nat Clin Pract Nephrol* 2008; **4**: 560-567 [PMID: 18695706 DOI: 10.1038/ncpneph0912]

7 **Hacihamdioglu DO**, Fidanci K, Kilic A, Gok F, Topaloglu R. QT and JT dispersion and cardiac performance in children with neonatal Bartter syndrome: a pilot study. *Pediatr Nephrol* 2013; **28**: 1969-1974 [PMID: 23760993 DOI: 10.1007/s00467-013-2517-5]

8 **Scognamiglio R**, Negut C, Calò LA. Aborted sudden cardiac death in two patients with Bartter's/Gitelman's syndromes. *Clin Nephrol* 2007; **67**: 193-197 [PMID: 17390745 DOI: 10.5414/CNP67193]

9 **Cruz AJ**, Castro A. Gitelman or Bartter type 3 syndrome? A case of distal convoluted tubulopathy caused by CLCNKB gene mutation. *BMJ Case Rep* 2013; **2013**: pii: bcr2012007929 [PMID: 23345488 DOI: 10.1136/bcr-2012-007929]

10 **Konrad M**, Weber S. Recent advances in molecular genetics of hereditary magnesium-losing disorders. *J Am Soc Nephrol* 2003; **14**: 249-260 [PMID: 12506158 DOI: 10.1097/01.ASN.0000049161.60740.CE]

11 **Rudin A**. Bartter's syndrome. A review of 28 patients followed for 10 years. *Acta Med Scand* 1988; **224**: 165-171 [PMID: 3421146 DOI: 10.1111/j.0954-6820.1988.tb16755.x]

12 **Rodríguez-Soriano J**, Vallo A, Pérez de Nanclares G, Bilbao JR, Castaño L. A founder mutation in the CLCNKB gene causes Bartter syndrome type III in Spain. *Pediatr Nephrol* 2005; **20**: 891-896 [PMID: 15875219 DOI: 10.1007/s00467-005-1867-z]

13 **Birkenhäger R**, Otto E, Schürmann MJ, Vollmer M, Ruf EM, Maier-Lutz I, Beekmann F, Fekete A, Omran H, Feldmann D, Milford DV, Jeck N, Konrad M, Landau D, Knoers NV, Antignac C, Sudbrak R, Kispert A, Hildebrandt F. Mutation of BSND causes Bartter syndrome with sensorineural deafness and kidney failure. *Nat Genet* 2001; **29**: 310-314 [PMID: 11687798 DOI: 10.1038/ng752]

14 **Rickheit G**, Maier H, Strenzke N, Andreescu CE, De Zeeuw CI, Muenscher A, Zdebik AA, Jentsch TJ. Endocochlear potential depends on Cl- channels: mechanism underlying deafness in Bartter syndrome IV. *EMBO J* 2008; **27**: 2907-2917 [PMID: 18833191 DOI: 10.1038/emboj.2008.203]

15 **Riazuddin S**, Anwar S, Fischer M, Ahmed ZM, Khan SY, Janssen AG, Zafar AU, Scholl U, Husnain T, Belyantseva IA, Friedman PL, Riazuddin S, Friedman TB, Fahlke C. Molecular basis of DFNB73: mutations of BSND can cause nonsyndromic deafness or Bartter syndrome. *Am J Hum Genet* 2009; **85**: 273-280 [PMID: 19646679 DOI: 10.1016/j.ajhg.2009.07.003]

16 **Vezzoli G**, Arcidiacono T, Paloschi V, Terranegra A, Biasion R, Weber G, Mora S, Syren ML, Coviello D, Cusi D, Bianchi G, Soldati L. Autosomal dominant hypocalcemia with mild type 5 Bartter syndrome. *J Nephrol* 2006; **19**: 525-528 [PMID: 17048213]

17 **Vargas-Poussou R**, Huang C, Hulin P, Houillier P, Jeunemaître X, Paillard M, Planelles G, Déchaux M, Miller RT, Antignac C. Functional characterization of a calcium-sensing receptor mutation in severe autosomal dominant hypocalcemia with a Bartter-like syndrome. *J Am Soc Nephrol* 2002; **13**: 2259-2266 [PMID: 12191970 DOI: 10.1097/01.ASN.0000025781.16723.68]

18 **Knoers NV**, Levtchenko EN. Gitelman syndrome. *Orphanet J Rare Dis* 2008; **3**: 22 [PMID: 18667063 DOI: 10.1186/1750-1172-3-22]

19 **Nakhoul F**, Nakhoul N, Dorman E, Berger L, Skorecki K, Magen D. Gitelman's syndrome: a pathophysiological and clinical update. *Endocrine* 2012; **41**: 53-57 [PMID: 22169961 DOI: 10.1007/s12020-011-9556-0]

20 **Urbanová M**, Reiterová J, Stěkrová J, Lněnička P, Ryšavá R. DNA analysis of renal electrolyte transporter genes among patients suffering from Bartter and Gitelman syndromes: summary of mutation screening. *Folia Biol* (Praha) 2011; **57**: 65-73 [PMID: 21631963]

21 **Peters M**, Jeck N, Reinalter S, Leonhardt A, Tönshoff B, Klaus G Gü, Konrad M, Seyberth HW. Clinical presentation of genetically defined patients with hypokalemic salt-losing tubulopathies. *Am J Med* 2002; **112**: 183-190 [PMID: 11893344 DOI: 10.1016/S0002-9343(01)01086-5]

22 **Brochard K**, Boyer O, Blanchard A, Loirat C, Niaudet P, Macher MA, Deschenes G, Bensman A, Decramer S, Cochat P, Morin D, Broux F, Caillez M, Guyot C, Novo R, Jeunemaître X, Vargas-Poussou R. Phenotype-genotype correlation in antenatal and neonatal variants of Bartter syndrome. *Nephrol Dial Transplant* 2009; **24**: 1455-1464 [PMID: 19096086 DOI: 10.1093/ndt/gfn689]

23 **Nozu K**, Iijima K, Kanda K, Nakanishi K, Yoshikawa N, Satomura K, Kaito H, Hashimura Y, Ninchoji T, Komatsu H, Kamei K, Miyashita R, Kugo M, Ohashi H, Yamazaki H, Mabe H, Otsubo A, Igarashi T, Matsuo M. The pharmacological characteristics of molecular-based inherited salt-losing tubulopathies. *J Clin Endocrinol Metab* 2010; **95**: E511-E518 [PMID: 20810575 DOI: 10.1210/jc.2010-0392]

24 **Colussi G**, Bettinelli A, Tedeschi S, De Ferrari ME, Syrén ML, Borsa N, Mattiello C, Casari G, Bianchetti MG. A thiazide test for the diagnosis of renal tubular hypokalemic disorders. *Clin J Am Soc Nephrol* 2007; **2**: 454-460 [PMID: 17699451 DOI: 10.2215/CJN.02950906]

25 **Vargas-Poussou R**, Dahan K, Kahila D, Venisse A, Riveira-Munoz E, Debaix H, Grisart B, Bridoux F, Unwin R, Moulin B, Haymann JP, Vantyghem MC, Rigothier C, Dussol B, Godin M, Nivet H, Dubourg L, Tack I, Gimenez-Roqueplo AP, Houillier P, Blanchard A, Devuyst O, Jeunemaitre X. Spectrum of mutations in Gitelman syndrome. *J Am Soc Nephrol* 2011; **22**: 693-703 [PMID: 21415153 DOI: 10.1681/ASN.2010090907]

26 **Jeck N**, Waldegger P, Doroszewicz J, Seyberth H, Waldegger S. A common sequence variation of the CLCNKB gene strongly activates ClC-Kb chloride channel activity. *Kidney Int* 2004; **65**: 190-197 [PMID: 14675050 DOI: 10.1111/j.1523-1755.2004.00363.x]

27 **Briet M**, Vargas-Poussou R, Lourdel S, Houillier P, Blanchard A. How Bartter's and Gitelman's syndromes, and Dent's disease have provided important insights into the function of three renal chloride channels: ClC-Ka/b and ClC-5. *Nephron Physiol* 2006; **103**: p7-13 [PMID: 16352917 DOI: 10.1159/000090218]

28 **Uchida S**. In vivo role of CLC chloride channels in the kidney. *Am J Physiol Renal Physiol* 2000; **279**: F802-F808 [PMID: 11053039]

29 **Dong Y**, Ji G, Feng QW, Zeng XT, Jiang GR. A novel splicing mutation in CLCNKB in a Chinese patient with Bartter syndrome type III. *Chin Med J* (Engl) 2010; **123**: 3151-3153 [PMID: 21162973]

30 **Nozu K**, Fu XJ, Nakanishi K, Yoshikawa N, Kaito H, Kanda K, Krol RP, Miyashita R, Kamitsuji H, Kanda S, Hayashi Y, Satomura K, Shimizu N, Iijima K, Matsuo M. Molecular analysis of patients with type III Bartter syndrome: picking up large heterozygous deletions with semiquantitative PCR. *Pediatr Res* 2007; **62**: 364-369 [PMID: 17622951 DOI: 10.1203/PDR.0b013e318123fb90]

31 **Coto E**, Rodriguez J, Jeck N, Alvarez V, Stone R, Loris C, Rodriguez LM, Fischbach M, Seyberth HW, Santos F. A new mutation (intron 9 +1 G& gt; T) in the SLC12A3 gene is linked to Gitelman syndrome in Gypsies. *Kidney Int* 2004; **65**: 25-29 [PMID: 14675033 DOI: 10.1111/j.1523-1755.2004.00388.x]

32 **Robitaille P**, Merouani A, He N, Pei Y. Bartter syndrome in two sisters with a novel mutation of the CLCNKB gene, one with deafness. *Eur J Pediatr* 2011; **170**: 1209-1211 [PMID: 21479528 DOI: 10.1007/s00431-011-1464-z]

33 **Zelikovic I**, Szargel R, Hawash A, Labay V, Hatib I, Cohen N, Nakhoul F. A novel mutation in the chloride channel gene, CLCNKB, as a cause of Gitelman and Bartter syndromes. *Kidney Int* 2003; **63**: 24-32 [PMID: 12472765 DOI: 10.1046/j.1523-1755.2003.00730.x]

34 **Fukuyama S**, Okudaira S, Yamazato S, Yamazato M, Ohta T. Analysis of renal tubular electrolyte transporter genes in seven patients with hypokalemic metabolic alkalosis. *Kidney Int* 2003; **64**: 808-816 [PMID: 12911530 DOI: 10.1046/j.1523-1755.2003.00163.x]

35 **Fukuyama S**, Hiramatsu M, Akagi M, Higa M, Ohta T. Novel mutations of the chloride channel Kb gene in two Japanese patients clinically diagnosed as Bartter syndrome with hypocalciuria. *J Clin Endocrinol Metab* 2004; **89**: 5847-5850 [PMID: 15531551 DOI: 10.1210/jc.2004-0775]

36 **Yu Y**, Xu C, Pan X, Ren H, Wang W, Meng X, Huang F, Chen N. Identification and functional analysis of novel mutations of the CLCNKB gene in Chinese patients with classic Bartter syndrome. *Clin Genet* 2010; **77**: 155-162 [PMID: 19807735 DOI: 10.1111/j.1399-0004.2009.01288.x]

37 **Xiumin W**, Zheng S, Meichun X, Junfen F, Li L. A Chinese Girl with Bartter Syndrome Type III due to a Novel Mutation and/or Single Nucleotide Polymorphisms (SNPs) in CLCNKB Gene. *Iran J Pediatr* 2013; **23**: 89-94 [PMID: 23550235]

38 **Gorgojo JJ**, Donnay S, Jeck N, Konrad M. A Spanish founder mutation in the chloride channel gene, CLCNKB, as a cause of atypical Bartter syndrome in adult age. *Horm Res* 2006; **65**: 62-68 [PMID: 16391491 DOI: 10.1159/000090601]

39 **Lee BH**, Cho HY, Lee H, Han KH, Kang HG, Ha IS, Lee JH, Park YS, Shin JI, Lee DY, Kim SY, Choi Y, Cheong HI. Genetic basis of Bartter syndrome in Korea. *Nephrol Dial Transplant* 2012; **27**: 1516-1521 [PMID: 21865213 DOI: 10.1093/ndt/gfr475]

40 **Tajima T**, Nawate M, Takahashi Y, Mizoguchi Y, Sugihara S, Yoshimoto M, Murakami M, Adachi M, Tachibana K, Mochizuki H, Fujieda K. Molecular analysis of the CLCNKB gene in Japanese patients with classic Bartter syndrome. *Endocr J* 2006; **53**: 647-652 [PMID: 16902263]

41 **Enríquez R**, Adam V, Sirvent AE, García-García AB, Millán I, Amorós F. Gitelman syndrome due to p.A204T mutation in CLCNKB gene. *Int Urol Nephrol* 2010; **42**: 1099-1102 [PMID: 20931281 DOI: 10.1007/s11255-010-9850-4]

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EDITORIAL

Neurally adjusted ventilator assist in very low birth weight infants: Current status

Hassib Narchi, Fares Chedid

Hassib Narchi, Department of Pediatrics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain PO Box 17666, United Arab Emirates
Fares Chedid, Department of Pediatrics, Al Jalila Hospital, Dubai Health Authority, Dubai PO Box 4545, United Arab Emirates

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Correspondence to: Fares Chedid, MD, Department of Pediatrics, Al Jalila Hospital, Dubai Health Authority, Dubai PO Box 4545, United Arab Emirates. fareschedid@hotmail.com
Telephone: +971-50-4474661

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Abstract

Continuous improvements in perinatal care have resulted in increased survival of premature infants. Their immature lungs are prone to injury with mechanical ventilation and this may develop into chronic lung disease (CLD) or bronchopulmonary dysplasia. Strategies to minimize the risk of lung injury have been developed and include improved antenatal management (education, regionalization, steroids, and antibiotics), exogenous surfactant administration and reduction of barotrauma by using exclusive or early noninvasive ventilatory support. The most frequently used mode of assisted ventilation is pressure support ventilation that may lead to patient-ventilator asynchrony that is associated with poor outcome. Ventilator-induced diaphragmatic dysfunction or disuse atrophy of diaphragm fibers may also occur. This has led to the development of new ventilation modes including neurally adjusted ventilatory assist (NAVA). This ventilation mode is controlled by electrodes embedded within a nasogastric catheter which detect the electrical diaphragmatic activity (Edi) and transmit it to trigger the ventilator in synchrony with the patient's own respiratory efforts. This permits the patient to control peak inspiratory pressure, mean airway pressure and tidal volume. Back up pressure control (PC) is provided when there is no Edi signal and no pneumatic trigger. Compared with standard conventional ventilation, NAVA improves blood gas regulation with lower peak inspiratory pressure and oxygen requirements in preterm infants. NAVA is safe mode of ventilation. The majority of studies have shown no significant adverse events in neonates ventilated with NAVA nor a difference in the rate of intraventricular hemorrhage, pneumothorax, or necrotizing enterocolitis when compared to conventional ventilation. Future large size randomized controlled trials should be established to compare NAVA with volume targeted and pressure controlled ventilation in newborns with mature respiratory drive. Most previous studies and trials were not sufficiently large and did not include long-term patient oriented outcomes. Multicenter, randomized, outcome trials are needed to determine whether NAVA is effective in avoiding intubation, facilitating extubation, decreasing time of ventilation, reducing the incidence of

CLD, decreasing length of stay, and improving long-term outcomes such as the duration of ventilation, length of hospital stay, rate of pneumothorax, CLD and other major complications of prematurity. In order to prevent barotrauma, next generations of NAVA equipment for neonatal use should enable automatic setting of ventilator parameters in the backup PC mode based on the values generated by NAVA. They should also include an upper limit to the inspiratory time as in conventional ventilation. The manufacturers of Edi catheters should produce smaller sizes available for extreme low birth weight infants. Newly developed ventilators should also include leak compensation and high frequency ventilation. A peripheral flow sensor is also essential to the proper delivery of all modes of conventional ventilation as well as NAVA.

Key words: Interactive ventilatory support; Positive-pressure respiration; Diaphragm; Premature; Very low birth weight; Respiratory distress syndrome; Electrical diaphragmatic activity; Synchrony; Neural triggering

Core tip: Neurally adjusted ventilator assist (NAVA) ventilation utilizes the patient's neural respiratory drive to synchronize ventilatory support on a breath-by-breath basis based on the infant's ongoing needs. It appears to work well in neonates but evidence that it makes a difference in outcomes in this population has not been established so far. The majority of studies have shown no significant adverse events in neonates ventilated with NAVA nor a difference in the rate of intraventricular hemorrhage, pneumothorax, or necrotizing enterocolitis when compared to conventional ventilation. The challenge for neonatal health care providers remains the steep and prolonged learning curve for the application of NAVA.

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INTRODUCTION

Survival of more and more premature infants has occurred as a result of continuous improvements in perinatal care. Their extremely immature lungs are prone to injury with mechanical ventilation because the gas volumes/kg body weight of the lungs are small[1]. Lung injury is inversely related to gestational age[2]. This injury may develop into chronic lung disease (CLD) or bronchopulmonary dysplasia (BPD). Strategies to minimize the risk of lung injury have been developed and include improved antenatal management (education, regionalization, steroids, and antibiotics), exogenous surfactant administration and reduction of barotrauma by using exclusive or early noninvasive ventilatory support[2,3].

The most frequently used mode of assisted ventilation is pressure support ventilation (PSV)[4-6]. Spontaneous breathing is detected by changes in airway flow or pressure in order to coordinate the ventilatory assist[7]. However, poor patient-ventilator interaction might result from conventional pneumatic triggering of the ventilator[8]. Furthermore, the potentially beneficial variability of the breathing pattern of the patient is not supported by PSV, as fixed pressure support is delivered regardless of the patients' needs[9]. This inability to provide synchrony between the patient and the assist delivered has also been demonstrated in children[10]. Patient–ventilator asynchrony has been associated with poor clinical outcome[11-15].

Changes in diaphragm structure occur following prolonged mechanical ventilation in animal models[14]. Rapid disuse atrophy of diaphragm fibers also occurs in mechanically ventilated humans[15]. This diaphragmatic muscle "disuse atrophy" "ventilator-induced diaphragmatic dysfunction" caused by sustained inactivity of the respiratory muscles (*i.e.*, passive ventilation) results in acute inflammation, loss of muscle mass, deconditioning and weakness in animal models and also in humans[6]. The preservation of spontaneous breathing during mechanical ventilation not only helps to preserve diaphragmatic function, but also to avoid atelectasis and improve oxygenation[11-15].

NEURALLY ADJUSTED VENTILATOR ASSIST

Why was neurally adjusted ventilator assist introduced and how does it work?

Over the past few years, new ventilation modes have been developed with different new strategies implemented to wean from mechanical ventilation early in order to reduce the occurrence of ventilator-induced lung injury. As patient-ventilator synchrony is essential, spontaneous patient's breathing with mechanical ventilation should be maintained whenever possible, with mechanical ventilation delivering exactly the support needed by the patient. When initiated by the patient each breath is supported immediately and that support is tailored breath-by-breath by the patient's current needs[16]. In addition, it is equally important that the level of mechanical assisted ventilation does not exceed the patient's needs. All these considerations have led to the development of neurally adjusted ventilatory assist (NAVA)[16-18]. This ventilation mode is controlled by an array of eight bipolar electrodes (sensors) electrodes embedded within a specialized nasogastric catheter positioned at the level of the crural diaphragm. These sensors detect the electrical diaphragmatic activity (Edi), and filter from electrical contamination from the heart, esophagus, and environment before

transmitting it to trigger the ventilator in synchrony with the patient's own respiratory efforts[18]. The ventilator breath is triggered and terminated by changes in this electrical activity, with the delivered inspiratory pressure proportional to the electrical signal, permitting the patient to control peak inspiratory pressure, mean airway pressure and tidal volume. In addition, as it is the patient who initiates and terminates the breaths, he also determines inspiratory (IT), expiratory times (ET) and respiratory rate, enabling flexible ventilation with breath-to-breath variability[17]. Breaths are generally initiated at 0.5 microvolt above the minimum Edi (Edi min) and terminated when the Edi signal has fallen to 70% of its peak value. The pressure curve in NAVA follows the Edi signal pattern. Back up pressure control (PC) is provided when there is no Edi signal and no pneumatic trigger. The pressure gradient (ΔP) is directly proportional to the ΔEdi following the equation (Δ P = ΔEdi × NAVA level) allowing a proportional assist mode of ventilation, which provides more support to distressed patients and permits spontaneous weaning once the lung compliance improves. NAVA levels between 1 and 4 cm H_2O/microvolt are generally used to augment respiratory support based on the size of the Edi signal aiming for normal Edi values between 5 and 10 microvolts. Therefore, if the Edi values are higher than normal, increasing ventilatory support may be considered for unloading the patient's diaphragm, while if the Edi values are lower than normal, reducing ventilatory support may be required instead in order to exercise diaphragmatic muscle fibers[18]. The tidal volume is lower than that of conventional ventilation potentially reducing lung injury and preventing disuse diaphragmatic atrophy. Failure to detect an Edi signal may result from respiratory center failure to deliver a signal (*e.g.*, apnea of prematurity, central hypoventilation syndrome, brain injury, sedation), diaphragmatic hernia, phrenic nerve conduction failure or chemical paralysis of the neuromuscular junction or the diaphragm[18]. Patients with diaphragmatic hernia are generally able to produce an Edi signal sufficient to allow assisted ventilation with NAVA[19,20].

Types of NAVA

Invasive NAVA is a complex mode of ventilation that combines NAVA, PS and PC in various proportions with short periods of apnea. Backup PC starts when the pneumatic and the neural trigger are both delayed for a period of time set by the clinician between 2 and 10 s. An automatic switch to the pressure support (PS) mode occurs in invasive NAVA modem when neural IT exceed 50% to 60% of the total respiratory time over 20 s. However, the clinician cannot set an upper limit to the IT for each breath.

Non-invasive NAVA (NIV-NAVA) is technically identical to invasive NAVA but it provides only NAVA and backup PC. Pneumatic triggers and PS are taken out of the loop because of the extremely high air leak that may reach 99% in non-invasive ventilation. Weaning occurs spontaneously but this is not the case in the backup PC mode where (PIP) is set manually. Frequent monitoring of the inspiratory pressure in NAVA should be used as a guide to select the appropriate PIP for the PC backup mode.

Advantages of NAVA

In neural triggering, the electrical trigger coming from the brain through the vagal nerve stimulates diaphragm as the same time as the ventilator, improving therefore patient-ventilator synchrony, permitting breath-to-breath variability and reducing the need for sedation[21,22]. In contrast, the pneumatic triggers used in conventional ventilation are delayed because, by definition, they occur only after the diaphragm has already contracted to generate a chest movement, a negative pressure or a positive flow. In addition neural triggering is independent of air leak around the endotracheal tube (ETT) while pneumatic triggering is sensitive to air leak. Multiple cross over trials between pressure control modes and NAVA have repeatedly shown that NAVA improves patient-ventilator synchrony in low birth weight infants, even in the presence of large air leaks[21,22]. The patient takes full control of the ventilator while receiving a timely support proportional to his own his efforts, unloading therefore the diaphragmatic muscle and reducing work of breathing[23]. This result in reduced infant fatigue and decreases the need for mandatory ventilation[16,24]. Compared with standard conventional ventilation in preterm infants, NAVA improves blood gas regulation while still using lower peak inspiratory pressure and oxygen requirements[25,26]. Edi may be used to determine optimal ventilatory support: if the infant is over-ventilated, his spontaneous respiratory drive will be suppressed resulting in a decrease of the Edi signal, while if he is under-ventilated an increased respiratory drive and higher Edi signals will result[18].

Feasibility of NAVA in very low birth weight infants

The feasibility of using NAVA in very low birth weight infants (VLBWI) was demonstrated in 2009 in a randomized crossover study on seven newborns between NAVA applied for 20 min and PSV[22]. Three other randomized crossover trials in VLBWI have compared NAVA to SIMV or PCV[23,25,27]. However they were of small sample size (n = 26, 5 and 10) and NAVA was applied for short time (4, 4 and 1 h respectively). Short-term benefits with NAVA were observed, including improved patient-ventilator synchrony and the need for a lower PIP to produce the same PaCO2. NAVA is safe mode of ventilation. The majority of studies have shown neither significant adverse events nor a difference in the rate of intraventricular hemorrhage, pneumothorax, or necrotizing enterocolitis when compared to conventional ventilation[26,28]. These were retrospective case series and randomized crossover studies with very small sample size. There are no up-to-date trials addressing

long-term outcomes.

Potential problems with NAVA

NAVA assumes that the respiratory center of preterm infants is mature enough to drive the ventilator at all times, with an appropriate rate, a sufficient magnitude and optimal IT and ET. This assumption may not hold true especially in extreme preterm infant, or during sepsis, intraventricular hemorrhage (IVH) or severe illnesses. Preterm infants demonstrate an immature response to hypercapnia[29,30], a paradoxical respiratory depression induced by hypoxemia[31] and a pronounced apneic response to laryngeal stimulation[32]. This immaturity of the respiratory drive in preterm infants very commonly results in apnea and periodic breathing in this group of neonates. These infants often produce a very small Edi signal that prevents backup ventilation with the PC mode but without producing a sufficient PIP to provide effective ventilation. This inability to generate a strong Edi may also prevent the termination of breath when the minimum Edi does not fall below 70% of the peak causing an extremely high inspiratory time. In invasive NAVA, a switch to PS takes place when the Edi signal is absent but the pneumatic trigger is still present. It occurs also when there is major discrepancy between the neural and pneumatic respiratory rates. As PS and PC are an integral part of NAVA, it is therefore incorrect to claim that NAVA is totally independent of air leak especially in VLBWI who switch frequently to PS and backup PC because of their immature respiratory drive.

A Cochrane meta-analysis demonstrated that the combination of volume targeted ventilation with PC or SIMV was associated with a statistically significant reduction of severe IVH, hypocarbia, pneumothorax, and the duration of ventilation[26]. VLBWI ventilated with NAVA keep moving back and forth between neutrally adjusted ventilation and PC backup. The non-availability of volume targeted ventilation and the fact that PIP during the backup periods is manually set by the clinician constitutes a major disadvantage of NAVA, considering the established benefits of volume targeted ventilation and the current lack of demonstrated long-term benefit of NAVA.

Capturing a strong and stable Edi signal is essential, but unfortunately, as Edi catheters for neonates are manufactured in three sizes only (6F/49 cm, 6F/50 cm and 8F/100 cm) while the length of newborns at birth is generally between 28 to 58 cm, NAVA effectiveness may not be as good as it should be. Capturing pneumatic triggers is also essential to the use of NAVA since pressure support and pressure control backup are required in case of apnea or discrepancy between pneumatic and neural triggers. Unfortunately, the flow sensor in Servo I and Servo U, which are the only ventilators providing NAVA, is located inside the machine and far from the ETT. As a result VLBWI are unable to constantly generate enough flow to compensate for the compliance of the tubing in order to trigger the ventilator. We have noticed that these patients frequently receive mandatory non-triggered breaths when they switch to the PC backup mode caused by either displacement of Edi catheter, or apnea or major discrepancy between pneumatic and neural triggers. Using a Y-peripheral flow sensor may provide a solution but must be purchased separately. The only two ventilators that offer the possibility of NAVA (servo I and Servo U) do not provide leak compensation or high frequency ventilation. This lack of versatility may become a limitation when volume targeted ventilation is required or when the clinical condition requires a change to high frequency oscillatory ventilation.

RECOMMENDATIONS

The place of NAVA in the management of respiratory distress in VLBWI is still not yet clear. We suggest that non-breathing infants should not be placed on NAVA because they do not produce a strong and consistent Edi signal to drive the ventilator. On the other hand, spontaneously breathing infants often do well on CPAP and do not require any type of ventilation. In our opinion, volume targeted ventilation in combination with PC should be the default mode of ventilation of VLBWI because it has been shown to reduce severe intraventricular hemorrhage, pneumothorax, hypocarbia and the duration of ventilation. As pressure control backup is very common in NAVA and since the long term benefits of NAVA have not yet been demonstrated in randomized trials, we recommend not to use NAVA in the first week of life in extreme low birth infants who have immature respiratory drive causing apnea and who already are at high risk of intraventricular hemorrhage and volume trauma. Larger and older infant can benefit from NAVA when they are able to generate a strong Edi activity. As NIV NAVA is presently the only ventilator mode that allows effective triggering despite the large air leak associated always present with nasal ventilation, we believe that it should be tried as a first option whenever possible. Patients with severe CLD and those who have received heavy sedation for long period may have a disuse atrophy of diaphragmatic muscle fibers and could benefit from progressive loading with NAVA to exercise their diaphragm and prepare them for possible extubation.

Future large size randomized controlled trials should be established to compare NAVA with volume targeted and pressure controlled ventilation in newborns with mature respiratory drive. Future studies should also compare NIV NAVA and biphasic CPAP or high flow nasal cannula to demonstrate if NIV-NAVA can prevent endotracheal intubation without causing abdominal distension or increasing the rate of necrotizing enterocolitis. As most previous studies and trials were not sufficiently large and did not include long-term patient oriented outcomes, multicenter, randomized, outcome trials are needed to determine whether NAVA is effective in avoiding intubation, facilitating extubation,

decreasing time of ventilation, reducing the incidence of CLD, decreasing length of stay, and improving long-term outcomes such as the duration of ventilation, length of hospital stay, rate of pneumothorax, CLD and other major complications of prematurity.

In order to prevent barotrauma, we recommend that the next generations of NAVA equipment for neonatal use should enable automatic setting of ventilatory parameters in the backup PC mode based on the values generated by NAVA. We believe that they should also include an upper limit to the IT as in conventional ventilation and that the manufacturers of Edi catheters should make smaller sizes available for extreme low birth weight infants. We also recommend that newly developed NAVA ventilators also include leak compensation, high frequency ventilation option as well as a peripheral flow sensor because it is essential for all modes of conventional ventilation as well as NAVA.

CONCLUSION

NAVA ventilation utilizes the patient's neural respiratory drive to synchronize ventilatory support on a breath-by-breath basis based on the infant's ongoing needs. It allows preterm neonates to use physiologic feedback to control ventilation and enhance comfort for each breath. The Edi signal provides the clinician with previously inaccessible information about central respiratory drive useful for both weaning and diagnostics, with infants informing the neonatologist of what support they need, directing both the timing and depth of their breathing pattern. NAVA appears to work well in neonates but if it makes a difference in outcomes in this population has not been established so far. The remaining challenge for neonatal health care providers is the steep and prolonged learning curve for the application of NAVA.

REFERENCES

1 **Jobe AH**, Ikegami M. Mechanisms initiating lung injury in the preterm. *Early Hum Dev* 1998; **53**: 81-94 [PMID: 10193929 DOI: 10.1016/j.prrv.2010.03.002]

2 **Donn SM**, Sinha SK. Minimising ventilator induced lung injury in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2006; **91**: F226-F230 [PMID: 16632652 DOI: 10.1136/adc.2005.082271]

3 **Chakraborty M**, McGreal EP, Kotecha S. Acute lung injury in preterm newborn infants: mechanisms and management. *Paediatr Respir Rev* 2010; **11**: 162-170; quiz 170 [PMID: 20692631]

4 **Esteban A**, Anzueto A, Alía I, Gordo F, Apezteguía C, Pálizas F, Cide D, Goldwaser R, Soto L, Bugedo G, Rodrigo C, Pimentel J, Raimondi G, Tobin MJ. How is mechanical ventilation employed in the intensive care unit? An international utilization review. *Am J Respir Crit Care Med* 2000; **161**: 1450-1458 [PMID: 10806138 DOI: 10.1164/ajrccm.161.5.9902018]

5 **Henzler D**, Pelosi P, Bensberg R, Dembinski R, Quintel M, Pielen V, Rossaint R, Kuhlen R. Effects of partial ventilatory support modalities on respiratory function in severe hypoxemic lung injury. *Crit Care Med* 2006; **34**: 1738-1745 [PMID: 16625116 DOI: 10.1097/01.CCM.0000218809.49883.54]

6 **Kallet RH**. Patient-ventilator interaction during acute lung injury, and the role of spontaneous breathing: part 1: respiratory muscle function during critical illness. *Respir Care* 2011; **56**: 181-189 [PMID: 21333178 DOI: 10.4187/respcare.00964]

7 **Goulet R**, Hess D, Kacmarek RM. Pressure vs flow triggering during pressure support ventilation. *Chest* 1997; **111**: 1649-1653 [PMID: 9187188]

8 **Beck J**, Campoccia F, Allo JC, Brander L, Brunet F, Slutsky AS, Sinderby C. Improved synchrony and respiratory unloading by neurally adjusted ventilatory assist (NAVA) in lung-injured rabbits. *Pediatr Res* 2007; **61**: 289-294 [PMID: 17314685 DOI: 10.1203/01.pdr.0000257324.22406.93]

9 **Spieth PM**, Carvalho AR, Pelosi P, Hoehn C, Meissner C, Kasper M, Hübler M, von Neindorff M, Dassow C, Barrenschee M, Uhlig S, Koch T, de Abreu MG. Variable tidal volumes improve lung protective ventilation strategies in experimental lung injury. *Am J Respir Crit Care Med* 2009; **179**: 684-693 [PMID: 19151194 DOI: 10.1164/rccm.200806-975OC]

10 **Beck J**, Tucci M, Emeriaud G, Lacroix J, Sinderby C. Prolonged neural expiratory time induced by mechanical ventilation in infants. *Pediatr Res* 2004; **55**: 747-754 [PMID: 14739354 DOI: 10.1203/01.PDR.0000119368.21770.33]

11 **Putensen C**, Zech S, Wrigge H, Zinserling J, Stüber F, Von Spiegel T, Mutz N. Long-term effects of spontaneous breathing during ventilatory support in patients with acute lung injury. *Am J Respir Crit Care Med* 2001; **164**: 43-49 [PMID: 11435237 DOI: 10.1164/ajrccm.164.1.2001078]

12 **Wrigge H**, Zinserling J, Neumann P, Defosse J, Magnusson A, Putensen C, Hedenstierna G. Spontaneous breathing improves lung aeration in oleic acid-induced lung injury. *Anesthesiology* 2003; **99**: 376-384 [PMID: 12883410]

13 **Neumann P**, Wrigge H, Zinserling J, Hinz J, Maripuu E, Andersson LG, Putensen C, Hedenstierna G. Spontaneous breathing affects the spatial ventilation and perfusion distribution during mechanical ventilatory support. *Crit Care Med* 2005; **33**: 1090-1095 [PMID: 15891341]

14 **Radell P**, Edström L, Stibler H, Eriksson LI, Ansved T. Changes in diaphragm structure following prolonged mechanical ventilation in piglets. *Acta Anaesthesiol Scand* 2004; **48**: 430-437 [PMID: 15025604 DOI: 10.1111/j.1399-6576.2004.00352.x]

15 **Levine S**, Nguyen T, Taylor N, Friscia ME, Budak MT, Rothenberg P, Zhu J, Sachdeva R, Sonnad S, Kaiser LR, Rubinstein NA, Powers SK, Shrager JB. Rapid disuse atrophy of diaphragm fibers in mechanically ventilated humans. *N Engl J Med* 2008; **358**: 1327-1335 [PMID: 18367735 DOI: 10.1056/NEJMoa070447]

16 **Bengtsson JA**, Edberg KE. Neurally adjusted ventilatory assist in children: an observational study. *Pediatr Crit Care Med* 2010; **11**: 253-257 [PMID: 19593241 DOI: 10.1097/PCC.0b013e3181b0655e]

17 **Sinderby C**, Beck J. Neurally adjusted ventilatory assist (NAVA): an update and summary of experiences. *Neth J Criti Care* 2007; **11**: 243-250. Available from: URL: http://njcc.nl/sites/default/files/Issue aanmaken/2007_NJCC_05 Sinderby.pdf

18 **Stein H**, Firestone K. Application of neurally adjusted ventilatory assist in neonates. *Semin Fetal Neonatal Med* 2014; **19**: 60-69 [PMID: 24238745]

19 **Durrani NU**, Chedid F, Rahmani A. Neurally adjusted ventilatory assist mode used in congenital diaphragmatic hernia. *J Coll Physicians Surg Pak* 2011; **21**: 637-639 [PMID: 22015130]

20 **Gentili A**, Masciopinto F, Mondardini MC, Ansaloni S, Reggiani ML, Baroncini S. Neurally adjusted ventilatory assist in weaning of neonates affected by congenital diaphragmatic hernia. *J Matern Fetal Neonatal Med* 2013; **26**: 598-602 [PMID: 23126605]

21 **Bordessoule A**, Emeriaud G, Morneau S, Jouvet P, Beck J. Neurally adjusted ventilatory assist improves patient–ventilator interaction in infants as compared with conventional ventilation. *Pediatr Res* 2012; **72**: 194-202 [PMID: 22580718 DOI: 10.1038/pr.2012.64]

22 **Beck J**, Reilly M, Grasselli G, Mirabella L, Slutsky AS, Dunn MS, Sinderby C. Patient-ventilator interaction during neurally adjusted ventilatory assist in low birth weight infants. *Pediatr Res* 2009; **65**: 663-668 [PMID: 19218884 DOI: 10.1203/PDR.0b013e31819e72ab]

23 **Chen Z**, Luo F, Ma XL, Lin HJ, Shi LP, Du LZ. Application of neurally adjusted ventilatory assist in preterm infants with respiratory distress syndrome. *Zhongguo Dangdai Erke Zazhi* 2013; **15**: 709-712 [PMID: 24034909]

24 **de la Oliva P**, Schüffelmann C, Gómez-Zamora A, Villar J,

Kacmarek RM. Asynchrony, neural drive, ventilatory variability and COMFORT: NAVA versus pressure support in pediatric patients. A non-randomized cross-over trial. *Intensive Care Med* 2012; **38**: 838-846 [PMID: 22481227 DOI: 10.1007/s00134-012-2535-y]

25 **Stein H**, Alosh H, Ethington P, White DB. Prospective crossover comparison between NAVA and pressure control ventilation in premature neonates less than 1500 grams. *J Perinatol* 2013; **33**: 452-456 [PMID: 23100042 DOI: 10.1038/jp.2012.136]

26 **Stein H**, Howard D. Neurally adjusted ventilatory assist in neonates weighing & lt; 1500 grams: a retrospective analysis. *J Pediatr* 2012; **160**: 786-9.e1 [PMID: 22137670 DOI: 10.1016/j.jpeds.2011.10.014]

27 **Lee J**, Kim HS, Sohn JA, Lee JA, Choi CW, Kim EK, Kim BI, Choi JH. Randomized crossover study of neurally adjusted ventilatory assist in preterm infants. *J Pediatr* 2012; **161**: 808-813 [PMID: 22658785 DOI: 10.1016/j.jpeds.2012.04.040]

28 **Alander M**, Peltoniemi O, Pokka T, Kontiokari T. Comparison of pressure-, flow-, and NAVA-triggering in pediatric and neonatal ventilatory care. *Pediatr Pulmonol* 2012; **47**: 76-83 [PMID: 21830318 DOI: 10.1002/ppul.21519]

29 **Krauss AN**, Klain DB, Waldman S, Auld PAM. Ventilatory Response to Carbon Dioxide in Newborn Infants. *Pediatr Res* 1975; **9**: 46-50 [DOI: 10.1203/00006450-197501000-00009]

30 **Connors G**, Hunse C, Carmichael L, Natale R, Richardson B. Control of fetal breathing in the human fetus between 24 and 34 weeks' gestation. *Am J Obstet Gynecol* 1989; **160**: 932-938 [PMID: 2496603]

31 **Rigatto H**, De La Torre Verduzco R, Gates DB. Effects of O2 on the ventilatory response to CO2 in preterm infants. *J Appl Physiol* 1975; **39**: 896-899 [PMID: 1213968]

32 **Marchal F**, Corke BC, Sundell H. Reflex apnea from laryngeal chemo-stimulation in the sleeping premature newborn lamb. *Pediatr Res* 1982; **16**: 621-627 [PMID: 7110784 DOI: 10.1203/00006450-198208000-00007]

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Accurate diagnosis of myalgic encephalomyelitis and chronic fatigue syndrome based upon objective test methods for characteristic symptoms

Frank NM Twisk

Frank NM Twisk, ME-de-patiënten Foundation, 1906 HB Limmen, The Netherlands

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Correspondence to: Frank NM Twisk, MBA, BEd, BEc, ME-de-patiënten Foundation, Zonnedauw 15, 1906 HB Limmen, The Netherlands. frank.twisk@hetnet.nl
Telephone: +31-72-5054775

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Abstract

Although myalgic encephalomyelitis (ME) and chronic fatigue syndrome (CFS) are considered to be synonymous, the definitional criteria for ME and CFS define two distinct, partially overlapping, clinical entities. ME, whether defined by the original criteria or by the recently proposed criteria, is not equivalent to CFS, let alone a severe variant of incapacitating chronic fatigue. Distinctive features of ME are: muscle weakness and easy muscle fatigability, cognitive impairment, circulatory deficits, a marked variability of the symptoms in presence and severity, but above all, post-exertional "malaise": a (delayed) prolonged aggravation of symptoms after a minor exertion. In contrast, CFS is primarily defined by (unexplained) chronic fatigue, which should be accompanied by four out of a list of 8 symptoms, *e.g.*, headaches. Due to the subjective nature of several symptoms of ME and CFS, researchers and clinicians have questioned the physiological origin of these symptoms and qualified ME and CFS as functional somatic syndromes. However, various characteristic symptoms, *e.g.*, post-exertional "malaise" and muscle weakness, can be assessed objectively using well-accepted methods, *e.g.*, cardiopulmonary exercise tests and cognitive tests. The objective measures acquired by these methods should be used to accurately diagnose patients, to evaluate the severity and impact of the illness objectively and to assess the positive and negative effects of proposed therapies impartially.

Key words: Myalgic encephalomyelitis; Chronic fatigue syndrome; Symptoms; Diagnosis; Disability; Impact

Core tip: The diagnostic criteria for myalgic encephalomyelitis (ME) and chronic fatigue syndrome (CFS) define two distinct clinical entities. Cognitive impairment and post-exertional "malaise" (a long-lasting aggravation of typical symptoms, *e.g.*, muscle weakness and cognitive "brain fog", after minor exertion) are obligatory for the diagnosis ME, while chronic fatigue is the only mandatory symptom for the diagnosis CFS. There is debate about the nature and severity of the symptoms in ME and CFS. For clinical and research purposes it is essential to accurately diagnose patients using objective tests for characteristic symptoms if possible. This article reviews accepted methods to assess various distinctive

symptoms of ME and CFS.

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INTRODUCTION

There is debate about various aspects of myalgic encephalomyelitis (ME) and chronic fatigue syndrome (CFS), including the nature of the symptoms, the etiology, the pathophysiology and presumed effective interventions, *e.g.,* cognitive behavioural therapy (CBT) and graded exercise therapies (GET)[1].

In light of the dispute about the origin of the symptoms, it is essential to assess the presence and severity of characteristic symptoms, and the impact and the disability in ME and CFS impartially as much as possible[2]. In the context of disability, it is important to establish physiological limitations in a specific patient objectively[3], independently of ones view on the etiology and the pathophysiology of ME and CFS.

To date diagnosis, symptom assessment and patient selection criteria of research studies of ME and CFS are often based upon self-report, questionnaires and subjective measures, *e.g.,* fatigue severity and impact. However, well-accepted methods can provide objective measures which can be used to diagnose patients more accurately. This article reviews relevant methods in this context.

ME/CFS is often initiated by an infection or another immunological insult[4]. Full recovery from ME/CFS seems rare (5%[5], 12%[6]). A long-term follow-up study[7] found that people who remitted from ME/CFS had non-significant differences in impairment on 17 out of 23 outcomes compared to those who maintained a CFS diagnosis. So, even patients who don't meet a CFS diagnosis anymore will not return to their premorbid level of functioning. ME/CFS has a greater negative impact on functional status and well-being than other chronic diseases, *e.g.,* cancer or lung diseases[8], and is associated with a drastic decrement in physical functioning[9]. In a comparison study[10] ME/CFS patients scored significantly lower than patients with hypertension, congestive heart failure, acute myocardial infarction, and multiple sclerosis (MS), on all of the eight Short Form Health Survey (SF-36)[11] subscales. As compared to patients with depression, ME/CFS patients scored significantly lower on all the scales, except for scales measuring mental health and role disability due to emotional problems, on which they scored significantly higher. Looking at several studies[12-16] the financial consequences of ME/CFS for the individual patient and the economic impact on society are often very profound.

This article aims to: (1) compares the diagnostic criteria for ME and CFS; and (2) reviews well-accepted methods to assess characteristic symptoms of ME and CFS objectively.

DIAGNOSIS

Although ME, CFS and post-viral fatigue syndrome are used interchangeably[17], the case criteria for ME[18] and CFS[19] define two distinctive clinical entities[1], delineating partially overlapping and partially disjoint patient populations (Figure 1).

ME, a neurological disease[20,21], has been described in the medical literature since 1934 under various names[22], *e.g.,* epidemic neuromyasthenia and atypical poliomyelitis, often on account of outbreaks[23-25]. Characteristic symptoms of ME, classified as a disease of the nervous system by the WHO since 1969[26], are: muscle weakness, neurological dysfunction, especially of cognitive, autonomic and neurosensory functions; variable involvement of the cardiac and other systems; a prolonged relapsing course; but above all general or local muscular fatigue after minimal exertion with prolonged recovery times (post-exertional "malaise")[20].

The clinical entity CFS was introduced in 1988[27] and redefined in 1994[19]. The diagnosis CFS is primarily based upon the ambiguous notion "chronic fatigue"[28,29]. According to commonly used criteria for CFS[19] "chronic fatigue" must be accompanied by at least 4 out of 8 symptoms, *e.g.,* tender lymph nodes and muscle and joint pain. However, 5 of the 8 "minor" symptoms, *i.e.*, headaches, lymph node pain, sore throat, joint pain, and muscle pain, do not differentiate people with melancholic depression group from healthy controls[30]. The CFS criteria[19] by definition select a heterogeneous population of people with "chronic fatigue"[31-34].

The diagnostic criteria for ME[18] and CFS[19] define distinctly nosological entities, since cognitive impairment and post-exertional "malaise", obligatory for the diagnosis ME, are not mandatory for the diagnosis CFS, and the diagnosis ME doesn't require "chronic fatigue". The distinction between ME and CFS is illustrated by a study[35] which found that 60% of the "less severe CFS" patents reported post-exertional "malaise" and 45% reported cognitive impairment. This implies that many "less severe" CFS patients don't fulfil the original[20] or new[18] criteria for ME. Looking at relevant studies[36-39] ± 30%-50% of subjects meeting the CFS criteria[19] seem to fulfil the more stringent International Consensus Criteria (ICC) for ME[18]. How many ME/ICC[18] patients don't meet the CFS[19] criteria is unknown, since almost all studies until now applied case definitions sequentially, *i.e.,* used other diagnostic criteria on a patient population preselected by chronic fatigue or CFS criteria[40]. In a recent study[41] ME/ICC[18] patients reported significantly more severe disability across all domains of the World Health Organisation Disability Adjustment Schedule 2.0[42] ($P < 0.05$), when compared to patients only fulfilling the criteria for

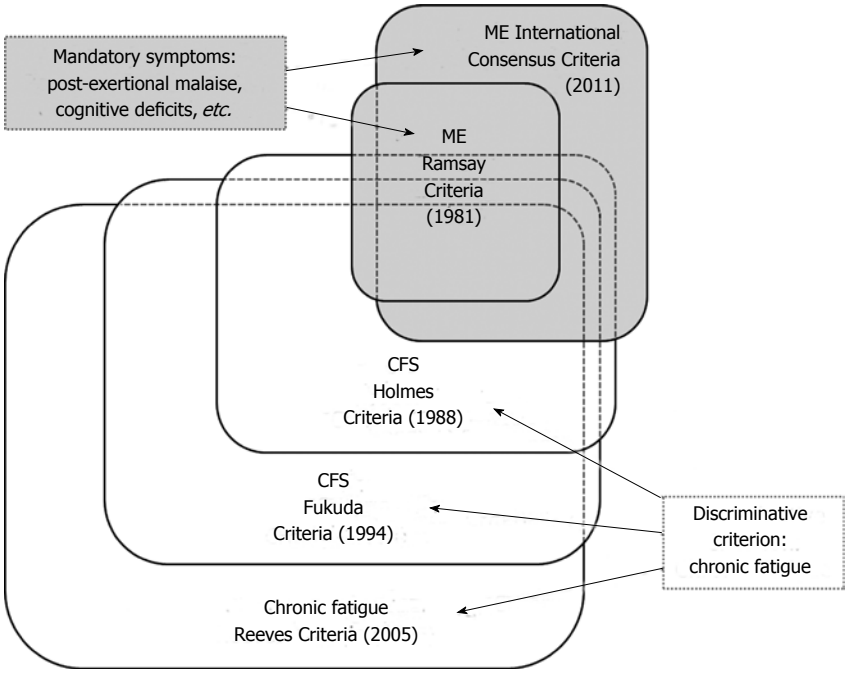

Figure 1 Myalgic encephalomyelitis *vs* chronic fatigue syndrome: Two distinct diagnostic entities. ME: Myalgic encephalomyelitis; CFS: Chronic fatigue syndrome.

Table 1 International Consensus Criteria for myalgic encephalomyelitis[18]

Post-exertional neuro-immune exhaustion:
A pathological inability to produce sufficient energy on demand with prominent symptoms primarily in the neuroimmune regions
Neurological impairments
At least one symptom from three of the following four symptom categories:
Cognitive impairments (information processing and short-term memory)
Pain (*e.g.*, headache, muscle, joint, abdominal and/or chest pain)
Sleep disturbance (disturbed sleep patterns and unrefreshing sleep)
Neurosensory, perceptual and motor disturbances
Immune, gastro-intestinal and genitourinary impairments
At least one symptom from three of the following five symptom categories:
Flu-like symptoms, *e.g.*, sore throat and tender lymph nodes
Susceptibility to viral infections with prolonged recovery periods
Gastro-intestinal tract complaints, *e.g.*, irritable bowel syndrome
Genitourinary complaints: *e.g.*, nocturia
Sensitivities to food, medications, odours or chemicals
Energy production and - transportation impairments
At least one of the following four symptoms:
Cardiovascular symptoms, *e.g.*, (delayed) orthostatic intolerance
Respiratory problems, *e.g.*, air hunger and fatigue of chest wall muscles
Loss of thermostatic stability, *e.g.*, sweating episodes or feverish feeling
Intolerance of extremes of temperature

CFS[19]. Another study[43] supports the notion that the ICC criteria for ME[18] identify patients with greater functional impairment and move severe physical, mental, and cognitive symptoms than patients who only meet the Fukuda criteria for CFS[19].

Diagnostic criteria applied (Figure 1) are crucial, not only because of the sensitivity and specificity[44-46] of the criteria[19,47], *e.g.*, resulting into the inclusion of people with psychiatric disorders[48], but also for a judgment about the effects of proposed effective interventions in specific patient populations[49-53].

Clinical assessment has shown to be essential for an accurate diagnosis and establishing prevalence rates. A recent study[54] for example observed that the pooled prevalence of CFS[19] was substantially higher for self-reporting assessment (3.28%, 95%CI: 2.24-4.33) than for clinical assessment (0.76%, 95%CI: 0.23-1.29).

In conclusion, ME[18] (Table 1) is not equivalent to CFS[19] (Table 2) or incapacitating chronic fatigue[55] (Table 3). While chronic fatigue is a common complaint, CFS[19] is a relatively rare condition (prevalence rate: 0.19%[38], 0.20%[58]). The prevalence of ME (CFS), as defined by more strict criteria[59], is even lower: 0.11%[38].

CHARACTERISTIC SYMPTOMS

ME/CFS patients often report a plethora of symptoms, which can vary in number and severity among individual patients and fluctuate within an individual over time, possibly as a result of daily activity[60]. Symptoms often reported by ME/CFS patients are post-exertional "malaise", cognitive deficits ("brain fog"), "fatigue" (lack of energy), muscle weakness, (muscle and/or joint) pain, impaired sleep, a new type of headaches, stress intolerance, orthostatic intolerance and visual symptoms[61].

Objective assessment of characteristic symptoms

Various typical symptoms, *e.g.,* post-exertional "malaise" and muscle weakness, can be quantified objectively using accepted, reproducible methods[1] (Table 4), while others symptoms, *e.g.,* fatigue, cannot be evaluated objectively due to their nature.

Due to the multi-systemic nature of ME/CFS, objective assessment of symptoms and disability in ME/CFS involves various medical specialists, *e.g.,* cardiologists, neuropsychologists, exercise physiologists,

Table 2 Fukuda *et al*[19] Diagnostic Criteria for chronic fatigue syndrome

Primary symptom:
Clinically evaluated, unexplained, persistent or relapsing chronic fatigue
That is of new or definite onset; is not the result of ongoing exertion
That is not substantially alleviated by rest; and
That results in substantial reduction in previous levels of occupational, educational, social, or personal activities
Secondary symptoms:
The concurrent occurrence of four or more of the following symptoms, all of which must have persisted or recurred during 6 or more consecutive months of illness and must not have predated the fatigue:
Self-reported impairment in short-term memory or concentration severe enough to cause substantial reduction in previous levels of occupational, educational, social, or personal activities
Sore throat
Tender cervical or axillary lymph nodes
Muscle pain
Multi-joint pain without joint swelling or redness
Headaches of a new type, pattern, or severity
Unrefreshing sleep
Post-exertional malaise lasting more than 24 h

Table 3 Empirical case definition for chronic fatigue (syndrome)[47]

Fatigue:
A score
⩾ 13 (out of 20) on the general fatigue or
⩾ 10 (out of 20) on the reduced activity
subscales of the multidimensional fatigue inventory[56]
Functional impairment:
A score
⩽ 70 (out of 100) on the physical function, or
⩽ 50 (out of 100) on role physical, or
⩽ 75 (out of 100) on the social function, or
⩽ 66.7 (out of 100) on the role emotional
subscales of the medical outcomes survey short form-36 (SF-36)[11]
Secondary symptoms:
⩾ 4 of the following 8 symptoms:
Impaired memory or concentration
Unrefreshing sleep
Headaches
Muscle pain
Joint pain
Sore throat
Tender cervical nodes and
Unusual post exertional fatigue
And
A score of ⩾ 25 (out of 128)
On the Symptom Inventory Case Definition subscale[57]

and endocrinologists. The exclusion of psychiatric diseases[18,19] and assessment of comorbid psychological disorders requires the input of psychologists/psychiatrists.

Table 4 denominates tests that have demonstrated to yield aberrant results in ME/CFS. However, Table 4 should not consider to be exhaustive. Due to the heterogeneity of the ME/CFS patients population[1,34,88], not all patients will experience all symptoms, *i.e.*, not all tests will show deviant results in all ME/CFS patients. Nevertheless, to assess the clinical status, the severity of the illness, and the disability of an individual patient impartially, patients should be subjected to the abovementioned and other objective tests as much is feasible.

Lack of energy: physical weakness and "fatigue"

ME/CFS is often incorrectly considered to be equivalent to chronic fatigue. "Fatigue" in ME/CFS is a multi-dimensional entity that is distinct from the generalized form of fatigue experienced by the general population[28]. Fatigue in ME/CFS encompasses at least five dimensions: a lack of energy resources needed for basic daily functioning, "brain fog", post-exertional "malaise", a "wired feeling" when very tired, and a flu-like feeling[28]. While these latter two aspects of "fatigue" are subjective due to their nature, the first three dimensions can be assessed more objectively. This paragraph focuses on "lack of energy", while "brain fog" (neurocognitive deficits) and post-exertional "malaise" will be discussed in the next paragraphs.

Cardiopulmonary exercise testing (CPET) is regarded to be an accurate method for assessing functional capacity[62,89]. The (maximum) oxygen uptake (O2) measured at a CPET is associated with the concept of Metabolic Equivalent of Task (MET): the energy cost of a physical activity compared to the energy produced by an average person seated at rest. By definition, 1 MET is equivalent to an oxygen utilization of 3.5 mL O_2/kg per minute. The functional capacity established by a CPET can be set against the metabolic requirements[90] of self-care tasks essential for fundamental functioning (Basic Activities of Daily Living, ADLs) and activities crucial to live independently from others (Instrumental Activities of Daily Living, IADLs)[91].

Although contradicted by some studies, *e.g.*,[92,93], various studies, *e.g.*,[94-98], implicate that the "lack of energy" experienced by ME/CFS patients is reflected in the performance levels at a CPET: a low maximum workload (Wmax) and oxygen uptake (VO2max) and a reduced anaerobic threshold (W AT) and corresponding oxygen uptake (VO2 AT), when compared to sedentary controls. When looking at the "high" mean performance levels of patients in some studies, *e.g.*,[92,99], contradictory findings are likely due to heterogeneity of the patient samples, *e.g.*, participation rates of "severe" *vs* "less severe cases".

Whether or not the exercise capacity is decreased or not in an individual patient should be assessed impartially by a CPET. Objective measures should be employed to establish the degree of effort during exercise, *e.g.*, the respiratory exchange ratio at peak exertion (RERmax). According to well-accepted criteria[62,63] a RERmax > 1.10 indicates excellent effort, while a RERmax < 1.0 reflects submaximal effort. Although (some) patients seem to be able to perform at a level comparable to that of sedentary controls once-off, a CPET often has profound negative effects on the

Table 4 Symptoms and tests to assess the disability in myalgic encephalomyelitis/chronic fatigue syndrome objectively

Symptoms	Tests	Ref.
Lack of energy: physical weakness and "fatigue"	CPET 1: workload and oxygen uptake at exhaustion and at the anaerobic threshold	[62,63]
Cognitive impairment	Specific neuropsychological tests	[64-67]
Post-exertional "malaise"		
Physical effects	Repeated CPETs 1, 24 h apart	
Cognitive effects	Specific neuropsychological tests before and after a CPET or before and during a tilt table test Repeated neuropsychological tests	
Muscle weakness	Examination of the muscles (power, endurance, recovery)	[68-71]
Orthostatic intolerance	Tilt-table test	[72-74]
Defective stress response	Hormonal investigation (HPA axis, thyroid) in rest, at specific moments, *e.g.*, at wakening, and during the day, after provocation, *e.g.*, by adrenocorticotropic hormone and insulin, and in response to an exercise test or psychological stress test	[75-78]
Sleep impairment	Polysomnographic investigation (EEG)	[79-81]
	Maintenance of wakefulness test	[79,82,83]
	Multiple sleep latency test	[79,82,83]
Visual symptoms	Useful field of view tests	[84,85]
	Eye movement tests	[86,87]

CPET: Cardiopulmonary exercise test.

exercise capacity 24 h later at a second CPET (see Post-exertional "malaise").

A "lack of energy" seems to be accompanied by hypovolemia (low blood volume)[98,100], low cardiac mass[101-103] and reduced cardiac function[100,104]. Some studies implicate interrelations between hypovolemia and low cardiac output[100] and between hypovolemia and (maximum) oxygen uptake[105]. A reduction of the exercise capacity seems to be associated with typical immunological abnormalities in ME/CFS, including immune activation and immune dysfunction[106-108].

In addition to a reduced exercise capacity, the "lack of energy" of ME/CFS patients seems to manifest itself in post-exertional malaise[109], muscle weakness[110] and orthostatic intolerance[111], which will be discussed in separate paragraphs.

Cognitive impairment

A second characteristic symptom of ME/CFS is cognitive impairment ("brain fog")[39]. Several studies, *e.g.*,[112-118], have established a wide range of neurocognitive deficits in ME/CFS. In addition, various studies have observed neurological aberrations[119-121], *e.g.*, reduced white[122-124] and grey[123,125,126] matter volume, electroencephalography (EEG) abnormalities[127], hypoperfusion of the brain[128-130], hypometabolism[131,132], neuro-inflammation of widespread brain regions[133], increased fractional anisotropy in the right arcuate fasciculus and, in right-handed patients, of the right inferior longitudinal fasciculus[124], and spinal fluid abnormalities[134,135]. A relationship between neurological anomalies and cognitive symptoms has also been observed[136-138]. Some findings indicate that the neurocognitive problems are induced or intensified by exercise[97,139] and an upright (orthostatic) position[140]. Cognitive impairment seems to be more severe in sudden onset-ME/CFS[141,142].

ME/CFS patients can present with moderate to large deficits in simple and complex information processing speed (attention, memory and reaction time)[143], in tasks which require working memory over a sustained period of time[143,144], in tasks which necessitate (simultaneous) processing of complex information[116,117] and in conflict-monitoring tasks (interference control)[145]. Specific cognitive deficits, reduced exercise capacity, decreased muscle power (strength and endurance) and immunological aberrations, *e.g.*, inflammation, seem to be interrelated[146,147].

Cognitive impairments can be identified, but only if the appropriate measures are used[114]. This important observation is confirmed by a meta review of 50 studies and 79 tests[143]. All tests for assessing attention, including attention span and working memory, showed significant deficits in ME/CFS. The effect sizes for most word list learning and recall tests were significant, but some tests seem more sensitive to memory deficits in ME/CFS than others. Reaction time is substantially impaired for responses to both simple and complex (choice) stimuli. Only two of the five tests used to assess movement times revealed significant group differences. Most tests for visuospatial ability, verbal abilities and language, cognitive reasoning and flexibility, and global functioning didn't yield significant group differences. In order to determine cognitive impairment objectively, ME/CFS patients should be subjected to neuropsychological tests[64-67] aimed at the abnormalities found in ME/CFS patients, *e.g.*, attention and memory[112,116,143].

Cognitive deficits don't seem to be related to "fatigue" or comorbid depression[148,149]. Goedendorp *et al*[150] have suggested that low cognitive test scores are due to underperformance, but this view is based upon the subjective premise that ME/CFS has not proven to be a cognitive disorder[151]. Objective measures indicate high levels of effort and an intention to do well during neurocognitive testing[152].

Post-exertional "malaise": physical and mental

Post-exertional "malaise" has been defined as "a pathological inability to produce sufficient energy on demand"[18], resulting into a (delayed) increase of typical symptoms, *e.g.*, weakness, muscular and/or joint pain, cognitive deficits, after a minor physical or mental exertion, with prolonged "recovery" times[109,153].

Looking at the research, post-exertional malaise in ME/CFS can present itself in several forms, including a decline in physiological exercise capacity at a second exercise test 24 h later[94,154], cognitive impairment

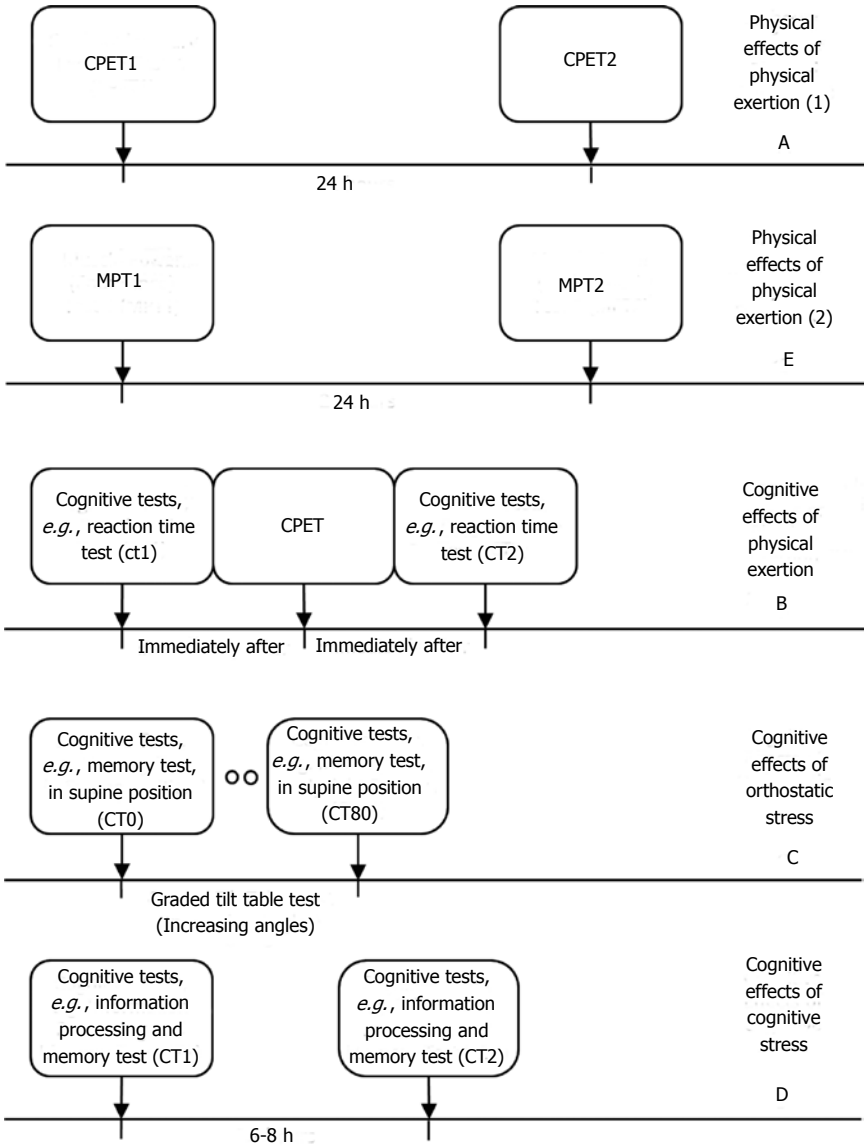

Figure 2 Objective tests for post-exertional malaise. CPET: Cardiopulmonary exercise test; MPT: Muscle power test.

induced or intensified by exercise[97,139] and orthostatic stress[140], and cognitive deficits due to mental exertion, *e.g.,* cognitive testing[155]. These various dimensions of post-exertional "malaise" can be assessed by combining CPETs, tilt-table and specific cognitive tests (Figure 2). The (long-lasting) physical effects of physical exertion (Figure 2A) can be evaluated objectively by subjecting a patient to two CPETs until exhaustion separated by 24 h[156]. The cognitive effects of physical exertion (Figure 2B) can be assessed impartially by comparing the cognitive performance, *e.g.,* simple and choice reaction times, before and immediately after a CPET, the cognitive effects of orthostatic stress (Figure 2C) can be established by subjecting patients to cognitive tests at various stages of a graded tilt table test, while the mental effects of cognitive exertion (Figure 2D) can be assessed objectively by exposing a patient twice to the same cognitive tests with several hours rest in-between. The prolonged negative effect of muscle contractions on muscular strength (Figure 2E), another aspect of the physical dimension of post-exertional "malaise", will be discussed in the paragraph Muscle weakness.

Physical effects of physical exertion: A profound decrease in the exercise capacity at a second CPET 24 h after the first CPET seems typical for ME/CFS and is neither observed in sedentary healthy controls nor in patients with other diseases[154,157]. The "exercise intolerance" in ME/CFS can be reflected in significantly lower oxygen uptake and performance levels at exhaustion (VO2max and Wmax) or at the anaerobic threshold (VO2 AT and W AT) at the second CPET[154]. In contrast, the first CPET appears to have a positive effect on the anaerobic threshold in sedentary controls at the second CPET[94]. Due to the first CPET the anaerobic threshold can decrease to a level below 5 METS; a level at or below that which is required by many job-related activities and IADLs[158]. Since many daily activities fall into the 3-5 MET energy range, persons with ME/CFS will exacerbate symptoms simply by completing normal daily activities[158]. A recent study[157] observed that VO2max at the first exercise test was reduced in

Table 5 Adverse effects of a CPET (CPET1) on the performance levels at a second CPET (CPET2) 24 h later: An example

	CPET Day 1	CPET Day 2
Rest		
Heart rate	88	80
Oxygen uptake (VO2min)	6	6
Anaerobic threshold		
Heart rate (HR AT)	105	89
Oxygen uptake (VO2 AT)	11	9
Workload (W AT)	54	35
Exhaustion		
Heart rate (HRmax)	151	131
Oxygen uptake (VO2max)	23	22
Workload (Wmax)	159	133

CPET: Cardiopulmonary exercise testing.

ME/CFS (mean ± SD: 21.9 ± 4.75 mL/k per min), that all patients showed clinically significant decreases in either VO2max and/or oxygen uptake at the ventilatory threshold (VO2 VT) at the second CPET, and that a classification of impairment[159] based on the VO2max or VO2 VT of the first CPET would result in overestimation of functional ability for 50% of the patients.

A real-life example of the effect of a CPET on the performance levels at a second CPET 24 h is summarized in Table 5 (male patient, 45 years, 65 kg).

The VO2max at day 1 was 23 mL/min per kilogram, while the anaerobic threshold was reached at a workload level of 54 Watt (W). The corresponding oxygen uptake (VO2 AT) was 11 mL/min per kilogram. Washing the floor requires 58 W and walking at a speed of 5 km/h 56 W[160], which implies that the anaerobic threshold (AT) is reached just by doing light household activities or low-speed walking. On the second CPET the AT declined to a workload of 35 W, which is equivalent to the energy cost of ironing (35 W) and walking at 3 km/h (32 W)[160]. The oxygen uptake at the anaerobic threshold (VO2 AT) has decreased to 9 mL/min per kilogram on day 2. The difference between the heart rate at the anaerobic threshold and the heart rate at rest is only 9 bpm at day 2. This example perfectly illustrates the (prolonged) negative effects of exertion in ME/CFS.

Mental (cognitive) effects of physical exertion: Although research studies into the effects of physical exercise on cognitive performance are scarce, there are several indications that exercise has a (durable) negative effects on cognitive functioning, *e.g.,* focused and sustained attention[161], simple reaction time and choice reaction times[97] and accuracy at the Symbol Digit Modalities Test, Stroop Word Test and Stroop Color Test[139]. This negative effect seems to be the opposite of the effect of exercise on cognitive performance in sedentary controls[139]. The negative impact of physical exertion on cognitive functioning could be mediated by reduced prefrontal cortex oxygenation during and after exercise[98] and/or diminished cardiovascular response to cognitive stress[162]. The effects of physical exertion on cognitive impairment can be assessed by subjecting a patient to specific cognitive tests[114,143] 0-24 h after a CPET and comparing the results on these tests with the pre-exercise test scores.

Mental (cognitive) effects of orthostatic stress: A subgroup of patients present with (delayed) orthostatic intolerance[163], as implicated by a substantially increased heart rate and/or reduced blood pressure in an upright position (POTS: postural tachycardia syndrome, respectively postural hypotension)[74]. Orthostatic symptoms are independently associated with functional impairment[164]. Orthostatic stress seems to impair working memory and information processing, as indicated by a deterioration of scores and reaction times on the N-back test as orthostasis progresses[140,165]. This phenomenon could be related to reduced neuronal activated cerebral blood flow velocity during orthostatic stress[165]. To assess the effects of orthostatic stress objectively, the patient should be subjected to cognitive tests at various angles during tilt-table testing. The N-back test could be a suitable cognitive test, since studies[140,165] have found that while N-back outcome in controls decreased with the value of N, the score was independent of tilt angle, while N-back outcome in ME/CFS patients also decreased with the value of N, but deteriorates as the tilt angle increases. A recent study[166] found that upright tilting caused a significant increase in the N-back normalized response time and a profound drop in cerebral blood flow velocity.

Mental (cognitive) effects of cognitive stress: Although scarcely investigated, there are indications that cognitive stress induces long-lasting mental/cognitive effect in ME/CFS. As mentioned in the paragraph Cognitive Impairment, "recovery" from a 3-h lasting cognitive test to pre-test levels of mental energy seems to take much longer in ME/CFS patients[155]. This phenomenon could be associated to a greater mental and neurological effort to process information as effectively as healthy controls[136-138,167]. A recent study[168] observed significant differences between self-reported levels of general, mental, and physical fatigue[58] and depression[169] before and two days after a cognitively fatiguing task[67]. Whether the effects of cognitive stress encompasses a mental dimension in a specific patient, could be assessed objectively by subjecting a ME/CFS patient to cognitive tasks 6-8 h after a fatiguing cognitive test battery and comparing the first and second scores on specific cognitive tests[114,143].

Muscle weakness: Many patients report muscle weakness[30,35,44]. According to a recent study[170] muscle recovery is closely related to cognitive deficits (see cognitive impairment). Several studies indicate reduced muscle strength and endurance and prolonged recovery from muscle contractions in ME/CFS. One study[171] for example found that the hand grip strength of patients

was significantly (26%) less than sedentary controls and that the maximum voluntary contraction (MVC) force in the patient group significantly reduced to 83% of the low baseline strength after 50 contractions with 10 s and 50 contractions with 5 s rest between trials. This study also observed deviant EEG-recorded brain signals in controlling voluntary muscle activities, especially when the activities induce fatigue. Another study[172] observed that ME/CFS patients were able to sustain a 10 kg handgrip contraction for less time than healthy controls and that the mean of handgrip contraction strength was substantially lower in ME/CFS patients when compared to healthy controls. A third study[110] found that the maximum twitch interpolated voluntary isometric contraction force of the quadriceps muscle of the dominant leg was significantly lower in ME/CFS patients (interquartile range, IQR: 234N-386N) than in sedentary controls (IQR: 364N-518N). In addition to reduced muscle strength and endurance, recovery of voluntary muscle contractions seem to be prolonged. This is illustrated by a study[173] in which patients and sedentary controls were subjected to an experiment involving 18 MVCs with a 50% duty cycle (10 s contraction, 10 s rest), followed by a recovery phase, lasting 200 min, in which the strength of the quadriceps muscle group was assessed, and a follow-up session 24 h post-exercise, involving three 10 s MVCs. The MVC forces in the control group were significantly higher than those of the ME/CFS patients, with a decline in force over the 18 contractions in both groups. Recovery was prolonged in the patient group, with a significant decline in the MVCs during the recovery phase and also at 24 h-post exercise (73% ± 9% of the initial force levels in patients *vs* 91% ± 7% in controls). In a recent study[174] patients exhibited lower isometric MVC levels for handgrip strength and slower and incomplete recovery in the 45 min after a fatiguing exercise (18 maximum contractions using a 50% duty cycle, 5 s contraction, 5 s rest), compared to both MS patients and sedentary healthy controls.

All in all, muscle weakness in ME/CFS seems to manifest itself into reduced muscle power, declining dynamic muscular endurance and the long-lasting recovery from repeated voluntary muscle contractions (Figure 2E). In order to assess muscle weakness and recovery from muscle exercise objectively and reliably the use of isokinetic and isometric dynamometers is essential[68-71,175-178].

Various studies have observed impaired skeletal muscle metabolism in ME/CFS, *e.g.,* decreased basal values of PCr/(PCr + Pi), increased pH levels during exercise, and low intracellular concentrations of ATP at exhaustion of a graded exercise of the right gastrocnemius muscle[179,180]. In addition to a significant prolongation (almost 4-fold) of the time taken by pH to recover to baseline after exercise, Jones *et al*[181] revealed the existence of two CFS[19] subgroups: patients with normal PCr depletion in response to a low-level voluntary contraction exercise, but with substantially increased intramuscular acidosis, and patients with low PCr depletion during exertion, generating abnormally low muscle power. An impaired cardiovascular response to standing, orthostatic intolerance, cardiac bioenergetic abnormalities, as implicated by low PCr/ATP values, and reduced muscle metabolism (longer PCr and ADP recovery times) seem to be interrelated[101].

Orthostatic intolerance: Orthostatic intolerance is accompanied by symptoms that arise or aggravate while standing, *e.g.,* light-headedness, blurred vision, fainting and syncope. Several studies[182-185] indicate orthostatic intolerance in ME/CFS patients or subgroups. Altered cardiovascular autonomic control and responses to orthostatic stress are associated with other typical symptoms, *e.g.,* cognitive deficits, and disability[186]. Since orthostatic intolerance is already present in the early stages of the disease[182,187] and CFS patients with POTS were have found to be significantly younger and to have a shorter length of illness than CFS patients without POTS[188], it seems unlikely that prolonged inactivity accounts for the orthostatic symptoms.

Orthostatic intolerance (in ME/CFS) seems associated with specific cardiovascular abnormalities in an upright position[111,189-191], *e.g.,* POTS and neurally mediated hypotension (NMH). These cardiovascular aberrations can be assessed using a tilt table test. The head-up tilt testing is considered a clinically useful diagnostic tool to assess susceptibility to orthostatic intolerance in patients with syncope, allowing reproduction of the patient's symptoms in a safe environment, under medical control[72,192]. With regard to deviant cardiovascular responses to orthostatic stress five types of abnormalities[74] can be distinguished (Table 6).

In a tilt table test a patient has to lie on a special table/bed, which gradually moves in posture from lying to an "upright position", *e.g.,* 70 degrees. Heart rate and blood pressures are monitored at various angles in order to establish hemodynamic abnormalities in a specific subject. It is relevant to note that the abovementioned aberrations seem to occur delayed and suddenly in ME/CFS[163,195]. So, in order to assess potential orthostatic abnormalities in a patient, the patient should remain in an "upright" position as long as possible, preferably longer than 15 min.

Defective stress response: Not only physical but psychological stress as well seems to intensify the symptoms in ME/CFS[196]. This phenomenon seems to be associated with hypothalamic-pituitary-adrenal (HPA) axis dysfunction[197,198], including hypocortisolism and deviant physiological responses to stress. HPA dysfunction in ME/CFS can manifest itself in reduced levels of stress hormones, *e.g.,* cortisol, at specific moments of the day[199,200] and aberrant diurnal production of specific hormones, *e.g.,* cortisol, cortisone and adrenocorticotropic hormone (ACTH)[201,202]; a blunted response to provocation, *e.g.,* by insulin[203], ACTH[204] or CRH[78]; a (long-lasting) deviant response

Table 6 Manifestations of orthostatic cardiovascular abnormalities

Abnormality	Definition
Orthostatic systolic hypotension	A fall in the systolic blood pressure of 20 mmHg or more[74,193]
Orthostatic diastolic hypotension	A fall in the diastolic blood pressure of 10 mmHg or more[74,193]
Orthostatic diastolic hypertension	A rise in dBP to 98 mmHg or more[74]
Orthostatic postural tachycardia	An increase in heart rate of 28[74]/30[194] beats per minute (bpm) or a pulse of more than 110[74]/120[194] bpm
Orthostatic narrowing of pulse pressure	A fall in the pulse pressure to 18 mmHg or less[74]

to psychological[205] or physical stress[205,206], and an enhanced sensitivity of the cellular immune system to glucocorticoids[207,208] and increased negative feedback of glucocorticoids to the HPA axis[209,210]. HPA axis dysfunction is not likely to be the primary cause of the illness, since HPA axis hypofunction, *e.g.,* hypocortisolism, is only present in a subgroup of patients[200,211], HPA axis abnormalities manifest themselves at a later stage of the illness[212-214] and hydrocortisone/fludrocortisone seem to have limited[215] or adverse[216] effects.

HPA axis dysfunction in ME/CFS can result into (1) low basal levels of ACTH; (free and total) cortisol (according to gas chromatography-mass spectrometry and high-performance liquid chromatography are considered to be the golden standard for assessing cortisol levels[78]), DHEA/DHEAS and noradrenalin at specific moments of the day, *e.g.,* at awakening; (2) reduced synthesis of ACTH and cortisol during the day; and (3) blunted HPA axis responses to "provocation", exercise or psychological stress. Tests[75,217] to assess HPA axis dysfunction objectively should be aimed at these aberrations.

ME/CFS has also been associated with thyroid dysfunction[218]. This finding is in line with inflammation-mediated loss of thyroid function[219-221]. Thyroid dysfunction can present itself in (1) low (free) thyroxine (T4) levels[222], due to decreased levels of thyroid stimulating hormone (TSH) secreted by the pituitary or a blunted response of the pituitary to TSH; (2) by reduced uptake of triiodothyronine (T3) and T4 by the cell[223,224]; (3) by diminished T4-T3-conversion, resulting into increased levels of reverse triiodothyronine (rT3)[225,226]; (4) by a diminished production of TSH and free T3 and T4 after administration of thyrotropin-releasing hormone (TRH)[227]; and/or (5) antithyroid microsomal antibodies[228]. Thyroid tests[229] could reveal if these aberrations are present in a particular patient.

Sleep impairment: Many patients report sleep disturbances[30,35,230,231], *e.g.,* insomnia, frequent awakenings, vivid dreams/nightmares and day/night reversal. Non-restorative sleep is the most specific and sensitive "minor" symptom[232] of CFS[19]. Sleep seems to be disturbed differently patterns in ME/CFS patients with and without comorbid fibromyalgia[233]. Abnormalities have been observed in reduced theta, sigma, and beta spectral power during the various sleep stages and shorter duration and higher frequency of transitions between the sleep stages[234-239].

Some methods to establish sleep dysfunction in ME/CFS objectively are: polysomnographic sleep investigation (EEG), aimed at the frequency of transitions between and the duration of sleep phases[233,237] and spectral power analysis[235,239], the maintenance of wakefulness and the multiple sleep latency test, although the latter two could be considered subjective, and not objective tests.

Visual symptoms: Patients often report visual symptoms, *e.g.,* problems with focusing, blurred vision and light insensitivity[240-242]. While various visual symptoms can be qualified as subjective, some aspects of the visual function can be assessed objectively. Abnormal visual attention, *e.g.,* conjunctive search (with divided and selective attention) and spatial cueing (selective attention with distraction)[243], can be assessed with the Useful Field of View test[84,85]. According to a recent study[244] dysfunctional eye movements in ME/CFS can present itself in reduced antisaccade focus accuracy and less precision and speed at smooth pursuing a target.

Subjective assessment of characteristic symptoms

While several symptoms can be assessed objectively, other characteristic symptoms[18,30] can't be quantified easily due to their nature. These symptoms include pain (muscle and joint pain, headaches, *etc.*), abdominal pain and other gastro-intestinal symptoms, "sickness behavior" (flu-like feeling, depression, *etc.*), intolerance of light (photophobia), sound (phonophobia) and odors (osmophobia), food and chemicals, and disturbed thermoregulation[18,30]. However, several of these symptoms could logically be explained by aberrations observed in ME/CFS patients or subgroups. Pain *e.g.,* could be the result of (1) inflammation[245,246]; (2) reduced oxidative metabolism[180,247], mitochondrial dysfunction[248,249] or damage[250,251]; (3) low cardiac output[101,185] and reduced blood and oxygen supply to the brain[98,129] and muscles[252,253], possibly leading to acidosis[181,254], accelerated glycolysis[180] and elevated lactate levels[132,255]; (4) central sensitisation[256], as a potential sequel of inflammation[257,258] and oxidative and nitrosative stress[259,260]; and (5) elevated pain receptors[261,262].

When assessing patients it is crucial to keep in mind that while various symptoms are obligatory for the diagnosis ME[18], they are not obligatory for to fulfil the CFS[19] diagnostic criteria. As argued, objective tests are to be preferred when possible. Nevertheless, when questionnaires and subjective measures are used to assess the clinical status of a patient, applying

minimum thresholds for the frequency and the severity of symptoms (*e.g.,* moderate severe about half of the time) can reduce the likelihood of possible misclassification of healthy persons and ME/CFS patients[61].

IMPACT

ME/CFS has a profound effect on the functional status[10,263] and life[264,265] of patients. As argued in this chapter, an objective assessment of the clinical status can quantify the severity of characteristic symptoms, *e.g.,* cognitive impairment, low physiological exercised capacity and the detrimental effects of exertion. However, next to the illness burden, patients experience serious medical, financial, social and psychological consequences of their illness, which can have a profound impact.

An objective assessment of symptoms could help to resolve the controversy around the nature and impact of ME/CFS within the medical profession. Patients for example often report negative experiences with health care workers[266]. Some medical professionals don't consider ME/CFS to be a legitimate illness[267]. Despite the neurological classification of ME/CFS[17,26] and various neuro-immunological abnormalities in ME/CFS observed repetitively[18,268], 84% of respondents in a survey of members of the Association of British Neurologists answered they did not consider ME/CFS to be a neurological condition[269]. According to a study[270] only half of the general practitioners (GPs) believed that ME/CFS actually exists. In a survey[271] patients reported that they felt that the doctors psychologized too much or trivialized the symptoms. Increasing physical activity had been recommend by doctors, but most of the respondents reported that this made them worse[271].

ME/CFS often has a huge impact on the occupational status[272] and income of patients[16], school attendance[273] and performance[265] of young patients, and the income of parents of children with ME/CFS[14]. A substantial proportion of ME/CFS patients, 50.1% according to[16], has to discontinue their employment due to their illness. Looking at the data of various studies[12,13,14] the average annual direct cost (medical costs, *etc.*) vary from $ 2342 to $ 8675, and the indirect cost (work productivity losses, disability reimbursements, services provided by family members, friends and others, *etc.*) vary from $ 8554 to $ 20000, for men: $ 23124[12,15,16]. Based upon the prevalence rates of[38] the direct and indirect cost of ME/CFS to the US society could be estimated at $ 8.5-$11 billion annually.

The prevalence of ME/CFS in children based upon a cross-sectional national sample among GPs was 0.111%[274], which is comparable with other prevalence figures[38]. The impact of ME/CFS on school attendance of children and adolescent is profound. On study for example found that 62% of children and young people aged under 18 years with ME/CFS, attended 40% of school or less[275]. Another study[274] observed that 45% of young patients with ME/CFS (aged 10 to 18 years) reported > 50% school absence during the previous 6 mo. A substantial subgroup of young patients, 29% of patients aged 12-18 years according to Bould *et al*[276], reports symptoms of comorbid depression, which seems to be associated with the degree of disability. The prognosis of children with CFS seems better in adolescents, *e.g.,* in CFS induced by infectious mononucleosis[277,278], but in both adults and adolescents the severity of the acute phase seems to be the sole predictor of the outcome[187,278].

ME/CFS also can have serious social and emotional consequences, *e.g.,* marginalisation, social isolation, stigmatisation and transformation of identity. Many patients with ME/CFS feel that their illness is not acknowledged as a legitimate illness within the social and medical context[271], and patients often report marginalization from family, friends, and medical professionals[279]. Not being able to be with friends or to attend school, makes adolescents with ME/CFS feel isolated, different and forgotten[280]. ME/CFS can also result into a transformation in identity[272,279] and values, expectations and life priorities[281]. In addition to destroying relationships and careers[272], ME/CFS also can disrupt self-perceptions[282]. Much of the stigma experienced by ME/CFS patients seems to originate from the associations with the name CFS[283], the lack of diagnostic biomarkers[284] and the absence of clear-cut etiologic models for ME and CFS[284]. Questioning the veracity of ME/CFS might represent a potent stressor in ME/CFS, and even coping methods thought to be useful in other conditions, are not associated with a reduction of distress among those with ME/CFS[284]. Doctors' beliefs can result into negative stereotyping of ME/CFS patients[285]. However, there don't seem to be major differences between the personalities of ME/CFS patients and patients with rheumatoid arthritis and the stereotype of ME/CFS patients as being "perfectionists with negative attitudes toward psychiatry" doesn't seem to be applicable[286].

DISCUSSION

ME/CFS is a serious disorder, which can have profound consequences on a patients' life and health status. In addition to the impact of the symptoms on everyday life, patients often disbelief, *e.g.,* when claiming disability related benefits[156]. Due to the fact that "chronic fatigue" is an ambiguous and subjective notion[28], that patients often report a plethora of symptoms which can fluctuate over time very rapidly, and that there are (yet) no clear-cut etiological models for ME and CFS, patients frequently encounter difficulties in proving their level of disability, which can have substantial financial consequences.

An objective assessment of core symptoms could not only impartially confirm the patients' self-reported disability[287], but could also contribute to reversal of other problems experienced by patients, *e.g.,* stigmatization and the attitude of medical professionals

towards the illness(es) and patients. In this context and in light of the controversy surrounding ME and CFS, it is essential to establish the functional (dis)abilities of a patient (output or functional consequences) objectively without an a priori judgment about the causes (the "black box": etiology and pathophysiology). However, establishing the functional impact objectively using well-accepted tests, *e.g.,* (repeated) exercise tests (CPETs), neurocognitive tests and tilt table tests, also could point towards the physiological origin of various symptoms, *e.g.,* post-exertional "malaise".

One very relevant limitation with regard to the objective assessment of symptoms relates to practical and ethical perspectives, since moderate and severe cases of ME/CFS may not be able to perform specific tests, *e.g.,* CPETs and tilt table testing, and the ethics of requiring patients to undertake a test likely to intensify pain and other symptoms could be questioned[154].

Assessing symptoms objectively, if possible, instead of using questionnaires and subjective measures, could also largely improve scientific progress. For example, the controversy about the claim that CBT and GET are effective interventions[49,288] without detrimental effects[289], which is challenged by others[50,290], could be resolved by subjecting the patients to objective tests, *e.g.,* CPETs and cognitive tests, before during and after CBT/GET. Especially since studies have shown that reduction in "fatigue" after behavioural interventions is not reflected by a clinical improvement in objective terms, *e.g.,* activity levels[291], distance walked in 6 min[49] or oxygen uptake[292]. Future trials into proposed effective pharmaceutical[52,53] and behavioural therapies[49,288] should be using objective measures to establish positive and negative effects in clear-defined patient populations impartially[293].

CONCLUSION

Although the labels ME and CFS are often used interchangeably, the diagnostic criteria for ME and CFS define two distinct, partially overlapping, clinical entities. ME, whether defined by the original[20] or the new consensus criteria[18], is not equivalent to CFS[19]. Muscle weakness, cognitive impairment, and above all, post-exertional "malaise", obligatory for the diagnosis ME, is not mandatory for the diagnosis CFS, while "chronic fatigue", the core feature of the diagnosis CFS, is not mandatory for ME.

Partly due to the subjective nature of the symptom-based definitions of ME and CFS and the use of self-report, questionnaires and subjective measures, some researchers and clinicians have questioned the physiological origin of the symptoms and qualified ME and CFS as functional somatic syndromes. The use of objective tests and measures to assess symptoms and functional limitations, *e.g.,* CPETs and cognitive tests, could resolve the controversy with regard to the nature of ME and CFS and the consequences for patients' lives and professional abilities. Looking at the medical, financial, social and emotional impact of ME and CFS on patients and society, and the future perspective of patients, an objective assessment of the symptoms and disability is a crucial step.

To explore the etiology and pathophysiology in well-defined ME and CFS patient subgroups research should employ objective test and biomarkers[1]. Therapies proposed to be effective for ME and/or CFS, should be evaluated by employing an objective assessment of the clinical status and biomarkers before, during and after the intervention in well-defined patient (sub)groups.

REFERENCES

1 **Twisk FN**. The status of and future research into Myalgic Encephalomyelitis and Chronic Fatigue Syndrome: the need of accurate diagnosis, objective assessment, and acknowledging biological and clinical subgroups. *Front Physiol* 2014; **5**: 109 [PMID: 24734022 DOI: 10.3389/fphys.2014.00109]

2 **Twisk FN**. A definition of recovery in myalgic encephalomyelitis and chronic fatigue syndrome should be based upon objective measures. *Qual Life Res* 2014; **23**: 2417-2418 [PMID: 24935018 DOI: 10.1007/s11136-014-0737-1]

3 **Klimas N**, Patarca R. Disability and chronic fatigue syndrome: clinical, legal and patient perspectives. Binghamton NY: Haworth Medical Press, 1997

4 **De Becker P**, McGregor N, de Meirleir K. Possible triggers and mode of onset of chronic fatigue syndrome. *J Chronic Fatigue Syndr* 2002; **10**: 3-18 [DOI: 10.1300/J092v10n02]

5 **Cairns R**, Hotopf M. A systematic review describing the prognosis of chronic fatigue syndrome. *Occup Med* (Lond) 2005; **55**: 20-31 [PMID: 15699087 DOI: 10.1093/occmed/kqi013]

6 **Pheley AM**, Melby D, Schenck C, Mandel J, Peterson PK. Can we predict recovery in chronic fatigue syndrome? *Minn Med* 1999; **82**: 52-56 [PMID: 10589213]

7 **Brown MM**, Bell DS, Jason LA, Christos C, Bell DE. Understanding long-term outcomes of chronic fatigue syndrome. *J Clin Psychol* 2012; **68**: 1028-1035 [PMID: 22753044 DOI: 10.1002/jclp.21880]

8 **Nacul LC**, Lacerda EM, Campion P, Pheby D, Drachler Mde L, Leite JC, Poland F, Howe A, Fayyaz S, Molokhia M. The functional status and well being of people with myalgic encephalomyelitis/chronic fatigue syndrome and their carers. *BMC Public Health* 2011; **11**: 402 [PMID: 21619607 DOI: 10.1186/1471-2458-11-402]

9 **Herrell R**, Goldberg J, Hartman S, Belcourt M, Schmaling K, Buchwald D. Chronic fatigue and chronic fatigue syndrome: a co-twin control study of functional status. *Qual Life Res* 2002; **11**: 463-471 [PMID: 12113393]

10 **Komaroff AL**, Fagioli LR, Doolittle TH, Gandek B, Gleit MA, Guerriero RT, Kornish RJ, Ware NC, Ware JE, Bates DW. Health status in patients with chronic fatigue syndrome and in general population and disease comparison groups. *Am J Med* 1996; **101**: 281-290 [PMID: 8873490 DOI: 10.1016/S0002-9343(96)00174-X]

11 **Ware JE**, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care* 1992; **30**: 473-483 [PMID: 1593914 DOI: 10.1097/00005650-199206000-00002]

12 **Lin JM**, Resch SC, Brimmer DJ, Johnson A, Kennedy S, Burstein N, Simon CJ. The economic impact of chronic fatigue syndrome in Georgia: direct and indirect costs. *Cost Eff Resour Alloc* 2011; **9**: 1 [PMID: 21251294 DOI: 10.1186/1478-7547-9-1]

13 **Jason LA**, Benton MC, Valentine L, Johnson A, Torres-Harding S. The economic impact of ME/CFS: individual and societal costs. *Dyn Med* 2008; **7**: 6 [PMID: 18397528 DOI: 10.1186/1476-5918-7-6]

14 **Missen A**, Hollingworth W, Eaton N, Crawley E. The financial and psychological impacts on mothers of children with chronic fatigue syndrome (CFS/ME). *Child Care Health Dev* 2012; **38**: 505-512 [PMID: 21880054 DOI: 10.1111/j.1365-2214.2011.01298.x]

15 **Reynolds KJ**, Vernon SD, Bouchery E, Reeves WC. The economic

impact of chronic fatigue syndrome. *Cost Eff Resour Alloc* 2004; **2**: 4 [PMID: 15210053 DOI: 10.1186/1478-7547-2-4]
16 **Collin SM**, Crawley E, May MT, Sterne JA, Hollingworth W. The impact of CFS/ME on employment and productivity in the UK: a cross-sectional study based on the CFS/ME national outcomes database. *BMC Health Serv Res* 2011; **11**: 217 [PMID: 21923897 DOI: 10.1186/1472-6963-11-217]
17 **World Health Organization.** International Classification of Diseases, Tenth Revision (ICD-10), Code G93.1. Geneva: Switzerland, 1992
18 **Carruthers BM**, van de Sande MI, De Meirleir KL, Klimas NG, Broderick G, Mitchell T, Staines D, Powles AC, Speight N, Vallings R, Bateman L, Baumgarten-Austrheim B, Bell DS, Carlo-Stella N, Chia J, Darragh A, Jo D, Lewis D, Light AR, Marshall-Gradisbik S, Mena I, Mikovits JA, Miwa K, Murovska M, Pall ML, Stevens S. Myalgic Encephalomyelitis International Consensus Criteria. *J Intern Med* 2011; **270**: 327-338 [PMID: 21777306 DOI: 10.1111/j.1365-2796.2011.02428.x]
19 **Fukuda K**, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. *Ann Intern Med* 1994; **121**: 953-959 [PMID: 7978722 DOI: 10.7326/0003-4819-121-12-199412150-00009]
20 **Dowsett EG**, Ramsay AM, McCartney RA, Bell EJ. Myalgic encephalomyelitis - a persistent enteroviral infection? *Postgrad Med J* 1990; **66**: 526-530 [PMID: 2170962 DOI: 10.1136/pgmj.66.777.526]
21 **Parish JG**. Early outbreaks of 'epidemic neuromyasthenia'. *Postgrad Med J* 1978; **54**: 711-717 [PMID: 370810 DOI: 10.1136/pgmj.54.637.711]
22 **Acheson ED**. The clinical syndrome variously called benign myalgic encephalomyelitis, Iceland disease and epidemic neuromyasthenia. *Am J Med* 1959; **26**: 569-595 [PMID: 13637100 DOI: 10.1016/0002-9343(59)90280-3]
23 **Gilliam AG**. Epidemiological study on an epidemic, diagnosed as poliomyelitis, occurring among the personnel of Los Angeles County General Hospital during the summer of 1934. Washington, DC: United States Treasury Department Public Health Service Public Health Bulletin, 1938: 1-90
24 **Crowley N**, Nelson M, Stovin S. Epidemiological aspects of an outbreak of encephalomyelitis at the Royal Free Hospital, London, in the summer of 1955. *J Hyg* (Lond) 1957; **55**: 102-122 [PMID: 13416578 DOI: 10.1017/S0022172400061295]
25 **Strickland PS**, Levine PH, Peterson DL, O'Brien K, Fears T. Neuromyasthenia and chronic fatigue syndrome (CFS) in Northern Nevada/California: a ten-year follow-up of an outbreak. *J Chronic Fatigue Syndr* 2001; **9**: 3-14 [DOI: 10.1300/J092v09n03_02]
26 **World Health Organization.** International Classification of Diseases, Eighth Revision (ICD-8): I (Code 323): 158. Geneva: Switzerland, 1967
27 **Holmes GP**, Kaplan JE, Gantz NM, Komaroff AL, Schonberger LB, Straus SE, Jones JF, Dubois RE, Cunningham-Rundles C, Pahwa S. Chronic fatigue syndrome: a working case definition. *Ann Intern Med* 1988; **108**: 387-389 [PMID: 2829679 DOI: 10.7326/0003-4819-108-3-387]
28 **Jason LA**, Jessen T, Porter N, Boulton A, Njoku MG, Friedberg F. Examining types of fatigue among individuals with ME/CFS. DSQ 2009; 29. Available from: URL: http://www.dsq-sds.org/article/view/938/1113
29 **Jason LA**, Boulton A, Porter NS, Jessen T, Njoku MG, Friedberg F. Classification of myalgic encephalomyelitis/chronic fatigue syndrome by types of fatigue. *Behav Med* 2010; **36**: 24-31 [PMID: 20185398 DOI: 10.1080/08964280903521370]
30 **Jason LA**, Torres-Harding SR, Carrico AW, Taylor RR. Symptom occurrence in persons with chronic fatigue syndrome. *Biol Psychol* 2002; **59**: 15-27 [PMID: 11790441 DOI: 10.1016/S0301-0511(01)00120-X]
31 **Wessely S**. Chronic fatigue syndrome. Summary of a report of a joint committee of the Royal Colleges of Physicians, Psychiatrists and General Practitioners. *J R Coll Physicians Lond* 1996; **30**: 497-504 [PMID: 8961200]
32 **Lane RJ**, Barrett MC, Taylor DJ, Kemp GJ, Lodi R. Heterogeneity in chronic fatigue syndrome: evidence from magnetic resonance spectroscopy of muscle. *Neuromuscul Disord* 1998; **8**: 204-209 [PMID: 9631403 DOI: 10.1016/S0960-8966(98)00021-2]
33 **Aslakson E**, Vollmer-Conna U, Reeves WC, White PD. Replication of an empirical approach to delineate the heterogeneity of chronic unexplained fatigue. *Popul Health Metr* 2009; **7**: 17 [PMID: 19804639 DOI: 10.1186/1478-7954-7-17]
34 **Wilson A**, Hickie I, Hadzi-Pavlovic D, Wakefield D, Parker G, Straus SE, Dale J, McCluskey D, Hinds G, Brickman A, Goldenberg D, Demitrack M, Blakely T, Wessely S, Sharpe M, Lloyd A. What is chronic fatigue syndrome? Heterogeneity within an international multicentre study. *Aust N Z J Psychiatry* 2001; **35**: 520-527 [PMID: 11531735 DOI: 10.1046/j.1440-1614.2001.00888.x]
35 **Peckerman A**, LaManca JJ, Dahl KA, Chemitiganti R, Qureishi B, Natelson BH. Abnormal impedance cardiography predicts symptom severity in chronic fatigue syndrome. *Am J Med Sci* 2003; **326**: 55-60 [PMID: 12920435 DOI: 10.1097/00000441-200308000-0000]
36 **Maes M**, Twisk FN, Johnson C. Myalgic Encephalomyelitis (ME), Chronic Fatigue Syndrome (CFS), and Chronic Fatigue (CF) are distinguished accurately: results of supervised learning techniques applied on clinical and inflammatory data. *Psychiatry Res* 2012; **200**: 754-760 [PMID: 22521895 DOI: 10.1016/j.psychres.2012.03.031]
37 **Brenu EW**, Johnston S, Hardcastle SL, Huth TK, Fuller K, Ramos SB, Ramos TK, Huth DR, Staines KF. Immune abnormalities in patients meeting new diagnostic criteria for chronic fatigue syndrome/Myalgic Encephalomyelitis. *J Mol Biomark Diagn* 2013; **4**: 152 [DOI: 10.4172/2155-9929.1000152]
38 **Nacul LC**, Lacerda EM, Pheby D, Campion P, Molokhia M, Fayyaz S, Leite JC, Poland F, Howe A, Drachler ML. Prevalence of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) in three regions of England: a repeated cross-sectional study in primary care. *BMC Med* 2011; **9**: 91 [PMID: 21794183 DOI: 10.1186/1741-7015-9-91]
39 **Jason LA**, Brown A, Clyne E, Bartgis L, Evans M, Brown M. Contrasting case definitions for chronic fatigue syndrome, Myalgic Encephalomyelitis/chronic fatigue syndrome and Myalgic Encephalomyelitis. *Eval Health Prof* 2012; **35**: 280-304 [PMID: 22158691 DOI: 10.1177/0163278711424281]
40 **Brurberg KG**, Fønhus MS, Larun L, Flottorp S, Malterud K. Case definitions for chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME): a systematic review. *BMJ Open* 2014; **4**: e003973 [PMID: 24508851 DOI: 10.1136/bmjopen-2013-003973]
41 **Johnston SC**, Brenu EW, Hardcastle SL, Huth TK, Staines DR, Marshall-Gradisnik SM. A comparison of health status in patients meeting alternative definitions for chronic fatigue syndrome/myalgic encephalomyelitis. *Health Qual Life Outcomes* 2014; **12**: 64 [PMID: 24886213 DOI: 10.1186/1477-7525-12-64]
42 **World Health Organization**. Disability Adjustment Schedule II (WHODAS II). [updated 2014 Nov 6]. Available from: URL: http://www.who.int/classifications/icf/whodasii/en/
43 **Jason LA**, Sunnquist M, Brown A, Evans M, Newton JL. Are Myalgic Encephalomyelitis and chronic fatigue syndrome different illnesses? A preliminary analysis. *J Health Psychol* 2014 Feb 7; Epub ahead of print [PMID: 24510231 DOI: 10.1177/1359105313520335]
44 **Kennedy G**, Abbot NC, Spence V, Underwood C, Belch JJ. The specificity of the CDC-1994 criteria for chronic fatigue syndrome: comparison of health status in three groups of patients who fulfill the criteria. *Ann Epidemiol* 2004; **14**: 95-100 [PMID: 15018881 DOI: 10.1016/j.annepidem.2003.10.004]
45 **Jason LA**, King CP, Richman JA, Taylor RR, Torres SR, Song S. U.S. case definition of chronic fatigue syndrome: diagnostic and theoretical issues. *J Chronic Fatigue Syndr* 1998; **5**: 3-33 [DOI: 10.1300/J092v05n03_02]
46 Jason LA, Evans M, Brown A, Brown M, Porter N, Hunnell J, Anderson V, Lerch A. Sensitivity and specificity of the CDC empirical chronic fatigue syndrome case definition. *Psychology* 2010; **1**: 9-16 [DOI: 10.4236/psych.2010.11002]

47 **Reeves WC**, Wagner D, Nisenbaum R, Jones JF, Gurbaxani B, Solomon L, Papanicolaou DA, Unger ER, Vernon SD, Heim C. Chronic fatigue syndrome--a clinically empirical approach to its definition and study. *BMC Med* 2005; **3**: 19 [PMID: 16356178 DOI: 10.1186/1741-7015-3-19]

48 **Jason LA**, Najar N, Porter N, Reh C. Evaluating the Centers for Disease Control's empirical chronic fatigue syndrome case definition. *J Disabil Policy Stud* 2009; **20**: 93-100 [DOI: 10.1177/1044207308325995]

49 **White PD**, Goldsmith KA, Johnson AL, Potts L, Walwyn R, DeCesare JC, Baber HL, Burgess M, Clark LV, Cox DL, Bavinton J, Angus BJ, Murphy G, Murphy M, O'Dowd H, Wilks D, McCrone P, Chalder T, Sharpe M. Comparison of adaptive pacing therapy, cognitive behaviour therapy, graded exercise therapy, and specialist medical care for chronic fatigue syndrome (PACE): a randomised trial. *Lancet* 2011; **377**: 823-836 [PMID: 21334061 DOI: 10.1016/S0140-6736(11)60096-2]

50 **Núñez M**, Fernández-Solà J, Nuñez E, Fernández-Huerta JM, Godás-Sieso T, Gomez-Gil E. Health-related quality of life in patients with chronic fatigue syndrome: group cognitive behavioural therapy and graded exercise versus usual treatment. A randomised controlled trial with 1 year of follow-up. *Clin Rheumatol* 2011; **30**: 381-389 [PMID: 21234629 DOI: 10.1007/s10067-010-1677-y]

51 **Twisk FN**, Maes M. A review on cognitive behavorial therapy (CBT) and graded exercise therapy (GET) in myalgic encephalomyelitis (ME) / chronic fatigue syndrome (CFS): CBT/GET is not only ineffective and not evidence-based, but also potentially harmful for many patients with ME/CFS. *Neuro Endocrinol Lett* 2009; **30**: 284-299 [PMID: 19855350]

52 **Fluge Ø**, Bruland O, Risa K, Storstein A, Kristoffersen EK, Sapkota D, Næss H, Dahl O, Nyland H, Mella O. Benefit from B-lymphocyte depletion using the anti-CD20 antibody rituximab in chronic fatigue syndrome. A double-blind and placebo-controlled study. *PLoS One* 2011; **6**: e26358 [PMID: 22039471 DOI: 10.1371/journal.pone.0026358]

53 **Watt T**, Oberfoell S, Balise R, Lunn MR, Kar AK, Merrihew L, Bhangoo MS, Montoya JG. Response to valganciclovir in chronic fatigue syndrome patients with human herpesvirus 6 and Epstein-Barr virus IgG antibody titers. *J Med Virol* 2012; **84**: 1967-1974 [PMID: 23080504 DOI: 10.1002/jmv.23411]

54 **Johnston S**, Brenu EW, Staines D, Marshall-Gradisnik S. The prevalence of chronic fatigue syndrome/ myalgic encephalomyelitis: a meta-analysis. *Clin Epidemiol* 2013; **5**: 105-110 [PMID: 23576883 DOI: 10.2147/CLEP.S39876]

55 **Reeves WC**, Jones JF, Maloney E, Heim C, Hoaglin DC, Boneva RS, Morrissey M, Devlin R. Prevalence of chronic fatigue syndrome in metropolitan, urban, and rural Georgia. *Popul Health Metr* 2007; **5**: 5 [PMID: 17559660 DOI: 10.1186/1478-7954-5-5]

56 **Smets EM**, Garssen B, Bonke B, De Haes JC. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. *J Psychosom Res* 1995; **39**: 315-325 [PMID: 7636775 DOI: 10.1016/0022-3999(94)00125-O]

57 **Wagner D**, Nisenbaum R, Heim C, Jones JF, Unger ER, Reeves WC. Psychometric properties of the CDC Symptom Inventory for assessment of chronic fatigue syndrome. *Popul Health Metr* 2005; **3**: 8 [PMID: 16042777 DOI: 10.1186/1477-7525-3-8]

58 **Steele L**, Dobbins JG, Fukuda K, Reyes M, Randall B, Koppelman M, Reeves WC. The epidemiology of chronic fatigue in San Francisco. *Am J Med* 1998; **105**: 83S-90S [PMID: 9790487 DOI: 10.1016/S0002-9343(98)00158-2]

59 **Carruthers BM**, Jain AK, de Meirleir K, Peterson DL, Klimas NG, Lerner AM, Bested AC, Flor-Henry P, Joshi P, Powles ACP, Sherkey JA, van de Sande MI. Myalgic encephalomyelitis/chronic fatigue syndrome: clinical working case definition, diagnostic and treatment protocols. *J Chronic Fatigue Syndr* 2003; **11**: 7-115 [DOI: 10.1300/J092v11n01_02]

60 **Meeus M**, van Eupen I, van Baarle E, De Boeck V, Luyckx A, Kos D, Nijs J. Symptom fluctuations and daily physical activity in patients with chronic fatigue syndrome: a case-control study. *Arch Phys Med Rehabil* 2011; **92**: 1820-1826 [PMID: 22032215 DOI: 10.1016/j.apmr.2011.06.023]

61 **Jason LA**, Sunnquist M, Brown A, Evans M, Vernon SD, Furst J, Simonis V. Examining case definition criteria for chronic fatigue syndrome and myalgic encephalomyelitis. *Fatigue* 2014; **2**: 40-56 [PMID: 24511456 DOI: 10.1080/21641846.2013.862993]

62 **American College of Sports Medicine**. ACSM's guidelines for exercise testing and prescription. 8th ed. Ambler, PA: Lippincott Williams and Wilkins, 2009

63 **Balady GJ**, Arena R, Sietsema K, Myers J, Coke L, Fletcher GF, Forman D, Franklin B, Guazzi M, Gulati M, Keteyian SJ, Lavie CJ, Macko R, Mancini D, Milani RV. Clinician's Guide to cardiopulmonary exercise testing in adults: a scientific statement from the American Heart Association. *Circulation* 2010; **122**: 191-225 [PMID: 20585013 DOI: 10.1161/CIR.0b013e3181e52e69]

64 **Strauss E**, Sherman EMS, Spreen O. A compendium of neuropsychological tests. 3rd ed. New York: Oxford University Press, 2006

65 **Lezak MD**, Howieson DB, Loring DW. Neuropsychological assessment. 4th ed. New York: Oxford University Press, 2004

66 **Wechsler D**. Wechsler adult intelligence scale-revised San Antonio, Texas: The Psychological Corporation, 1981

67 **Cambridge Cognition**. Cambridge Neuropsychological Test Automated Battery (CANTAB). Cambridge: Cambridge Cognition Limited, 2006

68 **Stark T**, Walker B, Phillips JK, Fejer R, Beck R. Hand-held dynamometry correlation with the gold standard isokinetic dynamometry: a systematic review. *PM R* 2011; **3**: 472-479 [PMID: 21570036 DOI: 10.1016/j.pmrj.2010.10.025]

69 **Wang CY**, Olson SL, Protas EJ. Test-retest strength reliability: hand-held dynamometry in community-dwelling elderly fallers. *Arch Phys Med Rehabil* 2002; **83**: 811-815 [PMID: 12048660 DOI: 10.1053/apmr.2002.32743]

70 **Andrews AW**, Thomas MW, Bohannon RW. Normative values for isometric muscle force measurements obtained with hand-held dynamometers. *Phys Ther* 1996; **76**: 248-259 [PMID: 8602410]

71 **van der Ploeg RJ**, Fidler V, Oosterhuis HJ. Hand-held myometry: reference values. *J Neurol Neurosurg Psychiatry* 1991; **54**: 244-247 [PMID: 2030353 DOI: 10.1136/jnnp.54.3.244]

72 **Moya A**, Sutton R, Ammirati F, Blanc JJ, Brignole M, Dahm JB, Deharo JC, Gajek J, Gjesdal K, Krahn A, Massin M, Pepi M, Pezawas T, Ruiz Granell R, Sarasin F, Ungar A, van Dijk JG, Walma EP, Wieling W. Guidelines for the diagnosis and management of syncope (version 2009). *Eur Heart J* 2009; **30**: 2631-2671 [PMID: 19713422 DOI: 10.1093/eurheartj/ehp298]

73 **Benditt DG**, Ferguson DW, Grubb BP, Kapoor WN, Kugler J, Lerman BB, Maloney JD, Raviele A, Ross B, Sutton R, Wolk MJ, Wood DL. Tilt table testing for assessing syncope. American College of Cardiology. *J Am Coll Cardiol* 1996; **28**: 263-275 [PMID: 8752825 DOI: 10.1016/0735-1097(96)00236-7]

74 **Streeten DH**. Orthostatic disorders of the circulation: mechanisms, manifestations and treatment. New York: Plenum Medical Book Publishin, 1987 [DOI: 10.1007/978-1-4684-8962-0]

75 **Melmed S**, Polonsky KS, Larsen RMD, Kronenberg HM. Williams Textbook of Endocrinology. 12th ed. Philadelphia: Elsevier/Saunders, 2011

76 **Kovacs WJ**, Ojeda SR. Textbook of endocrine physiology. 6th ed. Oxford, Oxfordshire: Oxford University Press, 2011

77 **Kirschbaum C**, Pirke KM, Hellhammer DH. The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 1993; **28**: 76-81 [PMID: 8255414 DOI: 10.1159/000119004]

78 **Holtorf K**. Diagnosis and treatment of hypothalamic-pituitary-adrenal (HPA) axis dysfunction in patients with chronic fatigue syndrome (CFS) and fibromyalgia (FM). *J Chronic Fatigue Syndr* 2008; **14**: 59-88 [DOI: 10.1300/J092v14n03_06]

79 **Iber C**, Ancoli-Israel S, Chesson A, Quan SF. The AASM manual for the scoring of sleep and associated events: rules, terminology, and technical specifications. 1st ed. Westchester, IL: American Academy of Sleep Medicine, 2007

80 **Dumermuth G**, Lange B, Lehmann D, Meier CA, Dinkelmann

R, Molinari L. Spectral analysis of all-night sleep EEG in healthy adults. *Eur Neurol* 1983; **22**: 322-339 [PMID: 6628461 DOI: 10.1159/000115579]

81 **Lo CC**, Nunes Amaral LA, Havlin S, Ivanov C, Penzel T, Peter JH, Stanley HE. Dynamics of sleep-wake transitions during sleep. *Europhys Lett* 2002; **57**: 625-631 [DOI: 10.1209/epl/i2002-00508-7]

82 **Littner MR**, Kushida C, Wise M, Davila DG, Morgenthaler T, Lee-Chiong T, Hirshkowitz M, Daniel LL, Bailey D, Berry RB, Kapen S, Kramer M. Practice parameters for clinical use of the multiple sleep latency test and the maintenance of wakefulness test. *Sleep* 2005; **28**: 113-121 [PMID: 15700727]

83 **Wise MS**. Objective measures of sleepiness and wakefulness: application to the real world? *J Clin Neurophysiol* 2006; **23**: 39-49 [PMID: 16514350 DOI: 10.1097/01.wnp.0000190416.62482.42]

84 **Ball K**, Owsley C, Sloane ME, Roenker DL, Bruni JR. Visual attention problems as a predictor of vehicle crashes in older drivers. *Invest Ophthalmol Vis Sci* 1993; **34**: 3110-3123 [PMID: 8407219]

85 **Ball K**, Owsley C. The useful field of view test: a new technique for evaluating age-related declines in visual function. *J Am Optom Assoc* 1993; **64**: 71-79 [PMID: 8454831]

86 **Rommelse NN**, Van der Stigchel S, Sergeant JA. A review on eye movement studies in childhood and adolescent psychiatry. *Brain Cogn* 2008; **68**: 391-414 [PMID: 18835079 DOI: 10.1016/j.bandc.2008.08.025]

87 **Hutton SB**, Ettinger U. The antisaccade task as a research tool in psychopathology: a critical review. *Psychophysiology* 2006; **43**: 302-313 [PMID: 16805870 DOI: 10.1111/j.1469-8986.2006.00403.x]

88 **Fischer DB**, William AH, Strauss AC, Unger ER, Jason L, Marshall GD Jr, Dimitrakoff JD. Chronic fatigue syndrome: the current status and future potentials of emerging biomarkers. *Fatigue* 2014; **2**: 93-109 [PMID: 24932428 DOI: 10.1080/21641846.2014.906066]

89 **Forman DE**, Myers J, Lavie CJ, Guazzi M, Celli B, Arena R. Cardiopulmonary exercise testing: relevant but underused. *Postgrad Med* 2010; **122**: 68-86 [PMID: 21084784 DOI: 10.3810/pgm.2010.11.2225]

90 **Ainsworth BE**, Haskell WL, Herrmann SD, Meckes N, Bassett DR, Tudor-Locke C, Greer JL, Vezina J, Whitt-Glover MC, Leon AS. 2011 Compendium of physical activities: A second update of codes and MET values. *Med Sci Sports Exerc* 2011; **43**: 1575-1581 [PMID: 21681120 DOI: 10.1249/MSS.0b013e31821ece12]

91 **Roley SS**, DeLany JV, Barrows CJ, Brownrigg S, Honaker D, Sava DI, Talley V, Voelkerding K, Amini DA, Smith E, Toto P, King S, Lieberman D, Baum MC, Cohen ES, Cleveland PA, Youngstrom MJ. Occupational therapy practice framework: domain & practice, 2nd edition. *Am J Occup Ther* 2008; **62**: 625-683 [PMID: 19024744 DOI: 10.5014/ajot.62.6.625]

92 **Sargent C**, Scroop GC, Nemeth PM, Burnet RB, Buckley JD. Maximal oxygen uptake and lactate metabolism are normal in chronic fatigue syndrome. *Med Sci Sports Exerc* 2002; **34**: 51-56 [PMID: 11782647 DOI: 10.1097/00005768-200201000-00009]

93 **Bazelmans E**, Bleijenberg G, Van Der Meer JW, Folgering H. Is physical deconditioning a perpetuating factor in chronic fatigue syndrome? A controlled study on maximal exercise performance and relations with fatigue, impairment and physical activity. *Psychol Med* 2001; **31**: 107-114 [PMID: 11200949 DOI: 10.1017/S0033291799003189]

94 **Vermeulen RC**, Kurk RM, Visser FC, Sluiter W, Scholte HR. Patients with chronic fatigue syndrome performed worse than controls in a controlled repeated exercise study despite a normal oxidative phosphorylation capacity. *J Transl Med* 2010; **8**: 93 [PMID: 20937116 DOI: 10.1186/1479-5876-8-93]

95 **De Becker P**, Roeykens J, Reynders M, McGregor N, De Meirleir K. Exercise capacity in chronic fatigue syndrome. *Arch Intern Med* 2000; **160**: 3270-3277 [PMID: 11088089 DOI: 10.1001/archinte.160.21.3270]

96 **Sisto SA**, LaManca J, Cordero DL, Bergen MT, Ellis SP, Drastal S, Boda WL, Tapp WN, Natelson BH. Metabolic and cardiovascular effects of a progressive exercise test in patients with chronic fatigue syndrome. *Am J Med* 1996; **100**: 634-640 [PMID: 8678084 DOI: 10.1016/S0002-9343(96)00041-1]

97 **VanNess JM**, Snell CR, Stevens SR, Stiles TL. Metabolic and neurocognitive responses to an exercise challenge in chronic fatigue syndrome (CFS). *Med Sci Sports Exerc* 2007; **39**: S445 [DOI: 10.1249/01.mss.0000274760.28522.df]

98 **Patrick Neary J**, Roberts AD, Leavins N, Harrison MF, Croll JC, Sexsmith JR. Prefrontal cortex oxygenation during incremental exercise in chronic fatigue syndrome. *Clin Physiol Funct Imaging* 2008; **28**: 364-372 [PMID: 18671793 DOI: 10.1111/j.1475-097X.2008.00822.x]

99 **LaManca JJ**, Sisto SA, Zhou XD, Ottenweller JE, Cook S, Peckerman A, Zhang Q, Denny TN, Gause WC, Natelson BH. Immunological response in chronic fatigue syndrome following a graded exercise test to exhaustion. *J Clin Immunol* 1999; **19**: 135-142 [PMID: 10226888]

100 **Hurwitz BE**, Coryell VT, Parker M, Martin P, Laperriere A, Klimas NG, Sfakianakis GN, Bilsker MS. Chronic fatigue syndrome: illness severity, sedentary lifestyle, blood volume and evidence of diminished cardiac function. *Clin Sci* (Lond) 2010; **118**: 125-135 [PMID: 19469714 DOI: 10.1042/CS20090055]

101 **Hollingsworth KG**, Hodgson T, Macgowan GA, Blamire AM, Newton JL. Impaired cardiac function in chronic fatigue syndrome measured using magnetic resonance cardiac tagging. *J Intern Med* 2012; **271**: 264-270 [PMID: 21793948 DOI: 10.1111/j.1365-2796.2011.02429.x]

102 **Miwa K**, Fujita M. Cardiovascular dysfunction with low cardiac output due to a small heart in patients with chronic fatigue syndrome. *Intern Med* 2009; **48**: 1849-1854 [PMID: 19881233 DOI: 10.2169/internalmedicine.48.2347]

103 **De Lorenzo F**, Xiao H, Mukherjee M, Harcup J, Suleiman S, Kadziola Z, Kakkar VV. Chronic fatigue syndrome: physical and cardiovascular deconditioning. *QJM* 1998; **91**: 475-481 [PMID: 9797930 DOI: 10.1093/qjmed/91.7.475]

104 **Hollingsworth KG**, Jones DE, Taylor R, Blamire AM, Newton JL. Impaired cardiovascular response to standing in chronic fatigue syndrome. *Eur J Clin Invest* 2010; **40**: 608-615 [PMID: 20497461 DOI: 10.1111/j.1365-2362.2010.02310.x]

105 **Farquhar WB**, Hunt BE, Taylor JA, Darling SE, Freeman R. Blood volume and its relation to peak O(2) consumption and physical activity in patients with chronic fatigue. *Am J Physiol Heart Circ Physiol* 2002; **282**: H66-H71 [PMID: 11748048]

106 **Nijs J**, Meeus M, McGregor NR, Meeusen R, de Schutter G, van Hoof E, de Meirleir K. Chronic fatigue syndrome: exercise performance related to immune dysfunction. *Med Sci Sports Exerc* 2005; **37**: 1647-1654 [PMID: 16260962 DOI: 10.1249/01.mss.0000181680.35503.ce]

107 **Snell CR**, Vanness JM, Strayer DR, Stevens SR. Physical performance and prediction of 2-5A synthetase/RNase L antiviral pathway activity in patients with chronic fatigue syndrome. *In Vivo* 2002; **16**: 107-109 [PMID: 12073768]

108 **Nijs J**, De Meirleir K, Meeus M, McGregor NR, Englebienne P. Chronic fatigue syndrome: intracellular immune deregulations as a possible etiology for abnormal exercise response. *Med Hypotheses* 2004; **62**: 759-765 [PMID: 15082102 DOI: 10.1016/j.mehy.2003.11.030]

109 **VanNess JM**, Stevens SR, Bateman L, Stiles TL, Snell CR. Postexertional malaise in women with chronic fatigue syndrome. *J Womens Health* (Larchmt) 2010; **19**: 239-244 [PMID: 20095909 DOI: 10.1089/jwh.2009.1507]

110 **Fulcher KY**, White PD. Strength and physiological response to exercise in patients with chronic fatigue syndrome. *J Neurol Neurosurg Psychiatry* 2000; **69**: 302-307 [PMID: 10945803 DOI: 10.1136/jnnp.69.3.302]

111 **Stewart JM**, Gewitz MH, Weldon A, Arlievsky N, Li K, Munoz J. Orthostatic intolerance in adolescent chronic fatigue syndrome. *Pediatrics* 1999; **103**: 116-121 [PMID: 9917448 DOI: 10.1542/peds.103.1.116]

112 **Dimitrov M**, Grafman J. Neuropsychological assessment of chronic fatigue syndrome. In: Klimas NG, Patarca R, editors. Disability and chronic fatigue syndrome: clinical, legal and patient perspectives. Binghamton NY: Haworth Medical Press, 1997: 31-42 [DOI:

10.1300/J092v03n04_05]
113 **Dickson A**, Toft A, O'Carroll RE. Neuropsychological functioning, illness perception, mood and quality of life in chronic fatigue syndrome, autoimmune thyroid disease and healthy participants. *Psychol Med* 2009; **39**: 1567-1576 [PMID: 19144216 DOI: 10.1017/S0033291708004960]
114 **Thomas M**, Smith A. An investigation into the cognitive deficits associated with chronic fatigue syndrome. *Open Neurol J* 2009; **3**: 13-23 [PMID: 19452031 DOI: 10.2174/1874205X00903010013]
115 **Constant EL**, Adam S, Gillain B, Lambert M, Masquelier E, Seron X. Cognitive deficits in patients with chronic fatigue syndrome compared to those with major depressive disorder and healthy controls. *Clin Neurol Neurosurg* 2011; **113**: 295-302 [PMID: 21255911 DOI: 10.1016/j.clineuro.2010.12.002]
116 **Tiersky LA**, Johnson SK, Lange G, Natelson BH, DeLuca J. Neuropsychology of chronic fatigue syndrome: a critical review. *J Clin Exp Neuropsychol* 1997; **19**: 560-586 [PMID: 9342690 DOI: 10.1080/01688639708403744]
117 **DeLuca J**, Johnson SK, Natelson BH. Information processing efficiency in chronic fatigue syndrome and multiple sclerosis. *Arch Neurol* 1993; **50**: 301-304 [PMID: 8442710 DOI: 10.1001/archneur.1993.00540030065016]
118 **Deluca J**, Christodoulou C, Diamond BJ, Rosenstein ED, Kramer N, Natelson BH. Working memory deficits in chronic fatigue syndrome: differentiating between speed and accuracy of information processing. *J Int Neuropsychol Soc* 2004; **10**: 101-109 [PMID: 14751012 DOI: 10.1017/S1355617704101124]
119 **Chen R**, Liang FX, Moriya J, Yamakawa J, Sumino H, Kanda T, Takahashi T. Chronic fatigue syndrome and the central nervous system. *J Int Med Res* 2008; **36**: 867-874 [PMID: 18831878 DOI: 10.1177/147323000803600501]
120 **Komaroff AL**, Cho TA. Role of infection and neurologic dysfunction in chronic fatigue syndrome. *Semin Neurol* 2011; **31**: 325-337 [PMID: 21964849 DOI: 10.1055/s-0031-1287654]
121 **Gonzalez MB**, Cousins JC, Doraiswamy PM. Neurobiology of chronic fatigue syndrome. *Prog Neuropsychopharmacol Biol Psychiatry* 1996; **20**: 749-759 [PMID: 8870062 DOI: 10.1016/0278-5846(96)00057-7]
122 **Barnden LR**, Crouch B, Kwiatek R, Burnet R, Mernone A, Chryssidis S, Scroop G, Del Fante P. A brain MRI study of chronic fatigue syndrome: evidence of brainstem dysfunction and altered homeostasis. *NMR Biomed* 2011; **24**: 1302-1312 [PMID: 21560176 DOI: 10.1002/nbm.1692]
123 **Puri BK**, Jakeman PM, Agour M, Gunatilake KD, Fernando KA, Gurusinghe AI, Treasaden IH, Waldman AD, Gishen P. Regional grey and white matter volumetric changes in myalgic encephalomyelitis (chronic fatigue syndrome): a voxel-based morphometry 3 T MRI study. *Br J Radiol* 2012; **85**: e270-e273 [PMID: 22128128 DOI: 10.1259/bjr/93889091]
124 **Zeineh MM**, Kang J, Atlas SW, Raman MM, Reiss AL, Norris JL, Valencia I, Montoya JG. Right arcuate fasciculus abnormality in chronic fatigue syndrome. *Radiology* 2015; **274**: 517-526 [PMID: 25353054 DOI: 10.1148/radiol.14141079]
125 **de Lange FP**, Kalkman JS, Bleijenberg G, Hagoort P, van der Meer JW, Toni I. Gray matter volume reduction in the chronic fatigue syndrome. *Neuroimage* 2005; **26**: 777-781 [PMID: 15955487 DOI: 10.1016/j.neuroimage.2005.02.037]
126 **Okada T**, Tanaka M, Kuratsune H, Watanabe Y, Sadato N. Mechanisms underlying fatigue: a voxel-based morphometric study of chronic fatigue syndrome. *BMC Neurol* 2004; **4**: 14 [PMID: 15461817 DOI: 10.1186/1471-2377-4-14]
127 **Duffy FH**, McAnulty GB, McCreary MC, Cuchural GJ, Komaroff AL. EEG spectral coherence data distinguish chronic fatigue syndrome patients from healthy controls and depressed patients--a case control study. *BMC Neurol* 2011; **11**: 82 [PMID: 21722376 DOI: 10.1186/1471-2377-11-82]
128 **Costa DC**, Tannock C, Brostoff J. Brainstem perfusion is impaired in chronic fatigue syndrome. *QJM* 1995; **88**: 767-773 [PMID: 8542261]
129 **Biswal B**, Kunwar P, Natelson BH. Cerebral blood flow is reduced in chronic fatigue syndrome as assessed by arterial spin labeling. *J Neurol Sci* 2011; **301**: 9-11 [PMID: 21167506 DOI: 10.1016/j.jns.2010.11.018]
130 **Yoshiuchi K**, Farkas J, Natelson BH. Patients with chronic fatigue syndrome have reduced absolute cortical blood flow. *Clin Physiol Funct Imaging* 2006; **26**: 83-86 [PMID: 16494597 DOI: 10.1111/j.1475-097X.2006.00649.x]
131 **Tirelli U**, Chierichetti F, Tavio M, Simonelli C, Bianchin G, Zanco P, Ferlin G. Brain positron emission tomography (PET) in chronic fatigue syndrome: preliminary data. *Am J Med* 1998; **105**: 54S-58S [PMID: 9790483 DOI: 10.1016/S0002-9343(98)00179-X]
132 **Murrough JW**, Mao X, Collins KA, Kelly C, Andrade G, Nestadt P, Levine SM, Mathew SJ, Shungu DC. Increased ventricular lactate in chronic fatigue syndrome measured by 1H MRS imaging at 3.0 T. II: comparison with major depressive disorder. *NMR Biomed* 2010; **23**: 643-650 [PMID: 20661876 DOI: 10.1002/nbm.1512]
133 **Nakatomi Y**, Mizuno K, Ishii A, Wada Y, Tanaka M, Tazawa S, Onoe K, Fukuda S, Kawabe J, Takahashi K, Kataoka Y, Shiomi S, Yamaguti K, Inaba M, Kuratsune H, Watanabe Y. Neuroinflammation in Patients with Chronic Fatigue Syndrome/Myalgic Encephalomyelitis: An 11C-(R)-PK11195 PET Study. *J Nucl Med* 2014; **55**: 945-950 [PMID: 24665088 DOI: 10.2967/jnumed.113.131045]
134 **Natelson BH**, Weaver SA, Tseng CL, Ottenweller JE. Spinal fluid abnormalities in patients with chronic fatigue syndrome. *Clin Diagn Lab Immunol* 2005; **12**: 52-55 [PMID: 15642984 DOI: 10.1128/CDLI.12.1.52-55.2005]
135 **Schutzer SE**, Angel TE, Liu T, Schepmoes AA, Clauss TR, Adkins JN, Camp DG, Holland BK, Bergquist J, Coyle PK, Smith RD, Fallon BA, Natelson BH. Distinct cerebrospinal fluid proteomes differentiate post-treatment lyme disease from chronic fatigue syndrome. *PLoS One* 2011; **6**: e17287 [PMID: 21383843 DOI: 10.1371/journal.pone.0017287]
136 **Lange G**, Steffener J, Cook DB, Bly BM, Christodoulou C, Liu WC, Deluca J, Natelson BH. Objective evidence of cognitive complaints in Chronic Fatigue Syndrome: a BOLD fMRI study of verbal working memory. *Neuroimage* 2005; **26**: 513-524 [PMID: 15907308 DOI: 10.1016/j.neuroimage.2005.02.011]
137 **Cook DB**, O'Connor PJ, Lange G, Steffener J. Functional neuroimaging correlates of mental fatigue induced by cognition among chronic fatigue syndrome patients and controls. *Neuroimage* 2007; **36**: 108-122 [PMID: 17408973 DOI: 10.1016/j.neuroimage.2007.02.033]
138 **Schmaling KB**, Lewis DH, Fiedelak JI, Mahurin R, Buchwald DS. Single-photon emission computerized tomography and neurocognitive function in patients with chronic fatigue syndrome. *Psychosom Med* 2003; **65**: 129-136 [PMID: 12554824 DOI: 10.1097/01.PSY.0000038942.33335.9B]
139 **LaManca JJ**, Sisto SA, DeLuca J, Johnson SK, Lange G, Pareja J, Cook S, Natelson BH. Influence of exhaustive treadmill exercise on cognitive functioning in chronic fatigue syndrome. *Am J Med* 1998; **105**: 59S-65S [PMID: 9790484 DOI: 10.1016/S0002-9343(98)00171-5]
140 **Ocon AJ**, Messer ZR, Medow MS, Stewart JM. Increasing orthostatic stress impairs neurocognitive functioning in chronic fatigue syndrome with postural tachycardia syndrome. *Clin Sci* (Lond) 2012; **122**: 227-238 [PMID: 21919887 DOI: 10.1042/CS20110241]
141 **Claypoole KH**, Noonan C, Mahurin RK, Goldberg J, Erickson T, Buchwald D. A twin study of cognitive function in chronic fatigue syndrome: the effects of sudden illness onset. *Neuropsychology* 2007; **21**: 507-513 [PMID: 17605583 DOI: 10.1037/0894-4105.21.4.507]
142 **DeLuca J**, Johnson SK, Ellis SP, Natelson BH. Sudden vs gradual onset of chronic fatigue syndrome differentiates individuals on cognitive and psychiatric measures. *J Psychiatr Res* 1997; **31**: 83-90 [PMID: 9201650 DOI: 10.1016/S0022-3956(96)00052-0]
143 **Cockshell SJ**, Mathias JL. Cognitive functioning in chronic fatigue syndrome: a meta-analysis. *Psychol Med* 2010; **40**: 1253-1267 [PMID: 20047703 DOI: 10.1017/S0033291709992054]
144 **Capuron L**, Welberg L, Heim C, Wagner D, Solomon L, Papanicolaou DA, Craddock RC, Miller AH, Reeves WC. Cognitive

dysfunction relates to subjective report of mental fatigue in patients with chronic fatigue syndrome. *Neuropsychopharmacology* 2006; **31**: 1777-1784 [PMID: 16395303 DOI: 10.1038/sj.npp.1301005]

145 **van de Putte EM**, Böcker KB, Buitelaar J, Kenemans JL, Engelbert RH, Kuis W, Kimpen JL, Uiterwaal CS. Deficits of interference control in adolescents with chronic fatigue syndrome. *Arch Pediatr Adolesc Med* 2008; **162**: 1196-1197 [PMID: 19047552 DOI: 10.1001/archpedi.162.12.1196]

146 **Ickmans K**, Clarys P, Nijs J, Meeus M, Aerenhouts D, Zinzen E, Aelbrecht S, Meersdom G, Lambrecht L, Pattyn N. Association between cognitive performance, physical fitness, and physical activity level in women with chronic fatigue syndrome. *J Rehabil Res Dev* 2013; **50**: 795-810 [PMID: 24203542 DOI: 10.1682/JRRD.2012.08.0156]

147 **Neu D**, Mairesse O, Montana X, Gilson M, Corazza F, Lefevre N, Linkowski P, Le Bon O, Verbanck P. Dimensions of pure chronic fatigue: psychophysical, cognitive and biological correlates in the chronic fatigue syndrome. *Eur J Appl Physiol* 2014; **114**: 1841-1851 [PMID: 24878689 DOI: 10.1007/s00421-014-2910-1]

148 **Togo F**, Lange G, Natelson BH, Quigley KS. Attention network test: assessment of cognitive function in chronic fatigue syndrome. *J Neuropsychol* 2015; **9**: 1-9 [PMID: 24112872 DOI: 10.1111/jnp.12030]

149 **Cockshell SJ**, Mathias JL. Cognitive deficits in chronic fatigue syndrome and their relationship to psychological status, symptomatology, and everyday functioning. *Neuropsychology* 2013; **27**: 230-242 [PMID: 23527651 DOI: 10.1037/a0032084]

150 **Goedendorp MM**, van der Werf SP, Bleijenberg G, Tummers M, Knoop H. Does neuropsychological test performance predict outcome of cognitive behavior therapy for Chronic Fatigue Syndrome and what is the role of underperformance? *J Psychosom Res* 2013; **75**: 242-248 [PMID: 23972413 DOI: 10.1016/j.jpsychores.2013.07.011]

151 **Twisk FN**. Underperformance of myalgic encephalomyelitis (ME)/chronic fatigue syndrome (CFS) patients at neurocognitive tests should be assessed objectively without an a priori judgment about the etiology. *J Psychosom Res* 2014; **76**: 339 [PMID: 24630187 DOI: 10.1016/j.jpsychores.2013.10.016]

152 **Cockshell SJ**, Mathias JL. Test effort in persons with Chronic Fatigue Syndrome when assessed using the Validity Indicator Profile. *J Clin Exp Neuropsychol* 2012; **34**: 679-687 [PMID: 22440059 DOI: 10.1080/13803395.2012.668176]

153 **Stiles TL**, Snell CR, Stevens SR, Moran M, VanNess JM. Post-exertional symptomology in chronic fatigue syndrome (CFS). *Med Sci Sports Exerc* 2007; **39**: S445 [DOI: 10.1249/01.mss.0000274761.36146.5f]

154 **Snell CR**, Stevens SR, Davenport TE, Van Ness JM. Discriminative validity of metabolic and workload measurements for identifying people with chronic fatigue syndrome. *Phys Ther* 2013; **93**: 1484-1492 [PMID: 23813081 DOI: 10.2522/ptj.20110368]

155 **Cockshell SJ**, Mathias JL. Cognitive functioning in people with chronic fatigue syndrome: a comparison between subjective and objective measures. *Neuropsychology* 2014; **28**: 394-405 [PMID: 24364389 DOI: 10.1037/neu0000025]

156 **Ciccolella ME**, Boone T, Davenport T. Part II - Legal aspects of aerobic capacity: objective evidence of the ability to work (Prof Exerc Physiol Online 2011; 14). Available from: URL: http://www.asep.org/asep/asep/PartIILegalMARGARET CICCOLELLA.pdf

157 **Keller BA**, Pryor JL, Giloteaux L. Inability of myalgic encephalomyelitis/chronic fatigue syndrome patients to reproduce VO_2peak indicates functional impairment. *J Transl Med* 2014; **12**: 104 [PMID: 24755065 DOI: 10.1186/1479-5876-12-104]

158 **Keller BA**, Micale FG. Exercise testing to quantify effects of fatigue on functional capacity in patients with CFS. IACFS/ME biennial conference: Translating evidence into practice. Ottawa (Ontario) Canada, 2011: 123-124

159 **Weber KT**, Janicki JS. Cardiopulmonary exercise testing for evaluation of chronic cardiac failure. *Am J Cardiol* 1985; **55**: 22A-31A [PMID: 3966407 DOI: 10.1016/0002-9149(85)90792-1]

160 **Jetté M**, Sidney K, Blümchen G. Metabolic equivalents (METS) in exercise testing, exercise prescription, and evaluation of functional capacity. *Clin Cardiol* 1990; **13**: 555-565 [PMID: 2204507 DOI: 10.1002/clc.4960130809]

161 **Blackwood SK**, MacHale SM, Power MJ, Goodwin GM, Lawrie SM. Effects of exercise on cognitive and motor function in chronic fatigue syndrome and depression. *J Neurol Neurosurg Psychiatry* 1998; **65**: 541-546 [PMID: 9771781 DOI: 10.1136/jnnp.65.4.541]

162 **LaManca JJ**, Peckerman A, Sisto SA, DeLuca J, Cook S, Natelson BH. Cardiovascular responses of women with chronic fatigue syndrome to stressful cognitive testing before and after strenuous exercise. *Psychosom Med* 2001; **63**: 756-764 [PMID: 11573024 DOI: 10.1097/00006842-200109000-00009]

163 **Streeten DH**, Anderson GH. The role of delayed orthostatic hypotension in the pathogenesis of chronic fatigue. *Clin Auton Res* 1998; **8**: 119-124 [PMID: 9613802 DOI: 10.1007/BF02267822]

164 **Costigan A**, Elliott C, McDonald C, Newton JL. Orthostatic symptoms predict functional capacity in chronic fatigue syndrome: implications for management. *QJM* 2010; **103**: 589-595 [PMID: 20534655 DOI: 10.1093/qjmed/hcq094]

165 **Stewart JM**, Medow MS, Messer ZR, Baugham IL, Terilli C, Ocon AJ. Postural neurocognitive and neuronal activated cerebral blood flow deficits in young chronic fatigue syndrome patients with postural tachycardia syndrome. *Am J Physiol Heart Circ Physiol* 2012; **302**: H1185-H1194 [PMID: 22180650 DOI: 10.1152/ajpheart.00994.2011]

166 **Medow MS**, Sood S, Messer Z, Dzogbeta S, Terilli C, Stewart JM. Phenylephrine alteration of cerebral blood flow during orthostasis: effect on n-back performance in chronic fatigue syndrome. *J Appl Physiol* (1985) 2014; **117**: 1157-1164 [PMID: 25277740 DOI: 10.1152/japplphysiol.00527.2014]

167 **Caseras X**, Mataix-Cols D, Giampietro V, Rimes KA, Brammer M, Zelaya F, Chalder T, Godfrey EL. Probing the working memory system in chronic fatigue syndrome: a functional magnetic resonance imaging study using the n-back task. *Psychosom Med* 2006; **68**: 947-955 [PMID: 17079703 DOI: 10.1097/01.psy.0000242770.50979.5f]

168 **Arroll MA**, Attree EA, O'Leary JM, Dancey CP. The delayed fatigue effect in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). *Fatigue* 2014; **2**: 57-63 [DOI: 10.1080/21641846.2014.892755]

169 **Zigmond AS**, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983; **67**: 361-370 [PMID: 6880820 DOI: 10.1111/j.1600-0447.1983.tb09716.x]

170 **Ickmans K**, Meeus M, De Kooning M, Lambrecht L, Pattyn N, Nijs J. Can recovery of peripheral muscle function predict cognitive task performance in chronic fatigue syndrome with and without fibromyalgia? *Phys Ther* 2014; **94**: 511-522 [PMID: 24363336 DOI: 10.2522/ptj.20130367]

171 **Siemionow V**, Fang Y, Calabrese L, Sahgal V, Yue GH. Altered central nervous system signal during motor performance in chronic fatigue syndrome. *Clin Neurophysiol* 2004; **115**: 2372-2381 [PMID: 15351380 DOI: 10.1016/j.clinph.2004.05.012]

172 **Lawrie SM**, MacHale SM, Cavanagh JT, O'Carroll RE, Goodwin GM. The difference in patterns of motor and cognitive function in chronic fatigue syndrome and severe depressive illness. *Psychol Med* 2000; **30**: 433-442 [PMID: 10824663 DOI: 10.1017/S0033291799001816]

173 **Paul L**, Wood L, Behan WM, Maclaren WM. Demonstration of delayed recovery from fatiguing exercise in chronic fatigue syndrome. *Eur J Neurol* 1999; **6**: 63-69 [PMID: 10209352 DOI: 10.1046/j.1468-1331.1999.610063.x]

174 **Meeus M**, Ickmans K, Struyf F, Kos D, Lambrecht L, Willekens B, Cras P, Nijs J. What is in a name? Comparing diagnostic criteria for chronic fatigue syndrome with or without fibromyalgia. *Clin Rheumatol* 2014 Oct 14; Epub ahead of print [PMID: 25308475 DOI: 10.1007/s10067-014-2793-x]

175 **van der Ploeg RJ**, Oosterhuis HJ. Physical examination--measurement of muscle strength. *Ned Tijdschr Geneeskd* 2001; **145**: 19-23 [PMID: 11198960]

176 **Schwartz S**, Cohen ME, Herbison GJ, Shah A. Relationship

between two measures of upper extremity strength: manual muscle test compared to hand-held myometry. *Arch Phys Med Rehabil* 1992; **73**: 1063-1068 [PMID: 1444773]

177 **Bohannon RW**. Reference values for extremity muscle strength obtained by hand-held dynamometry from adults aged 20 to 79 years. *Arch Phys Med Rehabil* 1997; **78**: 26-32 [PMID: 9014953 DOI: 10.1016/S0003-9993(97)90005-8]

178 **Bohannon RW**, Peolsson A, Massy-Westropp N, Desrosiers J, Bear-Lehman J. Reference values for adult grip strength measured with a Jamar dynamometer: a descriptive meta-analysis. *Physiotherapy* 2006; **92**: 11-15 [DOI: 10.1016/j.physio.2005.05.003]

179 **Block W,** Träber F, Kuhl CK, Keller E, Lamerichs R, Karitzky J, Rink H, Schild HH. 31P-mr spectroscopy of peripheral skeletal musculature under load: demonstration of normal energy metabolites compared with metabolic muscle diseases. *Rofo* 1998; **168**: 250-257 [DOI: 10.1055/s-2007-1015121]

180 **Wong R**, Lopaschuk G, Zhu G, Walker D, Catellier D, Burton D, Teo K, Collins-Nakai R, Montague T. Skeletal muscle metabolism in the chronic fatigue syndrome. In vivo assessment by 31P nuclear magnetic resonance spectroscopy. *Chest* 1992; **102**: 1716-1722 [PMID: 1446478 DOI: 10.1378/chest.102.6.1716]

181 **Jones DE**, Hollingsworth KG, Jakovljevic DG, Fattakhova G, Pairman J, Blamire AM, Trenell MI, Newton JL. Loss of capacity to recover from acidosis on repeat exercise in chronic fatigue syndrome: a case-control study. *Eur J Clin Invest* 2012; **42**: 186-194 [PMID: 21749371 DOI: 10.1111/j.1365-2362.2011.02567.x]

182 **Katz BZ**, Stewart JM, Shiraishi Y, Mears CJ, Taylor R. Autonomic symptoms at baseline and following infectious mononucleosis in a prospective cohort of adolescents. *Arch Pediatr Adolesc Med* 2011; **165**: 765-766 [PMID: 21810640 DOI: 10.1001/archpediatrics.2011.124]

183 **Newton JL**, Okonkwo O, Sutcliffe K, Seth A, Shin J, Jones DE. Symptoms of autonomic dysfunction in chronic fatigue syndrome. *QJM* 2007; **100**: 519-526 [PMID: 17617647 DOI: 10.1093/qjmed/hcm057]

184 **Rowe PC**, Bou-Holaigah I, Kan JS, Calkins H. Is neurally mediated hypotension an unrecognised cause of chronic fatigue? *Lancet* 1995; **345**: 623-624 [PMID: 7898182 DOI: 10.1016/S0140-6736(95)90525-1]

185 **Miwa K**. Cardiac dysfunction and orthostatic intolerance in patients with myalgic encephalomyelitis and a small left ventricle. *Heart Vessels* 2014 Apr 16; Epub ahead of print [PMID: 24736946 DOI: 10.1007/s00380-014-0510-y]

186 **Wyller VB**, Helland IB. Relationship between autonomic cardiovascular control, case definition, clinical symptoms, and functional disability in adolescent chronic fatigue syndrome: an exploratory study. *Biopsychosoc Med* 2013; **7**: 5 [PMID: 23388153 DOI: 10.1186/1751-0759-7-5]

187 **Jason LA**, Katz BZ, Shiraishi Y, Mears CJ, Im Y, Taylor R. Predictors of Post-Infectious Chronic Fatigue Syndrome in Adolescents. *Health Psychol Behav Med* 2014; **2**: 41-51 [PMID: 24660116 DOI: 10.1080/21642850.2013.869176]

188 **Reynolds GK**, Lewis DP, Richardson AM, Lidbury BA. Comorbidity of postural orthostatic tachycardia syndrome and chronic fatigue syndrome in an Australian cohort. *J Intern Med* 2014; **275**: 409-417 [PMID: 24206536 DOI: 10.1111/joim.12161]

189 **Galland BC**, Jackson PM, Sayers RM, Taylor BJ. A matched case control study of orthostatic intolerance in children/adolescents with chronic fatigue syndrome. *Pediatr Res* 2008; **63**: 196-202 [PMID: 18091356 DOI: 10.1203/PDR.0b013e31815ed612]

190 **Bou-Holaigah I**, Rowe PC, Kan J, Calkins H. The relationship between neurally mediated hypotension and the chronic fatigue syndrome. *JAMA* 1995; **274**: 961-967 [PMID: 7674527 DOI: 10.1001/jama.1995.03530120053041]

191 **Hoad A**, Spickett G, Elliott J, Newton J. Postural orthostatic tachycardia syndrome is an under-recognized condition in chronic fatigue syndrome. *QJM* 2008; **101**: 961-965 [PMID: 18805903 DOI: 10.1093/qjmed/hcn123]

192 **Forleo C**, Guida P, Iacoviello M, Resta M, Monitillo F, Sorrentino S, Favale S. Head-up tilt testing for diagnosing vasovagal syncope: a meta-analysis. *Int J Cardiol* 2013; **168**: 27-35 [PMID: 23041006 DOI: 10.1016/j.ijcard.2012.09.023]

193 Consensus statement on the definition of orthostatic hypotension, pure autonomic failure, and multiple system atrophy. The Consensus Committee of the American Autonomic Society and the American Academy of Neurology. *Neurology* 1996; **46**: 1470 [PMID: 8628505 DOI: 10.1212/WNL.46.5.1470]

194 **Karas B**, Grubb BP, Boehm K, Kip K. The postural orthostatic tachycardia syndrome: a potentially treatable cause of chronic fatigue, exercise intolerance, and cognitive impairment in adolescents. *Pacing Clin Electrophysiol* 2000; **23**: 344-351 [PMID: 10750135 DOI: 10.1111/j.1540-8159.2000.tb06760.x]

195 **De Lorenzo F**, Hargreaves J, Kakkar VV. Pathogenesis and management of delayed orthostatic hypotension in patients with chronic fatigue syndrome. *Clin Auton Res* 1997; **7**: 185-190 [PMID: 9292244 DOI: 10.1007/BF02267980]

196 **Lutgendorf SK**, Antoni MH, Ironson G, Fletcher MA, Penedo F, Baum A, Schneiderman N, Klimas N. Physical symptoms of chronic fatigue syndrome are exacerbated by the stress of Hurricane Andrew. *Psychosom Med* 1995; **57**: 310-323 [PMID: 7480560 DOI: 10.1097/00006842-199507000-00002]

197 **Papadopoulos AS**, Cleare AJ. Hypothalamic-pituitary-adrenal axis dysfunction in chronic fatigue syndrome. *Nat Rev Endocrinol* 2012; **8**: 22-32 [PMID: 21946893 DOI: 10.1038/nrendo.2011.153]

198 **Tomas C**, Newton J, Watson S. A review of hypothalamic-pituitary-adrenal axis function in chronic fatigue syndrome. *ISRN Neurosci* 2013; **2013**: 784520 [PMID: 24959566 DOI: 10.1155/2013/784520]

199 **Torres-Harding S**, Sorenson M, Jason L, Maher K, Fletcher MA, Reynolds N, Brown M. The associations between basal salivary cortisol and illness symptomatology in chronic fatigue syndrome. *J Appl Biobehav Res* 2008; **13**: 157-180 [PMID: 19701493 DOI: 10.1111/j.1751-9861.2008.00033.x]

200 **Tak LM**, Cleare AJ, Ormel J, Manoharan A, Kok IC, Wessely S, Rosmalen JG. Meta-analysis and meta-regression of hypothalamic-pituitary-adrenal axis activity in functional somatic disorders. *Biol Psychol* 2011; **87**: 183-194 [PMID: 21315796 DOI: 10.1016/j.biopsycho.2011.02.002]

201 **Jerjes WK**, Cleare AJ, Wessely S, Wood PJ, Taylor NF. Diurnal patterns of salivary cortisol and cortisone output in chronic fatigue syndrome. *J Affect Disord* 2005; **87**: 299-304 [PMID: 15922454 DOI: 10.1016/j.jad.2005.03.013]

202 **MacHale SM**, Cavanagh JT, Bennie J, Carroll S, Goodwin GM, Lawrie SM. Diurnal variation of adrenocortical activity in chronic fatigue syndrome. *Neuropsychobiology* 1998; **38**: 213-217 [PMID: 9813459 DOI: 10.1159/000026543]

203 **Gaab J**, Engert V, Heitz V, Schad T, Schürmeyer TH, Ehlert U. Associations between neuroendocrine responses to the Insulin Tolerance Test and patient characteristics in chronic fatigue syndrome. *J Psychosom Res* 2004; **56**: 419-424 [PMID: 15094026 DOI: 10.1016/S0022-3999(03)00625-1]

204 **De Becker P**, De Meirleir K, Joos E, Campine I, Van Steenberge E, Smitz J, Velkeniers B. Dehydroepiandrosterone (DHEA) response to i.v. ACTH in patients with chronic fatigue syndrome. *Horm Metab Res* 1999; **31**: 18-21 [PMID: 10077344 DOI: 10.1055/s-2007-978690]

205 **Gaab J**, Hüster D, Peisen R, Engert V, Heitz V, Schad T, Schürmeyer TH, Ehlert U. Hypothalamic-pituitary-adrenal axis reactivity in chronic fatigue syndrome and health under psychological, physiological, and pharmacological stimulation. *Psychosom Med* 2008; **64**: 951-962 [PMID: 12461200 DOI: 10.1097/01.PSY.0000038937.67401.61]

206 **Habib KE**, Negrao ABYMR, Deuster P, Gold PW. Altered interrelation among plasma stress hormones during treadmill exercise in females with chronic fatigue syndrome. The 84th Annual Meeting of The Endocrine Society; 2002 Jun 19-22; ENDO. San Francisco: California, 2002

207 **Visser J**, van Boxel-Dezaire A, Methorst D, Brunt T, de Kloet ER, Nagelkerken L. Differential regulation of interleukin-10 (IL-10) and IL-12 by glucocorticoids in vitro. *Blood* 1998; **91**: 4255-4264 [PMID: 9596674]

208 **Visser JT**, De Kloet ER, Nagelkerken L. Altered glucocorticoid regulation of the immune response in the chronic fatigue syndrome.

Ann N Y Acad Sci 2000; **917**: 868-875 [PMID: 11268418 DOI: 10.1111/j.1749-6632.2000.tb05453.x]
209 **Jerjes WK**, Taylor NF, Wood PJ, Cleare AJ. Enhanced feedback sensitivity to prednisolone in chronic fatigue syndrome. *Psychoneuroendocrinology* 2007; **32**: 192-198 [PMID: 17276605 DOI: 10.1016/j.psyneuen.2006.12.005]
210 **Van Den Eede F**, Moorkens G, Hulstijn W, Van Houdenhove B, Cosyns P, Sabbe BG, Claes SJ. Combined dexamethasone/corticotropin-releasing factor test in chronic fatigue syndrome. *Psychol Med* 2008; **38**: 963-973 [PMID: 17803834 DOI: 10.1017/S0033291707001444]
211 **Parker AJ**, Wessely S, Cleare AJ. The neuroendocrinology of chronic fatigue syndrome and fibromyalgia. *Psychol Med* 2001; **31**: 1331-1345 [PMID: 11722149 DOI: 10.1017/S0033291701004664]
212 **Cleare AJ**. The HPA axis and the genesis of chronic fatigue syndrome. *Trends Endocrinol Metab* 2004; **15**: 55-59 [PMID: 15036250 DOI: 10.1016/j.tem.2003.12.002]
213 **Candy B**, Chalder T, Cleare AJ, Peakman A, Skowera A, Wessely S, Weinman J, Zuckerman M, Hotopf M. Predictors of fatigue following the onset of infectious mononucleosis. *Psychol Med* 2003; **33**: 847-855 [PMID: 12877399 DOI: 10.1017/S0033291703007554]
214 **Katz BZ**, Zimmerman D, Gorman MRG, Mears CJ, Shiraishi Y, Taylor R. Normal salivary cortisol and NK cell function in adolescents with chronic fatigue syndrome following infectious mononucleosis. *Arch Pediatr Infect Dis* 2013; **1**: 211-216 [DOI: 10.5812/pedinfect.13107]
215 **Whiting P**, Bagnall AM, Sowden AJ, Cornell JE, Mulrow CD, Ramírez G. Interventions for the treatment and management of chronic fatigue syndrome: a systematic review. *JAMA* 2001; **286**: 1360-1368 [PMID: 11560542 DOI: 10.1001/jama.286.11.1360]
216 **Bagnall A**, Hempel S, Chambers D, Orton V, Forbes C. The treatment and management of chronic fatigue syndrome/myalgic encephalomyelitis in adults and children. Centre for Reviews and Dissemination (CRD), University of York. [accessed from 2007]. Available from: URL: http://www.york.ac.uk/inst/crd/CRD_Reports/crdreport35.pdf
217 **Pagana KD**, Pagana TJ. Mosby's diagnostic and laboratory test reference. 10th ed. St. Louis: Elsevier Mosby, 2011
218 **Fuite J**, Vernon SD, Broderick G. Neuroendocrine and immune network re-modeling in chronic fatigue syndrome: an exploratory analysis. *Genomics* 2008; **92**: 393-399 [PMID: 18775774 DOI: 10.1016/j.ygeno.2008.08.008]
219 **Nagaya T**, Fujieda M, Otsuka G, Yang JP, Okamoto T, Seo H. A potential role of activated NF-kappa B in the pathogenesis of euthyroid sick syndrome. *J Clin Invest* 2000; **106**: 393-402 [PMID: 10930442 DOI: 10.1172/JCI7771]
220 **Boelen A**, Kwakkel J, Wiersinga WM, Fliers E. Chronic local inflammation in mice results in decreased TRH and type 3 deiodinase mRNA expression in the hypothalamic paraventricular nucleus independently of diminished food intake. *J Endocrinol* 2006; **191**: 707-714 [PMID: 17170227 DOI: 10.1677/joe.1.07056]
221 **Bartalena L**, Bogazzi F, Brogioni S, Grasso L, Martino E. Role of cytokines in the pathogenesis of the euthyroid sick syndrome. *Eur J Endocrinol* 1998; **138**: 603-614 [PMID: 9678522 DOI: 10.1530/eje.0.1380603]
222 **Broderick G**, Craddock RC, Whistler T, Taylor R, Klimas N, Unger ER. Identifying illness parameters in fatiguing syndromes using classical projection methods. *Pharmacogenomics* 2006; **7**: 407-419 [PMID: 16610951 DOI: 10.2217/14622416.7.3.407]
223 **Englebienne P**, Verhas M, Herst CV, De Meirleir K. Type I interferons induce proteins susceptible to act as thyroid receptor (TR) corepressors and to signal the TR for destruction by the proteasome: possible etiology for unexplained chronic fatigue. *Med Hypotheses* 2003; **60**: 175-180 [PMID: 12606231 DOI: 10.1016/S0306-9877(02)00353-5]
224 **Tjørve E**, Tjørve KM, Olsen JO, Senum R, Oftebro H. On commonness and rarity of thyroid hormone resistance: a discussion based on mechanisms of reduced sensitivity in peripheral tissues. *Med Hypotheses* 2007; **69**: 913-921 [PMID: 17383828 DOI: 10.1016/j.mehy.2006.12.056]
225 **Chopra IJ**. An assessment of daily production and significance of thyroidal secretion of 3, 3', 5'-triiodothyronine (reverse T3) in man. *J Clin Invest* 1976; **58**: 32-40 [PMID: 932209 DOI: 10.1172/JCI108456]
226 **Wiersinga WM**, Fliers E. Determining the thyroid hormones T3 and T4 in the urine: an unreliable test for hypothyroidism. *Ned Tijdschr Geneeskd* 2007; **151**: 2813-2815 [PMID: 18237046]
227 **Moncayo H**, Dapunt O, Moncayo R. Diagnostic accuracy of basal TSH determinations based on the intravenous TRH stimulation test: an evaluation of 2570 tests and comparison with the literature. *BMC Endocr Disord* 2007; **7**: 5 [PMID: 17678551 DOI: 10.1186/1472-6823-7-5]
228 **Hilgers A**, Frank J. Chronic fatigue syndrome: evaluation of a 30-criteria score and correlation with immune activation. *J Chronic Fatigue Syndr* 1996; **2**: 35-47 [DOI: 10.1300/J092v02n04_04]
229 **Dunlap DB**. Thyroid function tests. In: Walker HK, Hall WD, Hurst JW, editors. The history, physical, and laboratory examinations. 3rd ed. Boston: Butterworths, 1990
230 **Unger ER**, Nisenbaum R, Moldofsky H, Cesta A, Sammut C, Reyes M, Reeves WC. Sleep assessment in a population-based study of chronic fatigue syndrome. *BMC Neurol* 2004; **4**: 6 [PMID: 15096280 DOI: 10.1186/1471-2377-4-6]
231 **Jackson ML**, Bruck D. Sleep abnormalities in chronic fatigue syndrome/myalgic encephalomyelitis: a review. *J Clin Sleep Med* 2012; **8**: 719-728 [PMID: 23243408 DOI: 10.5664/jcsm.2276]
232 **Hamaguchi M**, Kawahito Y, Takeda N, Kato T, Kojima T. Characteristics of chronic fatigue syndrome in a Japanese community population : chronic fatigue syndrome in Japan. *Clin Rheumatol* 2011; **30**: 895-906 [PMID: 21302125 DOI: 10.1007/s10067-011-1702-9]
233 **Kishi A**, Natelson BH, Togo F, Struzik ZR, Rapoport DM, Yamamoto Y. Sleep-stage dynamics in patients with chronic fatigue syndrome with or without fibromyalgia. *Sleep* 2011; **34**: 1551-1560 [PMID: 22043126 DOI: 10.5665/sleep.1396]
234 **Spitzer AR**, Broadman M. A retrospective review of the sleep characteristics in patients with chronic fatigue syndrome and fibromyalgia. *Pain Pract* 2010; **10**: 294-300 [PMID: 20230458 DOI: 10.1111/j.1533-2500.2009.00352.x]
235 **Decker MJ**, Tabassum H, Lin JM, Reeves WC. Electroencephalographic correlates of Chronic Fatigue Syndrome. *Behav Brain Funct* 2009; **5**: 43 [PMID: 19807920 DOI: 10.1186/1744-9081-5-43]
236 **Togo F**, Natelson BH, Cherniack NS, FitzGibbons J, Garcon C, Rapoport DM. Sleep structure and sleepiness in chronic fatigue syndrome with or without coexisting fibromyalgia. *Arthritis Res Ther* 2008; **10**: R56 [PMID: 18474105 DOI: 10.1186/ar2425]
237 **Kishi A**, Struzik ZR, Natelson BH, Togo F, Yamamoto Y. Dynamics of sleep stage transitions in healthy humans and patients with chronic fatigue syndrome. *Am J Physiol Regul Integr Comp Physiol* 2008; **294**: R1980-R1987 [PMID: 18417644 DOI: 10.1152/ajpregu.00925.2007]
238 **Krupp LB**, Jandorf L, Coyle PK, Mendelson WB. Sleep disturbance in chronic fatigue syndrome. *J Psychosom Res* 1993; **37**: 325-331 [PMID: 8510058 DOI: 10.1016/0022-3999(93)90134-2]
239 **Neu D**, Mairesse O, Verbanck P, Linkowski P, Le Bon O. Non-REM sleep EEG power distribution in fatigue and sleepiness. *J Psychosom Res* 2014; **76**: 286-291 [PMID: 24630178 DOI: 10.1016/j.jpsychores.2014.02.002]
240 **Leslie SB**. Chronic fatigue syndrome: optometric clinical presentation and management. *J Behav Optom* 1997; **8**: 155-161
241 **Vedelago LJ**. Visual dysfunction in chronic fatigue syndrome: behavioural optometric assessment and management. *J Behav Optom* 1997; **8**: 149-154
242 **Hutchinson CV**, Maltby J, Badham SP, Jason LA. Vision-related symptoms as a clinical feature of chronic fatigue syndrome/myalgic encephalomyelitis? Evidence from the DePaul symptom questionnaire. *Br J Ophthalmol* 2014; **98**: 144-145 [DOI: 10.1136/bjophthalmol-2013-304439]
243 **Hutchinson CV**, Badham SP. Patterns of abnormal visual attention in myalgic encephalomyelitis. *Optom Vis Sci* 2013; **90**: 607-614 [PMID: 23689679 DOI: 10.1097/OPX.0b013e318294c232]

244 **Badham SP**, Hutchinson CV. Characterising eye movement dysfunction in myalgic encephalomyelitis/chronic fatigue syndrome. *Graefes Arch Clin Exp Ophthalmol* 2013; **251**: 2769-2776 [PMID: 23918092 DOI: 10.1007/s00417-013-2431-3]

245 **Fletcher MA**, Zeng XR, Barnes Z, Levis S, Klimas NG. Plasma cytokines in women with chronic fatigue syndrome. *J Transl Med* 2009; **7**: 96 [PMID: 19909538 DOI: 10.1186/1479-5876-7-96]

246 **Klimas NG**, Koneru AO. Chronic fatigue syndrome: inflammation, immune function, and neuroendocrine interactions. *Curr Rheumatol Rep* 2007; **9**: 482-487 [PMID: 18177602 DOI: 10.1007/s11926-007-0078-y]

247 **McCully KK**, Natelson BH, Iotti S, Sisto S, Leigh JS. Reduced oxidative muscle metabolism in chronic fatigue syndrome. *Muscle Nerve* 1996; **19**: 621-625 [PMID: 8618560 DOI: 10.1002/(SICI)1097-4598(199605)19:]

248 **Vernon SD**, Whistler T, Cameron B, Hickie IB, Reeves WC, Lloyd A. Preliminary evidence of mitochondrial dysfunction associated with post-infective fatigue after acute infection with Epstein Barr virus. *BMC Infect Dis* 2006; **6**: 15 [PMID: 16448567 DOI: 10.1186/1471-2334-6-15]

249 **Booth NE**, Myhill S, McLaren-Howard J. Mitochondrial dysfunction and the pathophysiology of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). *Int J Clin Exp Med* 2012; **5**: 208-220 [PMID: 22837795]

250 **Behan WM**, More IA, Behan PO. Mitochondrial abnormalities in the postviral fatigue syndrome. *Acta Neuropathol* 1991; **83**: 61-65 [PMID: 1792865 DOI: 10.1007/BF00294431]

251 **Hokama Y**, Campora CE, Hara C, Kuribayashi T, Le Huynh D, Yabusaki K. Anticardiolipin antibodies in the sera of patients with diagnosed chronic fatigue syndrome. *J Clin Lab Anal* 2009; **23**: 210-212 [PMID: 19623655 DOI: 10.1002/jcla.20325]

252 **Javierre C**, Alegre J, Ventura JL, Garcia-Quintana A, Segura R, Suarez A, Morales A, Comella A, De Meirleir K. Physiological responses to arm and leg exercise in women patients with chronic fatigue syndrome. *J Chronic Fatigue Syndr* 2007; **1**: 43-53 [DOI: 10.1300/J092v14n01_05]

253 **McCully KK**, Natelson BH. Impaired oxygen delivery to muscle in chronic fatigue syndrome. *Clin Sci* (Lond) 1999; **97**: 603-608; discussion 611-613 [PMID: 10545311 DOI: 10.1042/CS19980372]

254 **Arnold DL**, Bore PJ, Radda GK, Styles P, Taylor DJ. Excessive intracellular acidosis of skeletal muscle on exercise in a patient with a post-viral exhaustion/fatigue syndrome. A 31P nuclear magnetic resonance study. *Lancet* 1984; **1**: 1367-1369 [PMID: 6145831 DOI: 10.1016/S0140-6736(84)91871-3]

255 **Lane RJ**, Barrett MC, Woodrow D, Moss J, Fletcher R, Archard LC. Muscle fibre characteristics and lactate responses to exercise in chronic fatigue syndrome. *J Neurol Neurosurg Psychiatry* 1998; **64**: 362-367 [PMID: 9527150 DOI: 10.1136/jnnp.64.3.362]

256 **Nijs J**, Meeus M, Van Oosterwijck J, Ickmans K, Moorkens G, Hans G, De Clerck LS. In the mind or in the brain? Scientific evidence for central sensitisation in chronic fatigue syndrome. *Eur J Clin Invest* 2012; **42**: 203-212 [PMID: 21793823 DOI: 10.1111/j.1365-2362.2011.02575.x]

257 **Samad TA**, Sapirstein A, Woolf CJ. Prostanoids and pain: unraveling mechanisms and revealing therapeutic targets. *Trends Mol Med* 2002; **8**: 390-396 [PMID: 12127725 DOI: 10.1016/S1471-4914(02)02383-3]

258 **Sommer C**, Kress M. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. *Neurosci Lett* 2004; **361**: 184-187 [PMID: 15135924 DOI: 10.1016/j.neulet.2003.12.007]

259 **Wu J**, Fang L, Lin Q, Willis WD. Nitric oxide synthase in spinal cord central sensitization following intradermal injection of capsaicin. *Pain* 2001; **94**: 47-58 [PMID: 11576744 DOI: 10.1016/S0304-3959(01)00340-2]

260 **Little JW**, Doyle T, Salvemini D. Reactive nitroxidative species and nociceptive processing: determining the roles for nitric oxide, superoxide, and peroxynitrite in pain. *Amino Acids* 2012; **42**: 75-94 [PMID: 20552384 DOI: 10.1007/s00726-010-0633-0]

261 **Light AR**, Bateman L, Jo D, Hughen RW, Vanhaitsma TA, White AT, Light KC. Gene expression alterations at baseline and following moderate exercise in patients with Chronic Fatigue Syndrome and Fibromyalgia Syndrome. *J Intern Med* 2012; **271**: 64-81 [PMID: 21615807 DOI: 10.1111/j.1365-2796.2011.02405.x]

262 **Light AR**, White AT, Hughen RW, Light KC. Moderate exercise increases expression for sensory, adrenergic, and immune genes in chronic fatigue syndrome patients but not in normal subjects. *J Pain* 2009; **10**: 1099-1112 [PMID: 19647494 DOI: 10.1016/j.jpain.2009.06.003]

263 **Buchwald D**, Pearlman T, Umali J, Schmaling K, Katon W. Functional status in patients with chronic fatigue syndrome, other fatiguing illnesses, and healthy individuals. *Am J Med* 1996; **101**: 364-370 [PMID: 8873506 DOI: 10.1016/S0002-9343(96)00234-3]

264 **Anderson VR**, Jason LA, Hlavaty LE. A qualitative natural history study of ME/CFS in the community. *Health Care Women Int* 2014; **35**: 3-26 [PMID: 23445264 DOI: 10.1080/07399332.2012.684816]

265 **Taylor RR**, O'Brien J, Kielhofner G, Lee SW, Katz B, Mears C. The occupational and quality of life consequences of chronic fatigue syndrome/myalgic encephalomyelitis in young people. *Br J Occup Ther* 2010; **73**: 524-530 [PMID: 22102767 DOI: 10.4276/030802210X12892992239233]

266 **Anderson JS**, Ferrans CE. The quality of life of persons with chronic fatigue syndrome. *J Nerv Ment Dis* 1997; **185**: 359-367 [PMID: 9205421 DOI: 10.1097/00005053-199706000-00001]

267 **Barsky AJ**, Borus JF. Functional somatic syndromes. *Ann Intern Med* 1999; **130**: 910-921 [PMID: 10375340 DOI: 10.7326/0003-4819-130-11-199906010-00016]

268 **Maes M**, Twisk FN. Chronic fatigue syndrome: Harvey and Wessely's (bio)psychosocial model versus a bio(psychosocial) model based on inflammatory and oxidative and nitrosative stress pathways. *BMC Med* 2010; **8**: 35 [PMID: 20550693 DOI: 10.1186/1741-7015-8-35]

269 **Wojcik W**, Armstrong D, Kanaan R. Chronic fatigue syndrome: labels, meanings and consequences. *J Psychosom Res* 2011; **70**: 500-504 [PMID: 21624573 DOI: 10.1016/j.jpsychores.2011.02.002]

270 **Thomas MA**, Smith AP. Primary healthcare provision and Chronic Fatigue Syndrome: a survey of patients' and General Practitioners' beliefs. *BMC Fam Pract* 2005; **6**: 49 [PMID: 16351714 DOI: 10.1186/1471-2296-6-49]

271 **Gilje AM**, Söderlund A, Malterud K. Obstructions for quality care experienced by patients with chronic fatigue syndrome (CFS)--a case study. *Patient Educ Couns* 2008; **73**: 36-41 [PMID: 18486415 DOI: 10.1016/j.pec.2008.04.001]

272 **Asbring P**. Chronic illness -- a disruption in life: identity-transformation among women with chronic fatigue syndrome and fibromyalgia. *J Adv Nurs* 2001; **34**: 312-319 [PMID: 11328436 DOI: 10.1046/j.1365-2648.2001.01767.x]

273 **Garralda ME**, Rangel L. Impairment and coping in children and adolescents with chronic fatigue syndrome: a comparative study with other paediatric disorders. *J Child Psychol Psychiatry* 2004; **45**: 543-552 [PMID: 15055373 DOI: 10.1111/j.1469-7610.2004.00244.x]

274 **Nijhof SL**, Maijer K, Bleijenberg G, Uiterwaal CS, Kimpen JL, van de Putte EM. Adolescent chronic fatigue syndrome: prevalence, incidence, and morbidity. *Pediatrics* 2011; **127**: e1169-1175 [PMID: 21502228 DOI: 10.1542/peds.2010-1147]

275 **Crawley E**, Sterne JA. Association between school absence and physical function in paediatric chronic fatigue syndrome/myalgic encephalopathy. *Arch Dis Child* 2009; **94**: 752-756 [PMID: 19001477 DOI: 10.1136/adc.2008.143537]

276 **Bould H**, Collin SM, Lewis G, Rimes K, Crawley E. Depression in paediatric chronic fatigue syndrome. *Arch Dis Child* 2013; **98**: 425-428 [PMID: 23619200 DOI: 10.1136/archdischild-2012-303396]

277 **Katz BZ**, Jason LA. Chronic fatigue syndrome following infections in adolescents. *Curr Opin Pediatr* 2013; **25**: 95-102 [PMID: 23263024 DOI: 10.1097/MOP.0b013e32835c1108]

278 **Hickie I**, Davenport T, Wakefield D, Vollmer-Conna U, Cameron B, Vernon SD, Reeves WC, Lloyd A. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. *BMJ* 2006; **333**: 575 [PMID: 16950834 DOI: 10.1136/bmj.38933.585764.AE]

279 **Donalek JG**. When a parent is chronically ill: chronic fatigue syndrome. *Nurs Res* 2009; **58**: 332-339 [PMID: 19752673 DOI:

10.1097/NNR.0b013e3181ac156f]
280 **Winger A**, Ekstedt M, Wyller VB, Helseth S. 'Sometimes it feels as if the world goes on without me': adolescents' experiences of living with chronic fatigue syndrome. *J Clin Nurs* 2014; **23**: 2649-2657 [PMID: 24354631 DOI: 10.1111/jocn.12522]
281 **Whitehead LC**. Quest, chaos and restitution: living with chronic fatigue syndrome/myalgic encephalomyelitis. *Soc Sci Med* 2006; **62**: 2236-2245 [PMID: 16236413 DOI: 10.1016/j.socscimed.2005.09.008]
282 **Tuck I**, Wallace D. Chronic fatigue syndrome: a woman's dilemma. *Health Care Women Int* 2000; **21**: 457-466 [PMID: 11261112 DOI: 10.1080/07399330050082263]
283 **Jason LA**, Taylor RR, Stepanek Z, Plioplys S. Attitudes regarding chronic fatigue syndrome: the importance of a name. *J Health Psychol* 2001; **6**: 61-71 [PMID: 22049238 DOI: 10.1177/135910530100600105]
284 **McInnis OA**, Matheson K, Anisman H. Living with the unexplained: coping, distress, and depression among women with chronic fatigue syndrome and/or fibromyalgia compared to an autoimmune disorder. *Anxiety Stress Coping* 2014; **27**: 601-618 [PMID: 24479644 DOI: 10.1080/10615806.2014.888060]
285 **Raine R**, Carter S, Sensky T, Black N. General practitioners' perceptions of chronic fatigue syndrome and beliefs about its management, compared with irritable bowel syndrome: qualitative study. *BMJ* 2004; **328**: 1354-1357 [PMID: 15169743 DOI: 10.1136/bmj.38078.503819.EE]
286 **Wood B**, Wessely S. Personality and social attitudes in chronic fatigue syndrome. *J Psychosom Res* 1999; **47**: 385-397 [PMID: 10616232 DOI: 10.1016/S0022-3999(99)00025-2]
287 **Ciccolella M**, Stevens SR, Snell CR, Mark VanNess J. Legal and scientific considerations of the exercise stress test. *Journ of Chr Fatigue Syndr* 2007; **14**: 61-75 [DOI: 10.1300/J092v14n02_06]
288 **Knoop H**, Bleijenberg G, Gielissen MF, van der Meer JW, White PD. Is a full recovery possible after cognitive behavioural therapy for chronic fatigue syndrome? *Psychother Psychosom* 2007; **76**: 171-176 [PMID: 17426416 DOI: 10.1159/000099844]
289 **Heins MJ**, Knoop H, Prins JB, Stulemeijer M, van der Meer JW, Bleijenberg G. Possible detrimental effects of cognitive behaviour therapy for chronic fatigue syndrome. *Psychother Psychosom* 2010; **79**: 249-256 [PMID: 20502065 DOI: 10.1159/000315130]
290 **Twisk FN**, Arnoldus RJ. Graded exercise therapy (GET)/cognitive behavioural therapy (CBT) is often counterproductive in myalgic encephalomyelitis (ME) and chronic fatigue syndrome (CFS). *Eur J Clin Invest* 2012; **42**: 1255-1256; author reply 1257-1258 [PMID: 23033954 DOI: 10.1111/j.13652362.2012.02718.x]
291 **Wiborg JF**, Knoop H, Stulemeijer M, Prins JB, Bleijenberg G. How does cognitive behaviour therapy reduce fatigue in patients with chronic fatigue syndrome? The role of physical activity. *Psychol Med* 2010; **40**: 1281-1287 [PMID: 20047707 DOI: 10.1017/S0033291709992212]
292 Akkoordraad in het kader van de revalidatieovereenkomsten inzake ten laste neming door Referentiecentra van patinten lijdend aan het Chronisch vermoeidheidssyndroom] [Dutch] Council of approval with regards to rehabilitation contracts with CFS reference centers. [Evaluation Report 2002-2004 with respect to rehabilitation contracts between the RIZIV and the CFS Reference Centers] [Dutch] Evaluation Report (2002- 2004) with respect to Rehabilitation Contracts between the RIZIV and the CFS Reference Centers. 2006, July
293 **Kindlon T**. Objective compliance and outcome measures should be used in trials of exercise interventions for Chronic Fatigue Syndrome. *Eur J Clin Invest* 2012; **42**: 1360-1361; author reply 1363-1365 [PMID: 22998769 DOI: 10.1111/j.1365-2362.2012.02724.x]

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REVIEW

Past, present and future of cyanide antagonism research: From the early remedies to the current therapies

Ilona Petrikovics, Marianna Budai, Kristof Kovacs, David E Thompson

Ilona Petrikovics, Kristof Kovacs, David E Thompson, Department of Chemistry, Sam Houston State University, Huntsville, TX 77341, United States
Marianna Budai, Department of Pharmaceutics, Semmelweis University, H-1092 Budapest, Hungary

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Correspondence to: Ilona Petrikovics, PhD, Department of Chemistry, Sam Houston State University, 1806 Avenue J, Huntsville, TX 77341, United States. ixp004@shsu.edu
Telephone: +1-936-2944389
Fax: +1-936-2944996

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Abstract

This paper reviews milestones in antidotal therapies for cyanide (CN) spanning early remedies, current antidotal systems and research towards next generation therapies. CN has been a part of plant defense mechanisms for millions of years. It became industrially important in the nineteenth century with the advent of CN assisted gold mining and the use of CN as a pest control agent. The biochemical basis of CN poisoning was actively studied and key mechanisms were understood as early as 1929. These fundamental studies led to a variety of antidotes, including indirect CN binders that generate methemoglobin, direct CN binders such as hydroxocobalamin, and sulfur donors that convert CN to the less toxic thiocyanate. Research on blood gases at the end of the twentieth century shed new light on the role of nitric oxide (NO) in the body. The discovery of NO's ability to compete with CN for enzymatic binding sites provided a previously missed explanation for the rapid efficacy of NO generating antidotes such as the nitrites. Presently used CN therapies include: methemoglobin/NO generators (*e.g.*, sodium nitrite, amyl nitrite, and dimethyl aminophenol), sulfur donors (*e.g.*, sodium thiosulfate and glutathione), and direct binding agents [(*e.g.*, hydroxocobalamin and dicobalt salt of ethylenediaminetetraacetic acid (dicobalt edetate)]. A strong effort is being made to explore novel antidotal systems and to formulate them for rapid administration at the point of intoxication in mass casualty scenarios. New antidotes, formulations, and delivery systems are enhancing bioavailability and efficacy and hold promise for a new generation of improved CN countermeasures.

Key words: Cyanide; Hydrocyanic acid; Antagonist; Antidote; Cobinamide; Sulfanegen; Sulfane sulfur donor

Core tip: This paper reviews milestones in antidotal

therapies for cyanide (CN) spanning early history, current antidotal systems, and research towards next generation therapies. Presently used CN therapies include: methemoglobin/nitric oxide generators (*e.g.*, sodium nitrite, amyl nitrite, and dimethyl aminophenol), sulfur donors (*e.g.*, sodium thiosulfate and glutathione), and direct binding agents (*e.g.*, hydroxocobalamin and dicobalt edetate). New antidotes, formulations, and delivery systems are presently being developed for rapid administration at the point of intoxication in mass casualty scenarios. These hold promise for a new generation of improved CN countermeasures.

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INTRODUCTION

Cyanide (CN) refers to both the anion CN^- and the undissociated form of hydrogen cyanide (HCN). It is a weak acid with a pKa of 9.2, therefore at the body's pH it exists mainly in the HCN form. HCN can easily cross cellular, and subcellular membranes such as the blood brain barrier, and mitochondrial membranes.

CN is formed and is present in various spheres of Nature, such as the cosmos, volcanic eruptions, and lightning[1]. Certain plants, fungi, bacteria, and animals synthesize CN as a component of cyanogenic glycosides to provide a source of nitrogen and for self-defense[2,3]. Consuming cyanogenic plants (*e.g.,* cassava roots, yams, sorghum, maize) without proper preparation can lead to CN intoxication. The cyanogenic glycoside amygdalin is present in some pitted fruits like bitter almond, and apricot cherries[4]. Cyanogenic glycosides can eliminate HCN through a hydrolytic reaction[5]. Metabolism of other cyanogenic chemicals such as the cyanogen halides, and nitriles also leads to CN intoxication[6].

CN is present in smoke from fires (especially burning acrylonitrile, polyurethane, polyamide, wool, silk, rubber) and cigarettes[7-9]; in the vasodilating agent Nitroprusside (formulated as a CN complex)[10,11]; and in industrial settings where CN is widely used to complex metals - for example, CN is widely used in industry for gold extraction from ores[12].

CN has been used as a poison from antiquity. It was first isolated from cherry laurel[13]. Early CN poisonings were reported more than 200 years ago by Wepfer in 1679, and by Fontana in 1795[13], however, the first attempt to antagonize CN was reported later, in the 19th century[14]. Theories, based on biochemical mechanisms of CN antagonism were reported in the mid-20th century[15-20] and therapeutic agents became available for clinical application. After the recent recognition of CN as a military weapon and terror agent, intense research has led to a deeper understanding of CN biochemistry, and has fostered the development of novel CN countermeasures.

CN was used as a chemical weapon for the first time in World War Ⅰ. In World War Ⅱ the Nazi's used CN in gas chambers[21]. In the 1980's Iraq used CN against the Kurds[22]. CN was also involved in the Tokyo subway attack in 1995[23], in the first World Trade Center attack in 1993[24], and in the Chicago Tylenol disaster in 1982[25].

FROM RESEARCH TO THERAPY

Mechanism of cyanide toxicity/search for diagnostics

The primary biochemical basis of CN poisoning was known and published as early as 1929[26]. CN binds and inactivates several metal-containing enzymes, but the most important effect is attributed to the binding of Cyt c Oxidase, which is the terminal oxidase of the mitochondrial electron transport chain. This results in histotoxic hypoxia, due to the inhibition of cell oxygen utilization. As the result of suppressing the aerobic metabolic pathway, the less efficient anaerobic pathway becomes dominant, and pyruvate is reduced to lactic acid. The resultant acidosis leads to central nervous system (CNS) and myocardial depressions.

Higher CN doses (higher than 5XLD50) generate more complex responses[27]. For example, at higher doses, CN induces pulmonary arteriolar and coronary vasoconstriction that can result in cardiogenic shock/pulmonary edema[28], and stimulates chemoreceptors in the carotid artery and aorta resulting in hyper apnea. The antidotal effects of the vasodilators (*e.g.,* nitrites) and alpha-adrenergic blockers (*e.g.*, phenoxybenzamine) confirmed the vascular effects of CN, that are present along with the earlier discovered biochemical enzyme binding effects[29].

Lower doses of CN cause responses such as dizziness, headache, nausea, and vomiting as a result of inhibiting and interfering with the cellular enzymes. Chronic low dose CN exposure can result in Parkinson-like syndromes, confusion, and intellectual deterioration[30]. It is suggested that the wide-spread pathologic condition of tropic ataxic neuropathy is associated with the intense consumption of cassava with high CN-Glycoside content[31].

HCN absorbs rapidly through the mucus membranes and the skin. Inhaling HCN results in rapid intoxication, and if concentrations are high enough, death. When salts are ingested, the absorption from the GI tract is slower. About 60% of the absorbed CN binds to protein. It has high affinity to Cobalt and Fe^{3+}, but it also reacts with sulfur containing molecules in the body (see next paragraphs).

The diagnosis of CN intoxication is difficult in the absence of a direct assay for CN; however, cardiovascular and CNS depressions in smoke inhalation victims always suggest CN involvement. Generally,

symptoms, such as metabolic acidosis, coma, shock, seizures, bradycardia, are not specific, but if the patient is randomly collapsed, and not responding to oxygen treatment, CN exposure should be suspected. Laboratory tests can show saturated hemoglobin (Hgb) (oxygen is not utilized), lactic acidosis (blocked oxidative phosphorylation), and hyperglycemia (toxic effects on pancreatic beta-cells)[32]. CN caused death is confirmed chemically using a colorimetric test at the scene of the crime[33], followed by analysis by GC-MS[34]. Due to the relatively short half-life of CN in the blood[35], detecting major metabolites such as thiocyanate (SCN) and 2-aminothiazoline-4-carboxylic acid (ATCA) are recommended[36,37]. ATCA, as a stable CN metabolite has been established as an important forensic CN biomarker[38]. A recent review of the analysis of CN and its metabolites was published by Logue *et al*[39] in 2010.

Cyanide metabolism

Small amounts of CN can be metabolized in the body by various endogenous metabolic pathways, however, the rate of detoxification is slow (about 0.017 mg CN/body weight/min). The body can metabolize CN at even the lethal dose, if that is given slowly over the course of several hours[40]. In the most important endogenous detoxification reaction CN is converted to the less toxic SCN by reaction with thiosulfate and other endogenous sulfur donors in the presence of a sulfurtransferase enzyme, Rhodanese (Rh). Since Rh is a mitochondrial enzyme, and the inorganic thiosulfate has limited cell penetration capability, the serum albumin-sulfane complex plays a major role in ferrying sulfur donors to Rh[41]. Endogenous sulfur donors are formed from cysteine and methionine in the presence of sulfurtransferases such as Rh, Mercaptopyruvate Sulfur Transferase and Cystathionase. The second most important metabolic pathway is the formation of the chemically stable CN metabolite, ATCA, when CN reacts with endogenous cysteine[37]. Another important metabolic pathway is the formation of cyanocobalamin from hydroxocobalamin. These metabolic end-products are excreted by the urine, while unreacted CN is excreted in breath, urine and sweat. The most recent understanding of CN metabolism, the role of nitric oxide (NO) will be discussed in the next section.

Milestones in CN antagonism

At the end of the 19th and beginning of the 20th century scientists began a systematic search for ways of neutralizing the toxic effects of the deadly chemical. These investigations of CN antagonism prior to the 1930s resulted in the development of the classic CN therapies such as of sodium thiosulfate and sodium nitrite that are still in use worldwide. Table 1 lists some important therapies and the countries where they are presently employed in treating CN intoxication.

Methemoglobin formers: Historically the first CN poisoning remedy was the methemoglobin former amyl nitrite[42]. In studies on the biochemical mechanism of CN antagonism, it was found that while CN has low affinity to Hgb, it has high affinity to the oxidized form of Hgb, methemoglobin (MetHgb), resulting in the formation of the relatively stable cyano-MetHgb complex[15,16]. Amyl nitrite, the first MetHgb former used for CN antagonism, was followed subsequently by sodium nitrite. As early as 1933, the combination of the MetHgb formers amyl nitrite, sodium nitrite and sodium thiosulfate was reported to show significant enhancement in antidotal protection[15,16]. To overcome the disadvantage of the slow MetHgb formation by nitrites, a fast MetHgb former, 4-dimethylaminophenol (DMAP), was developed and investigated[43-45]. When DMAP was administered intravenously, CN was entrapped within the red blood cells with relatively high efficiency[46]. This fast acting MetHgb former was developed and used in Germany in the military and civilian population. It produced 2-3 x LD50 protection in a dog model when the MetHgb level was kept around 30%[47]. Similar CN antidotal protections were found with other MetHgb formers such as p-aminopropiophenone (PAPP), p-aminoheptanoylphenone, and hydroxylamine[48].

Sulfane sulfurs and sulfur donors as CN antidotes: As early as 1894 Lang[49] reported that sodium thiosulfate antagonizes CN intoxication by converting it to a biologically less active metabolite in rabbits. Pascheles[50] and Kahn[51] pointed out that in the liver enzymes enhanced the transformation of CN to SCN. During that time other endogenous organo sulfur molecules, such as cysteine, cystine and glutathione were also investigated as CN antidotes[52-54].

In parallel with the MetHgb binding theory reported by Chen *et al*[15] in 1933, Lang[55] reported the biotransformation reaction between CN and thiosulfate, in which thiosulfate served as a sulfur donor substrate for the mitochondrial sulfurtransferase, Rh. Investigating the substrate specificity of Rh, Sorbo reported that sulfur donors, such as aliphatic and aromatic thiosulfonates are superior sulfur donors than thiosulfate[56]. Rh (E.C. 2.8.1.1; thiosulfate:cyanide sulfurtransferase) was the first sulfurtransferase that was studied in detail[57,58]. The utilization of thiosulfate as CN antidote was reported to be limited by its short biological half-life, small volume of distribution, and limited ability to penetrate the mitochondrial membranes to reach the Rh enzyme[41]. Frankenberg *et al*[59] reported in 1975 that the presence of CN enhanced the cell penetration capability of thiosulfate, making thiosulfate more efficient as a therapeutic agent, rather than as a prophylactic agent. Sulfane sulfur molecules (containing multiple divalent sulfur atoms bound to each other) can serve as a sulfur donor to the Rh reaction. In 1981, Westley[60] reported that a series of sulfane sulfur compounds, such as thiosulfonates, polythionates persulfides, polysulfides, and elementary sulfur serve as substrates for the Rh reaction, however, the pharmacokinetic parameters and toxicity of some of these compounds limited their usage

Table 1 Present cyanide therapies worldwide

Antidotal therapy	Country availability
Sodium nitrite, amyl nitrite and sodium thiosulfate	Europe, Asia (Lilly kit; Talor kit; Pasadena kit) United States (Nithiodote™)
Hydroxocobalamin	European Union/United States (Cyanokit®)
4-Dimethylaminophenol	Germany/Austria/Netherlands
Dicobalt edetate	Netherlands/France/United Kingdom/Australia/Israel (Kelocyanor)

as CN antidotes.

The Westley lab reported that the reaction between CN and mercaptopyruvate (another type of sulfur donor), was catalyzed by 3-Mercaptopyruvate sulfurtransferase (E.C. 2.8.1.2) in the cytosol and mitochondria[61]. When mercaptopyruvate reacts with CN, both SCN and cyanohydrin are formed. Mercaptopyruvate enhances the antidotal effect of thiosulfate, or the thiosulfate + nitrite combination[19]. Westley *et al*[57] reported that serum albumin may also act as an endogenous sulfane sulfur donor, and can react with CN to form SCN.

Sodium thiosulfate and nitrite are the active components of the present CN antidotal combination therapy in the United States (Nithiodote™).

Cobalt compounds: Because cobalt has high affinity to CN, cobalt containing compounds can be used as CN antidotes. Hydroxocobalamin can react with CN by forming cyanocobalamin, which is excreted in the urine[62]. In a dog model, dicobalt edetate is superior to the classic nitrite + thiosulfate combination[63]. When cobalt chloride was administered in combination with thiosulfate or nitrite, it was reported that there was a striking enhancement between cobalt and thiosulfate, but not between the cobalt and the nitrite[64]. Due to its toxicity, cobalt chloride is not used as an antidote for humans[65].

Carbonyl compounds: CN is a nucleophile that reacts with carbonyl containing molecules, such as aldehydes and ketones, to form cyanohydrines. Sodium pyruvate was reported first to antagonize CN in a mice model[66]. The advantage of sodium pyruvate over the classic thiosulfate + nitrite antidotal combination is that it is actively transported intracellularly and can distribute to sites of CN localizations[67]. Sodium pyruvate has been reported to significantly enhance the protective effects of nitrite, but with thiosulfate this enhancement is negligible. However, by adding pyruvate to the combination of nitrite and thiosulfate, a striking protection was found[19]. The strong CN antidotal effects of alpha-ketoglutaric acid were reported as a result of cyanohydrine formation with CN[68].

Clorpromazine: Chlorpromazine was reported as a CN antidote as early as 1958[69]. The mechanism of its action is not known. It does not form MetHgb, bind CN, nor serve as sulfur donor, but it does enhance the antidotal effects of thiosulfate, and the thiosulfate + nitrite combination[70].

Oxygen/hyperbaric oxygen: The Way lab reported first the CN antidotal effect of Oxygen/Hyperbaric oxygen[71-73]. Oxygen alone does not protect against CN, but it does enhance the effects of thiosulfate and the thiosulfate + nitrite combination[74].

Way - classification of CN antidotes: Way[19] summarized the mechanisms of CN antagonism and classified the CN antagonists known at that time. For example, he described the four major steps involved in the antagonism of CN by nitrites and thiosulfate: (1) CN binds to Cytochrome C Oxidase to form CN-Cytochrome C Oxidase; (2) nitrite reacts with Hgb to form MetHgb; (3) CN binds to MetHgb to form Cyano-MetHgb; and (4) thiosulfate reacts with CN to form SCN. This mechanism explained how the classic CN therapy (thiosulfate + nitrite) works, how CN partitions between Cytochrome c Oxidase and MetHgb until it is eventually converted to SCN and eliminated from the body by the urine.

Way described four basic classes of CN antagonists: (1) class Ⅰ: Cyanide Binders: Class IA: MetHgb formers (such as nitrites and DMAP); Class IB: Cobalt compounds (such as dicobalt edetate and Hydroxocobalamin); Class IC: Carbonyl compounds (such as pyruvate); (2) class Ⅱ: Sulfur Donors: Class ⅡA: Thiosulfate; Class ⅡB: Thiosulfonates; Class ⅡC: Other Sulfur sulfanes; (3) class Ⅲ: Cyanide Binders and Sulfur Donors: such as mercaptopyruvate; and (4) class Ⅳ: Unknown Mechanism: Class ⅣA: Oxygen; Class ⅣB: Chlorpromazine.

New antidotal approach and new sulfur donor combination studies in the 20th century: The lower *in vivo* antidotal efficacy of thiosulfate relative to its *in vitro* efficacy highlighted how the limited cell penetration capability of thiosulfate adversely impacted its ability to reach the mitochondrial Rh[75]. Early investigations indicated the importance of externally administered Rh directly to the circulation to enhance the CN antidotal effect of thiosulfate[43,76-78]. However, when proteins (enzymes) are injected directly to the bloodstream, they are rapidly destroyed by proteolytic enzymes and the body's immune system. Therefore, the efficiency of this approach is limited[79]. To minimize the adverse immunologic reactions, a protective

environment is needed for the externally administered enzymes. The two major challenges of this approach of placing sulfur donor and Rh in a close proximity are: finding an appropriate sulfur donor with high sulfur donor reactivity, and developing an appropriate scheme for protecting Rh against macrophage recognition in the circulation[80]. Early investigations following this approach focused on Rh encapsulation within Carrier Erythrocytes[79-81]. To enhance the antidotal efficacy of the CN antidotal system of sulfur donor + externally administered Rh, organic thiosulfonates with superior sulfur donor reactivity were employed. When butane thiosulfonate was encapsulated with Rh in Carrier Erythrocytes, and administered in combination of sodium nitrite, a 14 x LD50 prophylactic protection was found[81]. To overcome the disadvantage of carrier erythrocytes (labor demanding encapsulation, prior blood typing), biodegradable, synthetic polymeric nano-delivery systems were employed[82].

The importance of this approach became suppressed when sulfur donors with higher lipophilicity, and higher cell penetration capability were employed. In 1999 Baskin *et al*[83] reported results on the *in vitro* and *in vivo* efficacy studies with various synthetic sulfur donors with different chemical structures and greater lipophilicity than thiosulfate. In 2006 Ashani *et al*[84] reported that garlic, and its main component, allicin, were beneficial in acute CN intoxications. Allicin breaks down spontaneously to form a variety of organosulfur molecules, such as diallyl-sulfide, diallyl-disulfide and diallyl trisulfide. In the presence of oil, allicin is transformed to ajoene and vinyl-dithiins[85,86]. Investigations of these specific garlic components proved that they are not superior to thiosulfate *in vitro* nor *in vivo,* even when they were applied with nano-intercalated Rh[82]. More recent investigations have examined naturally occurring sulfur donors from garlic and onion that have lower Rh dependence. These sulfur donors demonstrate superior sulfur donor reactivity to thiosulfate. As a result of these investigations, an advanced formulation and superior therapeutic antidotal protection from intramuscularly administered methylpropyl trisulfide was reported in a mice model[87]. Very recent investigations are focused on other garlic compounds as sulfur donors that can improve the ancient, but still clinically used thiosulfate + nitrite combination (Nithiodote™). Publications of the results of these investigations are presently in progress.

Mystery of nitrite/nitric oxide: Turning point in mechanism of CN antagonism: The traditional nitrite theory of MetHgb formation provided a simple explanation of the role of nitrites (sodium nitrites and amyl nitrites) in CN antagonism: Nitrites produce MetHgb; since MetHgb has higher affinity to CN than Cyt c oxidase, MetHgb removes CN from the binuclear heme center (Fea3-CuB) of Cyt c Oxidase, and the mitochondrial electron transport chain is able to return to its job of transferring electrons to oxygen and generating ATP. In this picture nitrites act as indirect CN scavengers[19,88]. When the nitrite + thiosulfate combination is applied, the CN that nitrite displaces reacts with thiosulfate to form SCN that is excreted in the urine[89,90]. However, additional research revealed that the blood MetHgb content needed to be around 15% to effectively antagonize CN[91]. However, when the recommended amyl nitrite dose is applied, only about 5%-7% of Hgb is oxidized to MetHgb, and at these low doses amyl nitrite still acts as an efficacious CN antidote.

The fact that rapid onset of antidotal efficacy by nitrites could not be explained by MetHgb formation suggested one or more additional therapeutic mechanisms. A turning point in understanding this mystery came when the mitochondrial NO synthase was discovered and the function of NO as a regulator in the electron transport chain was characterized[92].

NO regulates the conversion of oxygen to water by Cyt c Oxidase as follows: Ferri-heme a3 takes up electrons and is reduced to ferro-heme a3, NO enters its active site pocket before oxygen does, a nitrosyl-ferro-heme a3 derivative is formed that reacts with dioxygen. NO then dissociates from ferro-heme a3 (rate limiting step), and in the presence of an additional electron donor, nitrite is formed *via* an intermediate peroxynitrite. The cycle's last step is the conversion of peroxynitrite to nitrite and water. In this way, the cycle results in the reduction of oxygen by NO to form water and nitrite. When CN enters the mitochondria, it binds to Cyt c Oxidase. NO can alter the CN binding to Cyt c Oxydase and displace CN from the Cyt c Oxidase's binding site thus restoring its availability for Oxygen binding[93,94]. Pearce *et al*[95] 2003, suggested the reversal of CN inhibition of Cyt c Oxidase by NO occurs in the presence of excess reduced (ferro cyt c Oxidase) and oxygen. They followed the CN substitution by NO in the ferri-heme a3, through a 5-coordinate structure by electron paramagnetic resonance spectroscopy. They stated that NO does not simply act as a reversibly bound competitive inhibitor, but it is also an auxiliary substrate that is consumed and converted to readily releasable nitrite. The displaced CN may then be converted to SCN, or be scavenged by the circulating MetHgb. When external nitrite is added, it boosts the availability of the auxiliary substrate for the Cyt c Oxidase. Along with the NO and MetHgb mechanisms, the vasodilation effects of nitrites also contribute to the their antidotal effects against CN. These results by the Pearce *et al*[95] have provided strong evidence toward solving the puzzle of the mechanisms by which nitrite/NO antagonize CN.

The regulatory effect of NO on Cyt c Oxidase became a well-studied area[96,97]. The signaling molecule NO is generated endogenously from L-Arginine by nitric oxide synthase (NOS)[98]. In CN intoxication, due to the histotoxic hypoxia and lactic acidosis, the NOS activity is decreased, and an exogenous NO source is necessary to protect the cellular respiration. Exogenous nitrites can be transformed to NO even in the condition of

hypoxia[99,100].

Studies on isolated cells (where no Hgb/MetHgb) confirmed that NO inhibits CN by displacing it from Cyt c Oxidase[94]. When the NO donor S-nitroso-N-acetyl-DL-penicillamine was applied to the CN inhibited Cyt c Oxydase, CN was replaced by NO. However, when a NO scavenger 2-phenyl-4,4,5,5-teramethylimidazoline-1-oxy-3 oxide (PTIO) was present, the CN antagonism was blocked, providing strong experimental support for the role of NO as a CN displacement agent[96]. In 2010, Leavesley *et al*[101] used a selective NO scavenger, PTIO to explore CN inhibition by nitrites. In the presence of CN, both the PTIO consumption and the Cyt c Oxidase were inhibited. However, nitrite pretreatment reversed the CN inhibition of NO production and Cyt c Oxidase activity. Conversely, pretreatment with the NO scavenger PTIO negated the ability of the nitrites to antagonize CN. Cumulatively, these studies conclude that a key mechanism of CN antagonism by nitrites involves the generation of NO that competitively displaces CN from Cyt c Oxydase. The NO mechanism runs parallel to the MetHgb mechanism. The formation of each of these species, NO and MetHgb, plays an independent role in the *in vivo* antagonism of CN by nitrites.

Modern theory for old molecules (Nitrites): In 2013 Cambal *et al*[102] compared CN antidotal effects, and blood NO concentration when equimolar amounts of sodium nitrite and amyl nitrite were given to mice intraperitoneally or by inhalation after sub lethal CN doses. They reported that the toxic effects of iso-amyl alcohol formation from amyl nitrite, and the more efficient antidotal effects by sodium nitrite favored the use of sodium nitrite over iso-amyl nitrite. Agreeing with prior studies the authors noted that MetHgb formation was insufficiently rapid to explain the full antidotal effects of amyl nitrite[103]. They also noted that the sodium nitrite maintained efficacy even when MetHgb formation was suppressed. These studies suggested that the NO mechanism was primary and MetHgb formation secondary to the antidotal action of these nitrites. The authors also discussed the complexity of experimental interpretation when interferences due to anesthesia are present.

When the CN antidotal effects of the clinically used anti-angina drug isosorbide dinitrate (ISDN) were reported, NO formation was declared as the major mechanism of its CN antidotal efficacy. It was reported that ISDN has potential advantages over sodium nitrite because it is relatively non-toxic, and is less likely to form MetHgb[104].

Present therapies and recent research investigations in the United States

For treating CN poisoning, multiple antidotes exist and vary in regional availability: MetHgb generators (*e.g.,* sodium nitrite, amyl nitrite, and dimethyl aminophenol), sulfur donors (*e.g.,* sodium thiosulfate and glutathione), and direct binding agents (*e.g.,* hydroxocobalamin and dicobalt edetate). All currently marketed antidotes appear to be effective. In the United States, sodium nitrite and sodium thiosulfate and hydroxocobalamin are used as cyanide antidotes, while in France and several other European countries, only hydroxocobalamin is favored[105]. Antidotal mechanisms include chelation, formation of stable, less toxic complexes, MetHgb induction, and sulfane sulfur reaction with endogenous Rh enzyme. Research with the goal of finding new, safer and more effective cyanide antidotes continues[106].

Table 2 Novel cyanide therapies in development for rapid IM delivery in mass casualty situations

Novel therapy	Mechanism of action	Older analog
Cobinamide	Direct CN Scavenger	Hydroxocobalamin
Sulfanegen	CN Transformer (Leverages the mercaptopyruvate sulfur transferase enzyme)	Mercaptopyruvate
Next generation sulfane sulfur donors	CN Transformer (Leverages the rhodanese enzyme)	Thiosulfate

CN: Cyanide.

The two currently FDA approved CN countermeasures, Nithiodote™ (sodium nitrite and sodium thiosulfate) and Cyanokit® (hydroxocobalamin), each have limitations [*e.g.,* sodium nitrite: intravenous (IV) administration, hypotension, methemoglobinemia; thiosulfate: IV administration, slow onset of action; hydroxocobalamin: IV administration, large volume]. Furthermore, the common requirement for IV administration renders broad use of these CN countermeasures unrealistic in a mass CN exposure event. The distinct need remains to develop a CN countermeasure suitable for mass casualties. Recent investigation efforts focus on developing efficient, easy to administer (*e.g.,* intramuscular) CN countermeasures, which may also be used in combination with other new generation countermeasures. Table 2 shows the classification of recent CN antidotes under development in the United States related to the two present CN therapies of Cyanokit® and Nithiodote™.

Hydroxocobalamin

Hydroxocobalamin is a vitamin B derivative known as vitamin B_{12a}. The compound is a hygroscopic, odorless, dark red crystalline powder which is freely soluble in water and ethanol, and practically insoluble in acetone and diethyl ether. It is the hydroxylated active form of vitamin B_{12} differing in that hydroxocobalamin has a hydroxo moiety linked to a cobalt ion while cyanocobalamin, known as vitamin B_{12} has a cyano moiety. The latter is used to treat pernicious anemia while hydroxocobalamin, based on its strong cyanide binding ability is a potent antidote against cyanide. Hydroxocobalamin has a large molecular weight and a trivalent

cobalt ion that is coordinated by a tetrapyrrol ring. Its mode of action is attributed to its ability to form cyanocobalamin through binding cyanide ion by substituting the aforementioned hydroxo ligand[107,108].

Its pharmacokinetic properties can be characterized by significant plasma protein binding and the formation of various cobalamin-(Ⅲ) complexes after intravenous administration. Free and total cobalamin-(Ⅲ) complexes have half-lives of 26-31 h and overall urinary excretion accounts for 60%-70% of the administered dose[108].

The first reports of its antidotal efficacy were documented in 1952 when an experimental cyanide poisoning of mice was conducted. Ever since, hydroxocobalamin has proved its efficacy in combating cyanide intoxication both in animal models and humans. Mice, rats, guinea pigs, beagle dogs and Yorkshire pigs are some of the species that were included in hydroxocobalamin studies. Alongside the large variety of species included in the investigations various administration methods, such as intravenous, intraperitoneal and intracerebral micro dialysis were also tested. The studies also included pre- and post-poisoning set-ups and cyanide intoxications of various natures including inhalation and parenteral[109-114].

The efficacy of hydroxocobalamin was also seen in human poisonings. In these cases the antidotal effect of the compound, although originally developed as a monotherapy was seen either alone or in combination with other agents, such as 100% oxygen, sodium nitrite and sodium thiosulfate. The therapy was applied in cyanide poisonings originating from various sources including ingestion (*e.g.,* cyanide salts and hydrogen cyanide) and inhalation (*e.g.,* smoke). Survival rate depended on many factors but most notably hydroxocobalamin therapy was especially helpful when administered before anoxic brain damage occurred due to the cardiac arrest of the patients[114-116].

Hydroxocobalamin is available as an United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) approved formulation. The development of the medication was initiated in the 1980s based on request from the Fire Brigade of Paris and antipoisoning center of Paris. The product was approved in France in 1996 and approved in the United States fast track in 2006, followed by EMA approval in 2007. The antidotal kit comprises two glass vials. Each vial contains 2.5 g of hydroxocobalamin in the form of lyophilized powder, a transfer spike for reconstitution, and an intravenous tubing system. Prior to administration the product is reconstituted with 100 mL of preferably 0.9% sodium chloride solution for injection, or lactated Ringer or 5% glucose solution for injection. The mixture is shaken and then administered intravenously over a period of 7.5 min. Hydroxocobalamin has shown itself to be an effective CN antidote *via* intravenous administration. In the pivotal studies which due to ethical reasons were not controlled studies but included patients with suspected or known cyanide-poisoning a survival rate of 58% was seen[108,117].

Cobinamide

Cobinamide is the penultimate precursor in the biosynthesis of cobalamin, lacking the dimethylbenzimidazole nucleotide tail coordinated to the cobalt atom in the lower axial position.

Thus, whereas cobalamin has only an upper ligand binding site, cobinamide has two - both an upper and a lower - ligand binding sites. Moreover, the dimethylbenzimidazole group has a negative trans effect on the upper binding site, thereby reducing cobalamin's affinity for ligands. As a result of these cobinamide has a higher affinity with an overall K_a of 10^{22} mol/L for cyanide ion (binding affinity for first cyanide ion is 10^{14} mol/L and for second ion is 10^{8} mol/L) than cobalamin; for the sake of comparison K_a is 10^{12} mol/L for cobalamin. On the basis of K_a values cobinamide should be a more effective cyanide detoxifying agent than cobalamin[118]. Furthermore, from the aspect of antidote formulation an advantageous feature of cobinamide is that it is at least five times more water soluble (as aquohydroxocobinamide) than hydroxocobalamin[119].

As *in vitro* affinities are not always in line with the *in vivo* conditions (*e.g.,* intracellular proteins may bind cobinamide and cobalamin to varying degrees), many *in vivo* investigations on cell lines and animal models were carried out to examine the cyanide detoxifying properties of cobinamide and to compare it with cobalamin and other antidotes.

The ability of equimolar doses of cobinamide and hydroxocobalamin to reverse the effects of cyanide exposure in New Zealand white rabbits (n = 16) was investigated by Brenner *et al*[119]. CN toxicity was induced by intravenous infusion and the animals were monitored continuously noninvasively by diffuse optical spectroscopy.

Rabbits were divided into three groups: controls (n = 5) received intravenous saline, 6 rabbits were treated with intravenous hydroxocobalamin, and 5 rabbits with intravenous cobinamide following CN exposure. At the end of the sodium cyanide (NaCN) infusion (10 mg in 60 cc normal saline for 60 min), 5 cc of normal saline or 0.0816 millimoles of cobinamide or hydroxocobalamin dissolved in 5 cc of normal saline were infused over 30 s. Cobinamide caused significantly faster and more complete recovery of oxy- and deoxyhemoglobin concentrations in CN-exposed animals over hydroxocobalamin with a recovery time constant of 13.8 ± 7.1 min.

Broderick *et al*[118] found cobinamide to be several-fold more effective than cobalamin in reversing CN inhibition of oxidative phosphorylation in mammalian cells; in rescuing mammalian cells and Drosophila melanogaster from cyanide toxicity; and in reducing CN inhibition of Drosophila Malpighian tubule secretion. Cobinamide was as effective when administered up to 5 min post-CN exposure as when used pre-exposure for prophylaxis[118].

Currently marketed antidotal formulations for CN poisoning must be given by IV administration, limiting their use in treating mass casualties. It is advantageous that cobinamide could be delivered by other routes, *e.g.,* oral ingestion, inhalation, too[118,120].

Cobinamide was studied in both an inhaled and intraperitoneal model of CN poisoning in mice by Chan *et al*[120]. They found cobinamide more effective than hydroxocobalamin, sodium thiosulfate, sodium nitrite, and the combination of sodium thiosulfate-sodium nitrite. Compared to hydroxocobalamin, cobinamide was 3 and 11 times more potent in the intraperitoneal and inhalation models, respectively.

The use of intramuscular cobinamide sulfite to reverse CN toxicity-induced physiologic changes in a sub lethal CN exposure animal model was investigated by Brenner *et al*[121]. New Zealand white rabbits (n = 11) were given 10 mg sodium cyanide intravenously over 60 min. To follow the effect of antidote, tissue oxy- and deoxyhemoglobin concentrations were monitored using quantitative diffuse optical spectroscopy and continuous-wave near-infrared spectroscopy. After completion of the CN infusion, the rabbits were treated intramuscularly with cobinamide sulfite (n = 6) or vehicle (controls, n = 5). Intramuscular administration led to rapid absorption of cobinamide and the molecule was extremely effective at reversing the physiologic effects of CN. Recovery time to 63% of their baseline values in the central nervous system occurred within a mean of 1032 min in the control group and 9 min in the cobinamide group, with a difference of 1023 min. In muscle tissue, recovery times were 76 and 24 min, with a difference of 52 min[121].

When hydroxocobalamin and cobinamide were compared on a nonventillated swine model it was reported that both hydroxocobalamin and cobinamide rescued severely CN-poisoned swine from apnea, however, the dose of cobinamide was one fifth that of hydroxocobalamin to get the same protection[122].

In another study in which cobinamide sulfite was administered *via* intramuscular injection, cobinamide was rapidly absorbed. Mice recovered from a lethal dose of CN even when cobinimide was injected after they had been apneic for over 2 min. Cobinamide sulfite at doses up to 2000 mg/kg exhibited no clinical toxicity[120].

Broderick *et al*[123] observed that cobinamide is highly effective in neutralizing CN ions released by nitroprusside in cultured mammalian cells, Drosophila melanogaster, and mice. Sodium nitroprusside is used to treat hypertensive emergencies and acute heart failure. It acts by releasing NO, a highly potent vasodilator, but for each NO molecule released, five cyanide ions are released, too; thus limiting the safe use of this therapy. To avoid this side effect a CN scavenger could be beneficial when administering nitroprusside. It was reported that cobinamide could neutralize nitroprusside-released CN without having any effect on nitroprusside-released NO, thus it could be a valuable adjunct to nitroprusside therapy[123].

It is worth mentioning that in addition to cyanide, cobinamide is capable of reacting with NO. However, the binding constant of cobinamide is substantially lower for NO than it is for cyanide ($\approx 10^{22}$ mol/L for cyanide; $\approx 10^{10}$ mol/L for NO)[124]. On this basis when given in excess of available CN, cobinamide may potentially induce vasoconstriction and increase blood pressure, as has also been observed with hydroxocobalamin[125].

It can be concluded that cobinamide and its sulfite-salt are effective cyanide detoxifying agents that have the potential to serve as CN antidotes for smoke inhalation victims and persons exposed to CN used as a weapon of mass destruction.

Sulfanegen

Sulfanegen is the water-soluble prodrug of 3-mercaptopyruvate that was developed in the early 1990's to overcome the problem of the relatively low serum stability of the sulfur donor 3-mercaptopyruvate[126]. Sulfanegen is a dimer that dissociates non-enzymatically in physiological systems to two equivalents of the monomer 3-mercaptopyruvate. The endogenous enzyme 3-mercaptopyruvate sulfur transferase (3-MPST) catalyzes the transfer of reactive sulfane sulfur from 3-mercaptopyruvate to CN resulting in the formation of thiocyanate and pyruvate[127,128]. Compared to Rh, the 3-MPST enzyme is available in both the mitochondria and cytoplasm, whereas rhodanese is present only in the mitochondria of hepatic and renal tissues. It was shown that a high amount of 3-MPST exists in the tissue of the brain, specifically in the cerebellum. This strong presence of detoxifying enzyme in the brain holds therapeutic promise, because much of the damage from CN intoxication is localized in the heart and brain.

The other potential advantage with sulfanegen is the fact that it exerts its effects in less than 3 min. With hydroxocobalamin, a fifteen-minute intravenous infusion is required to deliver a standard dose. The discovery of the highly water-soluble sulfanegen triethanolamine for development as an intramuscular injectable antidote was reported by Patterson *et al*[129]. The potential of intramuscular and intravenous sulfanegen sodium treatment to reverse CN effects was evaluated in a rabbit model (n = 35). Changes associated with CN exposure and reversal were monitored by diffuse optical spectroscopy and continuous wave near infrared spectroscopy. Sulfanegen sodium was shown to reverse the effects of CN exposure on oxy- and deoxyHgb rapidly, significantly faster than in case of control animals. Red blood cell CN levels also returned to normal levels faster with both intramuscular and intravenous sulfanegen sodium treatment than with control treatments[126].

Severe CN toxicity - occurrence of severe lactic acidosis accompanied by significant elevation in blood CN levels - was induced in juvenile pig models to demonstrate the CN antagonism capability of sulfanegen[127]. Anesthetized pigs (n = 8) received a high-dose intravenous infusion of sodium nitroprusside SNP (100 mg/h) for 2 h to induce CN toxicity. Then, four

pigs received 3 doses of sulfanegen sodium (2.5 g *i.v.*) and four pigs received placebos. Administration of the sulfanegen antidote resulted in progressively significant reduction in blood lactate and CN levels with 100% survival ($P < 0.05$), whereas the placebo-treated pigs deteriorated and did not survive ($P < 0.05$). In another group of pigs ($n = 6$) severe CN toxicity was induced by NaCN and at peak toxicity (value determined during preliminary measurements), the animals were given sodium sulfanegen (2.5 g *i.v.*) followed by a repeat dose 60 min later in surviving animals. Without sulfanegen the NaCN injection used in this study resulted in CN toxicity, accompanied by severe lactic acidosis, and mortality in all the pigs. Sodium sulfanegen reversed NaCN-toxicity and prevented mortality in all the pigs treated with this antidote[127].

The combination of cobinamide and sulfanegen was explored by Chan *et al*[120] using a non-lethal and two different lethal models - a CN injection and a CN inhalation - of CN poisoning in mice. The effect of the two antidotes was found to be at least additive when used together in all the models used in this study. At doses where all animals died with either drug alone, the combination yielded 80% and 40% survival in the injection and inhalation models, respectively. Similarly, drug doses that yielded only 40% survival with either drug alone, yielded 80% and 100% survival with the combination therapy in the injection and inhalation models, respectively[120].

New sulfur donors

As indicated earlier, very recent investigations in the Petrikovics lab have focused on naturally occurring sulfur donors with lower Rh dependence (scavenger type mechanism), that have superior sulfur donor reactivity to thiosulfate. These sulfur donors were first extracted from garlic compounds and show great promise as successors to the ancient antidote thiosulfate, which is still clinically used in the thiosulfate + nitrite combination (Nithiodote™). Publications of the results of these investigations are presently in progress.

CONCLUSION

Table 1 shows the recent therapies in the US and some European countries, and Table 2 indicates the relations of the new generation CN antidotes to the recent CN therapies in the US. The roots of the present clinically employed therapies reach back to research efforts initiated in the early 20th century. CN intoxication from suicides, homicides, fires, industrial accidents, and potential terrorist attacks, presents a tremendous need for new, rapidly acting and efficient tools to antagonize/treat the toxic and lethal effects of CN in both isolated and mass casualty scenarios.

The lead new generation CN countermeasure cobinamide is a successor of the ancient, but still employed therapy of hydroxocobalamin. Sulfanegen is a next generation successor of mercaptopyruvate. The new generation of sulfane sulfur donors are successors of the classic sulfur donor sodium thiosulfate. These novel sulfane sulfur donors are organo-sulfur molecules that were originally found in garlic and onion. They have other potentially positive health effects[130] and work efficiently against CN intoxication without requiring the catalytic mediation of sulfurtransferases.

New combinations, formulations and nano-delivery systems of these next generation antidotes with enhanced bioavailability, and efficacy, provide strong hope for the gradual replacement of present therapies with improved new drugs and therapies for CN intervention.

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REFERENCES

1 **Solomon P**, Vanden Bout P, Carilli C, Guelin M. The essential signature of a massive starburst in a distant quasar. *Nature* 2003; **426**: 636-638 [PMID: 14668856 DOI: 10.1038/nature02149]
2 **Lieberei R**, Biehl B, Giesemann A, Junqueira NT. Cyanogenesis inhibits active defense reactions in plants. *Plant Physiol* 1989; **90**: 33-36 [PMID: 16666758 DOI: 10.1104/pp.90.1.33]
3 **Møller BL**. Functional diversifications of cyanogenic glucosides. *Curr Opin Plant Biol* 2010; **13**: 338-347 [PMID: 20197238 DOI: 10.1016/j.pbi.2010.01.009]
4 **Zagrobelny M**, Bak S, Olsen CE, Møller BL. Intimate roles for cyanogenic glucosides in the life cycle of Zygaena filipendulae (Lepidoptera, Zygaenidae). *Insect Biochem Mol Biol* 2007; **37**: 1189-1197 [PMID: 17916505 DOI: 10.1016/j.ibmb.2007.07.008]
5 **Sadoff L**, Fuchs K, Hollander J. Rapid death associated with laetrile ingestion. *JAMA* 1978; **239**: 1532 [PMID: 633565 DOI: 10.1001/jama.1978.03280420068022]
6 **Eisler R**. Handbook of Chemical Risk Assessment: Health Hazards to Humans, Plants, and Animals. *Organics* 2000; **2**: 903-959
7 **Hall AH**, Kulig KW, Rumack BH. Suspected cyanide poisoning in smoke inhalation: complications of sodium nitrite therapy. *J Toxicol Clin Exp* 1989; **9**: 3-9 [PMID: 2746547]
8 **Alarie Y**. The toxicity of smoke from polymeric materials during thermal decomposition. *Annu Rev Pharmacol Toxicol* 1985; **25**: 325-347 [PMID: 3890706 DOI: 10.1146/annurev.pa.25.040185.001545]
9 **Baud FJ**, Barriot P, Toffis V, Riou B, Vicaut E, Lecarpentier Y, Bourdon R, Astier A, Bismuth C. Elevated blood cyanide concentrations in victims of smoke inhalation. *N Engl J Med* 1991; **325**: 1761-1766 [PMID: 1944484 DOI: 10.1056/NEJM199112193252502]
10 **Lockwood A**, Patka J, Rabinovich M, Wyatt K, Abraham P. Sodium nitroprusside- associated cyanide toxicity in adult patients- fact or fiction? A critical review of the evidence and clinical relevance. *Open Access J Clin Trials* 2010; **2**: 133-148 [DOI: 10.2147/OAJCT.S7573]
11 **Aitken D**, West D, Smith F, Poznanski W, Cowan J, Hurtig J, Peterson E, Benoit B. Cyanide toxicity following nitroprusside induced hypotension. *Can Anaesth Soc J* 1977; **24**: 651-660 [PMID: 589503 DOI: 10.1007/BF03006709]
12 **Fleming CA**, Cromberg G. The extraction of gold from cyanide solutions by strong- and weak-base anion-exchange resins. *J S Afr Inst Min Metall* 1984; **84**: 125-137
13 **Borowitz JL**, Kanthasamy AG, Isom GE. Toxicodynamics of cyanide. In: Chemical Warfare Agents. Somani SM, editor. San Diego: Academic Press, 1992: 209-236
14 **Blake J**. Observations on the physiological effects of various agents introduced into the circulation as indicated by the hemodynamometer. *Edin Med Surg J* 1839; **51**: 330-345
15 **Chen KK**, Rose CL, Clowes GHA. Methylene Blue, Nitrites and

Sodium Thiosulfate Against Cyanide Poisoning. *Proc Soc Exp Biol Med* 1933; **31**: 250-252 [DOI: 10.3181/00379727-31-7079P]
16 **Chen KK**, Rose CL. Nitrite and thiosulfate therapy in cyanide poisoning. *J Am Med Assoc* 1952; **149**: 113-119 [PMID: 14917568 DOI: 10.1001/jama.1952.02930190015004]
17 **Sorbo BH**. Crystalline rhodanese. I. Purification and physicochemical examination. *Acta Chem Scand* 1953; **7**: 1129-1136
18 **Westley J**. Rhodanese and the sulfane pool. In: Enzymatic Basis of Detoxification. Jakoby WB, editor. London: Academic Press, 1980: 245-262
19 **Way JL**. Cyanide antagonism. *Fundam Appl Toxicol* 1983; **3**: 383-386 [PMID: 6416915 DOI: 10.1016/S0272-0590(83)80009-8]
20 **Schubert J**, Brill WA. Antagonism of experimental cyanide toxicity in relation to the in vivo acitivity of cytochrome oxidase. *J Pharmacol Exp Ther* 1968; **162**: 352-359 [PMID: 4299107]
21 **Baskin SI**. Zyklon. Baumel JT, Laqueur W, editors. The Holocaust Encyclopedia. Yale: Yale University Press, 2001: 716-719
22 **Baskin SI**. Cyanide Poisoning. Zajtchuk MC, Bellamy RF, editors. Medical Aspects of Chemical and Biological Warfare. Washington, DC: Office of the Surgeon General, Department of the Army, 1997: 271-282
23 **Sidell FR**, Takafuji ET, Franz DR, editors. Medical Aspects of Chemical and Biological Warfare. Washington, DC: Office of the Surgeon General, Department of the Army, 1997
24 **Brennan RJ**, Waeckerle JF, Sharp TW, Lillibridge SR. Chemical warfare agents: emergency medical and emergency public health issues. *Ann Emerg Med* 1999; **34**: 191-204 [PMID: 10424921 DOI: 10.1016/S0196-0644(99)70229-5]
25 **Wolnik KA**, Fricke FL, Bonnin E, Gaston CM, Satzger RD. The Tylenol tampering incident - tracing the source. *Anal Chem* 1984; **56**: 466A-470A, 474A [PMID: 6711821]
26 **Keilin D**. Cytochrome and respiratory enzymes. *Proc R Soc Ser B* 1929; 104: 206-252 [DOI: 10.1098/rspb.1929.0009]
27 **Borak J**. Pharmacologic mechanism of antidotes in cyanide and nitrile poisoning. *J Occup Environ Med* 1995; **37**: 793-794 [PMID: 7552462]
28 **Okolie NP**, Osagie AU. Differential effects of chronic cyanide intoxication on heart, lung and pancreatic tissues. *Food Chem Toxicol* 2000; **38**: 543-548 [PMID: 10828506 DOI: 10.1016/S0278-6915(00)00020-X]
29 **Vick JA**, Froehlich HL. Studies of cyanide poisoning. *Arch Int Pharmacodyn Ther* 1985; **273**: 314-322 [PMID: 2860882]
30 **Karalliedde L**, Wheeler H, Maclehose R, Murray V. Possible immediate and long-term health effects following exposure to chemical warfare agents. *Public Health* 2000; **114**: 238-248 [PMID: 10962584 DOI: 10.1038/sj.ph.1900659]
31 **Osuntokun BO**. A degenerative neuropathy with blindness and chronic cyanide intoxication of dietary origin. The evidence in Nigerians. In: Toxicology in the Tropics. Smith RL, Bababunmi EA, editors. London: Taylor and Francis Publishers, 1980: 16-79
32 **Hall AH**, Rumack BH. Clinical toxicology of cyanide. *Ann Emerg Med* 1986; **15**: 1067-1074 [PMID: 3526995 DOI: 10.1016/S0196-0644(86)80131-7]
33 **Laforge M**, Buneaux F, Houeto P, Bourgeois F, Bourdon R, Levillain P. A rapid spectrophotometric blood cyanide determination applicable to emergency toxicology. *J Anal Toxicol* 1994; **18**: 173-175 [PMID: 8065128 DOI: 10.1093/jat/18.3.173]
34 **Darr RW**, Capson TL, Hileman FD. Determination of hydrogen cyanide in blood using gas chromatography with alkali thermionic detection. *Anal Chem* 1980; **52**: 1379-1381 [PMID: 6255825 DOI: 10.1021/ac50058a058]
35 **Moriya F**, Hashimoto Y. Potential for error when assessing blood cyanide concentrations in fire victims. *J Forensic Sci* 2001; **46**: 1421-1425 [PMID: 11714154 DOI: 10.1520/JFS15165J]
36 **Ballantyne B**. In vitro production of cyanide in normal human blood and the influence of thiocyanate and storage temperature. *Clin Toxicol* 1977; **11**: 173-193 [PMID: 891111 DOI: 10.3109/15563657708989832]
37 **Baskin SI**, Petrikovics I, Platoff GE, Rockwood GA, Logue BA. Spectrophotometric Analysis of the Cyanide Metabolite 2-Aminothiazoline-4-Carboxylic Acid (ATCA). *Toxicol Mech Methods* 2006; **16**: 339-345 [PMID: 20021033 DOI: 10.1080/15376520600616933]
38 **Yu JC**, Martin S, Nasr J, Stafford K, Thompson D, Petrikovics I. LC-MS/MS analysis of 2-aminothiazoline-4-carboxylic acid as a forensic biomarker for cyanide poisoning. *World J Methodol* 2012; **2**: 33-41 [PMID: 25237615 DOI: 10.4329/wjm.v2.i5.1]
39 **Logue B**, Hinkens D, Baskin S, Rockwood G. The analysis of cyanide and its breakdown products in biological samples. *Crit Rev Anal Chem* 2010; **40**: 122-147 [DOI: 10.1080/10408340903535315]
40 **MacNamara BP**. Estimation of the toxicity of hydrocyanide acid vapors in man (Edgewood Arsenal Technical Report Number EB-TR-76023). Aberdeen Proving Ground, MD: U.S. Biomedical Laboratory, 1976
41 **Sylvester DM**, Hayton WL, Morgan RL, Way JL. Effects of thiosulfate on cyanide pharmacokinetics in dogs. *Toxicol Appl Pharmacol* 1983; **69**: 265-271 [PMID: 6868088 DOI: 10.1016/0041-008X(83)90307-1]
42 **Moore SJ**, Norris JC, Walsh DA, Hume AS. Antidotal use of methemoglobin forming cyanide antagonists in concurrent carbon monoxide/cyanide intoxication. *J Pharmacol Exp Ther* 1987; **242**: 70-73 [PMID: 2886639]
43 **Christel D**, Eyer P, Hegemann M, Kiese M, Lörcher W, Weger N. Pharmacokinetics of cyanide in poisoning of dogs, and the effect of 4-dimethylaminophenol or thiosulfate. *Arch Toxicol* 1977; **38**: 177-189 [PMID: 578721]
44 **Kiese M**, Weger N. Formation of ferrihaemoglobin with amino phenols in humans for the treatment of cyanide poisoning. *Eur J Pharmacol* 1969; **7**: 97-105 [DOI: 10.1016/0014-2999(69)90170-8]
45 **Szinicz L**, Weger N, Schneiderhan W, Kiese M. Nephrotoxicity of aminophenols: effects of 4-dimethylaminophenol on isolated rat kidney tubules. *Arch Toxicol* 1979; **42**: 63-73 [PMID: 454186 DOI: 10.1007/BF00351825]
46 **Klimmek R**, Fladerer H, Weger N. Circulation, respiration and blood homeostasis in cyanide poisoned dogs after treatment with 4-dimethylaminophenol or cobaltous compounds. *Arch Toxicol* 1979; **43**: 121-133 [DOI: 10.1007/BF00333619]
47 **Lorcher W**, Wegner N. Optimal concentration of ferrihemoglobin for treatment of cyanide poisoning. *Arch Exp Patol Pharmacol* 1971; **270** Suppl: R88
48 **Bhattacharya R**, Jeevaratnam K, Raza SK, Das Gupta S. Protection against cyanide poisoning by the co-administration of sodium nitrite and hydroxylamine in rats. *Hum Exp Toxicol* 1993; **12**: 33-36 [PMID: 8094969 DOI: 10.1177/096032719301200107]
49 **Lang S.** Uber die Umwandlung des Acetonitrils und seiner homologen im Thierkörper. Naunyn- Schmeidebergs. *Arch Pathol Pharmakol* 1894; **34**: 247-258 [DOI: 10.1007/BF01824916]
50 **Pascheles W.** Versuche über die Unwandlung der Cyanverbindungen im Thierkörper. *Arch Pathol Pharmacol* 1984; **34**: 281-288 [DOI: 10.1007/BF01824919]
51 **Kahn M.** Biochemical studies of sulfocyanate. Dissertation: Columbia University, 1912
52 **Lang S.** Studien über Entgiftungs Therapie. I. Über Entgiftung der Blausäure. Naunyn-Schmeidebergs. *Arch Pathol Pharmakol* 1895; **36**: 75-99 [DOI: 10.1007/BF01825016]
53 **Hebting J.** Versuche über Entgiftung der Blausäure durch schwefelabspaltende Substanzen. *Biochem Zeitschri* 1910; **28**: 208-212
54 **Voegtlin C,** Johnson JM, Dyer HA. Biological significance of cystine and glutathione. I. On the mechanism of the cyanide action. *J Pharmacol Exp Ther* 1926; **27**: 467-483
55 **Lang K.** Die rhodanbildung in tierkorper. *Biochem Z* 1933; **259**: 243-256
56 **Sörbo BH.** On the substrate specificity of rhodanese. *Acta Chem Scand* 1953; **7**: 32-37 [DOI: 10.3891/acta.chem.scand.07-0032]
57 **Westley J,** Adler A, Westley L, Nishida C. The sulfur transferases. *Fund Appl Toxicol* 1983; **3**: 377-382 [DOI: 10.1016/S0272-0590(83)80008-6]

58 **Himwich WA**, Saunders JP. Enzymatic conversion of cyanide to thiocyanate. *Am J Physiol* 1948; **153**: 348-354 [PMID: 18872649]

59 **Frankenberg L**, Sörbo B. Effect of cyanide antidotes on the metabolic conversion of cyanide to thiocyanate. *Arch Toxicol* 1975; **33**: 81-89 [PMID: 810115 DOI: 10.1007/BF00353233]

60 **Westley J**. Cyanide and sulfane sulfur. In: Cyanide in Biology. Vennesland B, Conn EC, Knowles CJ, Westley J, Wissing F, editors. London: Academic Press, 1981: 61-76

61 **Jarabak R**, Westley J. Steady-state kinetics of 3-mercaptopyruvate sulfurtransferase from bovine kidney. *Arch Biochem Biophys* 1978; **185**: 458-465 [PMID: 564663 DOI: 10.1016/0003-9861(78)90189-3]

62 **Mushett CW**, Kelley KL, Boxer GE, Rickards JC. Antidotal efficacy of vitamin B12 (hydroxocobalamine) in experimental cyanide poisoning. *Proc Soc Exp Biol Med* 1952; **81**: 234-237 [DOI: 10.3181/00379727-81-19831]

63 **Paulet GL**. Intoxication Cyanhydrique et son Traitment. Pairs: Masson, 1960

64 **Isom G**, Way JL. Cyanide intoxication: protection with cobaltous chloride. *Toxicol Appl Pharmacol* 1973; **24**: 449-456 [PMID: 4704816 DOI: 10.1016/0041-008X(73)90051-3]

65 **Antal J**. Experimentelle Untersuchungen zur Therapie die Cyanvergiftungen. *Ungar Arch Med* 1894; **3**: 117-128

66 **Cittadini A**, Galeotti T, Terranova T. The effect of pyruvate on cyanide-inhibited respiration in intact ascites tumor cells. *Experientia* 1971; **27**: 633-635 [PMID: 5556436 DOI: 10.1007/BF02136930]

67 **Schwartz C**, Morgan RL, Way LM, Way JL. Antagonism of cyanide intoxication with sodium pyruvate. *Toxicol Appl Pharmacol* 1979; **50**: 437-441 [PMID: 516056 DOI: 10.1016/0041-008X(79)90396-X]

68 **Norris JC**, Utley WA, Hume AS. Mechanism of antagonizing cyanide-induced lethality by alpha-ketoglutaric acid. *Toxicology* 1990; **62**: 275-283 [PMID: 2167518 DOI: 10.1016/0300-483X(90)90051-H]

69 **Guth PS**, Sprites MA. Antagonism of cyanide intoxication by chlorpromazine. *Fed Proc* 1958; **17**: 374

70 **Way JL**, Burrows G. Cyanide intoxication: protection with chlorpromazine. *Toxicol Appl Pharmacol* 1976; **36**: 93-97 [PMID: 1273841 DOI: 10.1016/0041-008X(76)90029-6]

71 **Way JL**, Gibbon SL, Sheehy M. Effect of Oxygen on Cyanide Intoxication I. Prophylactic Protection. *J Pharmacol Exp Ther* 1966; **153**: 381-385

72 **Burrows GE**, Liu DH, Way JL. Effect of oxygen on cyanide intoxication. V. Physiologic effects. *J Pharmacol Exp Ther* 1973; **184**: 739-748 [PMID: 4687234]

73 **Isom GE**, Way JL. Effect of oxygen on cyanide intoxication. VI. Reactivation of cyanide-inhibited glucose metabolism. *J Pharmacol Exp Ther* 1974; **189**: 235-243 [PMID: 4823293]

74 **Sheehy M**, Way JL. Effect of oxygen on cyanide intoxication. 3. Mithridate. *J Pharmacol Exp Ther* 1968; **161**: 163-168 [PMID: 5648494]

75 **Way JL**, Sylvester D, Morgan RL, Isom GE, Burrows GE, Tamulinas CB, Way JL. Recent perspectives on the toxicodynamic basis of cyanide antagonism. *Fundam Appl Toxicol* 1984; **4**: S231-S239 [PMID: 6327443 DOI: 10.1016/0272-0590(84)90157-X]

76 **Clemedson CJ**, Hultman HI, Sorbo B. The antidote effect of some sulfur compounds and rhodanese in experimental cyanide poisoning. *Acta Physiol Scand* 1954; **32**: 245-251 [PMID: 13228113 DOI: 10.1111/j.1748-1716.1954.tb01171.x]

77 **Atkinson A**, Rutter DA, Sargeant K. Letter: Enzyme antidote for experimental cyanide poisoning. *Lancet* 1974; **2**: 1446 [PMID: 4140348 DOI: 10.1016/S0140-6736(74)90093-2]

78 **Frankenberg L**. Enzyme therapy in cyanide poisoning: effect of rhodanese and sulfur compounds. *Arch Toxicol* 1980; **45**: 315-323 [PMID: 6934715 DOI: 10.1007/BF00293812]

79 **Way JL**, Leung P, Ray L, Sander C. Erythrocyte encapsulated thiosulfate sulfurtransferase. *Bibl Haematol* 1985; **(51)**: 75-81 [PMID: 3859292]

80 **Way JL**, Cannon EP, Leung P, Davis R, McGuinn DW, Yao C, Chiou C, Naqi A. Antagonism of cyanide intoxication with rhodanese encapsulated within resealed erythrocytes. *Adv Biosci* 1991; **81**: 207-211

81 **Petrikovics I**, Cannon EP, McGuinn WD, Pei L, Pu L, Linder LE, Way JL. Cyanide antagonism with carrier erythrocytes and organic thiosulfonates. *Fund Appl Toxicol* 1995; **24**: 86-93 [DOI: 10.1006/faat.1995.1010]

82 **Petrikovics I**, Baskin SI, Beigel KM, Schapiro BJ, Rockwood GA, Manage AB, Budai M, Szilasi M. Nano-intercalated rhodanese in cyanide antagonism. *Nanotoxicology* 2010; **4**: 247-254 [PMID: 20795898 DOI: 10.3109/17435390903528254]

83 **Baskin SI**, Porter DW, Rockwood GA, Romano JA, Patel HC, Kiser RC, Cook CM, Ternay AL. In vitro and in vivo comparison of sulfur donors as antidotes to acute cyanide intoxication. *J Appl Toxicol* 1999; **19**: 173-183 [PMID: 10362268 DOI: 10.1002/(SICI)1099-1263(199905/06)19:]

84 **Ashani MR**, Mohri M, Chekani M. Effects of garlic (Allium sativum) and its chief compound, allicin, on acute lethality of cyanide in rat. *Comp Clin Pathol* 2006; **15**: 211-213 [DOI: 10.1007/s00580-006-0633-3]

85 **Iciek M**, Bilska A, Ksiazek L, Srebro Z, Włodek L. Allyl disulfide as donor and cyanide as acceptor of sulfane sulfur in the mouse tissues. *Pharmacol Rep* 2005; **57**: 212-218 [PMID: 15886420]

86 **Block E**. The chemistry of garlic and onions. *Sci Am* 1985; **252**: 114-119 [PMID: 3975593 DOI: 10.1038/scientificamerican0385-114]

87 **Kovacs K**, Ancha M, Jane M, Lee S, Angalakurthi S, Negrito M, Rasheed S, Nwaneri A, Petrikovics I. Identification, solubility enhancement and in vivo testing of a cyanide antidote candidate. *Eur J Pharm Sci* 2013; **49**: 352-358 [PMID: 23602996 DOI: 10.1016/j.ejps.2013.04.007]

88 **Isom GE**, Way JL. Effects of oxygen on the antagonism of cyanide intoxication: cytochrome oxidase, in vitro. *Toxicol Appl Pharmacol* 1984; **74**: 57-62 [PMID: 6328698 DOI: 10.1016/0041-008X(84)90269-2]

89 **Hall AH**, Saiers J, Baud F. Which cyanide antidote? *Crit Rev Toxicol* 2009; **39**: 541-552 [PMID: 19650716 DOI: 10.1080/10408440802304944]

90 **Way JL**. Cyanide intoxication and its mechanism of antagonism. *Annu Rev Pharmacol Toxicol* 1984; **24**: 451-481 [PMID: 6428300 DOI: 10.1146/annurev.pa.24.040184.002315]

91 **Bhattacharya R**. Antidotes to cyanide poisoning: present status. *Indian J Pharmacol* 2000; **32**: 94-101

92 **Kanai AJ**, Pearce LL, Clemens PR, Birder LA, VanBibber MM, Choi SY, de Groat WC, Peterson J. Identification of a neuronal nitric oxide synthase in isolated cardiac mitochondria using electrochemical detection. *Proc Natl Acad Sci USA* 2001; **98**: 14126-14131 [PMID: 11717466 DOI: 10.1073/pnas.241380298]

93 **Torres J**, Darley-Usmar V, Wilson MT. Inhibition of cytochrome c oxidase in turnover by nitric oxide: mechanism and implications for control of respiration. *Biochem J* 1995; **312** (Pt 1): 169-173 [PMID: 7492308]

94 **Leavesley HB**, Li L, Prabhakaran K, Borowitz JL, Isom GE. Interaction of cyanide and nitric oxide with cytochrome c oxidase: implications for acute cyanide toxicity. *Toxicol Sci* 2008; **101**: 101-111 [PMID: 17906319 DOI: 10.1093/toxsci/kfm254]

95 **Pearce LL**, Bominaar EL, Hill BC, Peterson J. Reversal of cyanide inhibition of cytochrome c oxidase by the auxiliary substrate nitric oxide: an endogenous antidote to cyanide poisoning? *J Biol Chem* 2003; **278**: 52139-52145 [PMID: 14534303 DOI: 10.1074/jbc.M310359200]

96 **Collman JP**, Dey A, Decreau RA, Yang Y, Hosseini A, Solomon EI, Eberspacher TA. Interaction of nitric oxide with a functional model of cytochrome c oxidase. *Proc Natl Acad Sci USA* 2008; **105**: 9892-9896 [PMID: 18632561 DOI: 10.1073/pnas.0804257105]

97 **Cooper CE**, Brown GC. The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: chemical mechanism and physiological significance. *J Bioenerg Biomembr* 2008; **40**: 533-539 [PMID: 18839291 DOI: 10.1007/s10863-008-9166-6]

98 **Lundberg JO**, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov* 2008; **7**: 156-167 [PMID: 18167491 DOI: 10.1038/nrd2466]

99 **Feelisch M**, Fernandez BO, Bryan NS, Garcia-Saura MF, Bauer S, Whitlock DR, Ford PC, Janero DR, Rodriguez J, Ashrafian H. Tissue processing of nitrite in hypoxia: an intricate interplay of nitric oxide-generating and -scavenging systems. *J Biol Chem* 2008; **283**: 33927-33934 [PMID: 18835812 DOI: 10.1074/jbc.M806654200]

100 **Webb A**, Bond R, McLean P, Uppal R, Benjamin N, Ahluwalia A. Reduction of nitrite to nitric oxide during ischemia protects against myocardial ischemia-reperfusion damage. *Proc Natl Acad Sci USA* 2004; **101**: 13683-13688 [PMID: 15347817 DOI: 10.1073/pnas.0402927101]

101 **Leavesley HB**, Li L, Mukhopadhyay S, Borowitz JL, Isom GE. Nitrite-mediated antagonism of cyanide inhibition of cytochrome c oxidase in dopamine neurons. *Toxicol Sci* 2010; **115**: 569-576 [PMID: 20335280 DOI: 10.1093/toxsci/kfq084]

102 **Cambal LK**, Weitz AC, Li HH, Zhang Y, Zheng X, Pearce LL, Peterson J. Comparison of the relative propensities of isoamyl nitrite and sodium nitrite to ameliorate acute cyanide poisoning in mice and a novel antidotal effect arising from anesthetics. *Chem Res Toxicol* 2013; **26**: 828-836 [PMID: 23536974 DOI: 10.1021/tx400103k]

103 **Cambal LK**, Swanson MR, Yuan Q, Weitz AC, Li HH, Pitt BR, Pearce LL, Peterson J. Acute, sublethal cyanide poisoning in mice is ameliorated by nitrite alone: complications arising from concomitant administration of nitrite and thiosulfate as an antidotal combination. *Chem Res Toxicol* 2011; **24**: 1104-1112 [PMID: 21534623 DOI: 10.1021/tx2001042]

104 **Sun P**, Borowitz JL, Kanthasamy AG, Kane MD, Gunasekar PG, Isom GE. Antagonism of cyanide toxicity by isosorbide dinitrate: possible role of nitric oxide. *Toxicology* 1995; **104**: 105-111 [PMID: 8560488 DOI: 10.1016/0300-483X(95)03152-6]

105 **Amizet L**, Pruvot G, Remy S, Kfoury M. Occupational cyanide poisoning. *BMJ Case Rep* 2011; **2011**: [PMID: 22674698 DOI: 10.1136/bcr.09.2011.4865]

106 **Fincham SM**, Hill GB, Hanson J, Wijayasinghe C. Epidemiology of prostatic cancer: a case-control study. *Prostate* 1990; **17**: 189-206 [PMID: 2235728 DOI: 10.2174/138920112802273182]

107 **Thompson JP**, Marrs TC. Hydroxocobalamin in cyanide poisoning. *Clin Toxicol* (Phila) 2012; **50**: 875-885 [PMID: 23163594 DOI: 10.3109/15563650.2012.742197]

108 **Sanofi Pasteur MSD Ltd**. Summary of Product Characteristics ViATIM. Updated. [accessed 2013 Sept 23]. Available from: URL: http://emc.medicines.org.uk/medicine/7684/SPC/ViATIM/

109 **Reade MC**, Davies SR, Morley PT, Dennett J, Jacobs IC. Review article: management of cyanide poisoning. *Emerg Med Australas* 2012; **24**: 225-238 [PMID: 22672162 DOI: 10.1111/j.1742-6723.2012.01538.x]

110 **Fahmy NR**. Consumption of vitamin B12 during sodium nitroprusside administration in humans. *Anesthesiology* 1981; **54**: 305-309 [PMID: 7212330 DOI: 10.1097/00000542-198104000-00009]

111 **Hatch RC**, Laflamme DP, Jain AV. Effects of various known and potential cyanide antagonists and a glutathione depletor on acute toxicity of cyanide in mice. *Vet Hum Toxicol* 1990; **32**: 9-16 [PMID: 2301155]

112 **Chan A**, Balasubramanian M, Blackledge W, Mohammad OM, Alvarez L, Boss GR, Bigby TD. Cobinamide is superior to other treatments in a mouse model of cyanide poisoning. *Clin Toxicol* (Phila) 2010; **48**: 709-717 [PMID: 20704457 DOI: 10.3109/15563650.2010.505197]

113 **Lawson-Smith P**, Olsen NV, Hyldegaard O. Hyperbaric oxygen therapy or hydroxycobalamin attenuates surges in brain interstitial lactate and glucose; and hyperbaric oxygen improves respiratory status in cyanide-intoxicated rats. *Undersea Hyperb Med* 2011; **38**: 223-237 [PMID: 21877551]

114 **Borron SW**, Baud FJ, Mégarbane B, Bismuth C. Hydroxocobalamin for severe acute cyanide poisoning by ingestion or inhalation. *Am J Emerg Med* 2007; **25**: 551-558 [PMID: 17543660 DOI: 10.1016/j.ajem.2006.10.010]

115 **Breton D**, Jouvet P, de Blic J, Delacourt C, Hubert P. Toxicity of fire smoke. Apropos of 2 pediatric cases. *Arch Fr Pediatr* 1993; **50**: 43-45 [PMID: 8389538]

116 **Houeto P**, Hoffman JR, Imbert M, Levillain P, Baud FJ. Relation of blood cyanide to plasma cyanocobalamin concentration after a fixed dose of hydroxocobalamin in cyanide poisoning. *Lancet* 1995; **346**: 605-608 [PMID: 7651005 DOI: 10.1016/S0140-6736(95)91437-4]

117 **Levy F.** Cyanide poisoning Therapy in the field. CTIF Heatlth Commission International Scientific Journey Mulhouse. [accessed 2013 Jul 18]. Available from: URL: http://www.google.com.hk/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CB4QFjAA&url=http%3A%2F%2Fwww.pompiers68.fr%2Fdownload.php%3Ffil_id%3D219%26nom%3Dctif_cyanide_poisoning_teatment_in_the_field.pdf%26fichier%3Dprivate_files%2Ffile%2Fctif_cyanide_poisoning_teatment_in_the_field.pdf&ei=tYa3VNLdI8mqggTUnIPQBg&usg=AFQjCNEPWgM7D1X_j2nX3jqgYRZDA5op5Q&bvm=bv.83829542,d.eXY&cad=rjt

118 **Broderick KE**, Potluri P, Zhuang S, Scheffler IE, Sharma VS, Pilz RB, Boss GR. Cyanide detoxification by the cobalamin precursor cobinamide. *Exp Biol Med* (Maywood) 2006; **231**: 641-649 [PMID: 16636313]

119 **Brenner M**, Mahon SB, Lee J, Kim J, Mukai D, Goodman S, Kreuter KA, Ahdout R, Mohammad O, Sharma VS, Blackledge W, Boss GR. Comparison of cobinamide to hydroxocobalamin in reversing cyanide physiologic effects in rabbits using diffuse optical spectroscopy monitoring. *J Biomed Opt* 2010; **15**: 017001 [PMID: 20210475 DOI: 10.1117/1.3290816]

120 **Chan A**, Crankshaw DL, Monteil A, Patterson SE, Nagasawa HT, Briggs JE, Kozocas JA, Mahon SB, Brenner M, Pilz RB, Bigby TD, Boss GR. The combination of cobinamide and sulfanegen is highly effective in mouse models of cyanide poisoning. *Clin Toxicol* (Phila) 2011; **49**: 366-373 [PMID: 21740135 DOI: 10.3109/15563650.2011.584879]

121 **Brenner M**, Kim JG, Mahon SB, Lee J, Kreuter KA, Blackledge W, Mukai D, Patterson S, Mohammad O, Sharma VS, Boss GR. Intramuscular cobinamide sulfite in a rabbit model of sublethal cyanide toxicity. *Ann Emerg Med* 2010; **55**: 352-363 [PMID: 20045579 DOI: 10.1016/j.annemergmed.2009.12.002]

122 **Bebarta VS**, Tanen DA, Boudreau S, Castaneda M, Zarzabal LA, Vargas T, Boss GR. Intravenous cobinamide versus hydroxocobalamin for acute treatment of severe cyanide poisoning in a swine (Sus scrofa) model. *Ann Emerg Med* 2014; **64**: 612-619 [PMID: 24746273 DOI: 10.1016/j.annemergmed.2014.02.009]

123 **Broderick KE**, Balasubramanian M, Chan A, Potluri P, Feala J, Belke DD, McCulloch A, Sharma VS, Pilz RB, Bigby TD, Boss GR. The cobalamin precursor cobinamide detoxifies nitroprusside-generated cyanide. *Exp Biol Med* (Maywood) 2007; **232**: 789-798 [PMID: 17526771]

124 **Sharma VS**, Pilz RB, Boss GR, Magde D. Reactions of nitric oxide with vitamin B12 and its precursor, cobinamide. *Biochemistry* 2003; **42**: 8900-8908 [PMID: 12873151 DOI: 10.1021/bi034469t]

125 **Borron SW**, Stonerook M, Reid F. Efficacy of hydroxocobalamin for the treatment of acute cyanide poisoning in adult beagle dogs. *Clin Toxicol* (Phila) 2006; **44** Suppl 1: 5-15 [PMID: 16990189 DOI: 10.1080/15563650600811672]

126 **Brenner M**, Kim JG, Lee J, Mahon SB, Lemor D, Ahdout R, Boss GR, Blackledge W, Jann L, Nagasawa HT, Patterson SE. Sulfanegen sodium treatment in a rabbit model of sub-lethal cyanide toxicity. *Toxicol Appl Pharmacol* 2010; **248**: 269-276 [PMID: 20705081 DOI: 10.1016/j.taap.2010.08.002]

127 **Belani KG**, Singh H, Beebe DS, George P, Patterson SE, Nagasawa HT, Vince R. Cyanide toxicity in juvenile pigs and its reversal by a new prodrug, sulfanegen sodium. *Anesth Analg* 2012; **114**: 956-961 [PMID: 22392971]

128 **Nagasawa HT**, Goon DJ, Crankshaw DL, Vince R, Patterson SE. Novel, orally effective cyanide antidotes. *J Med Chem* 2007; **50**: 6462-6464 [PMID: 18038966 DOI: 10.1021/jm7011497]

129 **Patterson SE**, Monteil AR, Cohen JF, Crankshaw DL, Vince R,

Nagasawa HT. Cyanide antidotes for mass casualties: water-soluble salts of the dithiane (sulfanegen) from 3-mercaptopyruvate for intramuscular administration. *J Med Chem* 2013; **56**: 1346-1349 [PMID: 23301495 DOI: 10.1021/jm301633x]

130 **Rivlin RS**. Historical perspective on the use of garlic. *J Nutr* 2001; **131**: 951S-954S [PMID: 11238795]

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Current *Helicobacter pylori* treatment in 2014

Fatih Ermis, Elif Senocak Tasci

Fatih Ermis, Department of Gastroenterology, Duzce University Faculty of Medicine, 81620 Duzce, Turkey
Elif Senocak Tasci, Department of Internal Medicine, Duzce University Faculty of Medicine, 81620 Duzce, Turkey

Author contributions: Ermis F and Senocak Tasci E contributed equally to this work, generated the tables and figures and wrote the manuscript.

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Correspondence to: Fatih Ermis, MD, Associate Professor, Department of Gastroenterology, Duzce University Faculty of Medicine, Beciyorukler Street, Konuralp, 81620 Duzce, Turkey. fatihermis2@hotmail.com
Telephone: +90-53-34689404

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Abstract

Helicobacter pylori is one of the most commonly seen bacterium worldwide. It's in the etiology of multiple gastrointestinal diseases, ranging from gastritis to gastric carcinoma. The antimicrobial therapies, which are frequently prescribed empirically, are losing their effectivity as a result of the increasing antimicrobial resistance. As the standard triple therapy is now left especially in areas with high-clarithromycin resistance due to decreased eradication rates, quadruple therapies are recommended in most regions of the world. Alternatively, concomitant, sequential and hybrid therapies are used. There is still a debate going on about the use of levofloxacin-based therapy in order to prevent the increase in quinolone resistance. If no regimen can achieve the desired eradication rate, culture-guided individualized therapies are highly recommended. Probiotics, statins and n-acetylcysteine are helpful as adjuvant therapies in order to increase the effectiveness of the eradication therapy. Herein, we focused on different eradication regimens in order to highlight the current *Helicobacter pylori* treatment.

Key words: *Helicobacter pylori*; Eradication; Treatment; Bismuth-quadruple therapy; Sequential therapy; Concomitant therapy; Hybrid therapy

Core tip: In this review, we focused on different treatment regimens used for *Helicobacter pylori* eradication. The worldwide increase in antibiotic resistance, especially clarithromycin, caused change in the preferred initial treatments. The efficiency of bismuth-quadruple therapy, sequential, concomitant and hybrid therapies are emphasized in relation to each other. In addition, adjuvant therapies to increase the efficiency are reviewed. In conclusion, the optimal approach for eradication was found to be the individualized therapy.

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INTRODUCTION

Helicobacter pylori (*H. pylori*), a gram-negative

Table 1 Regimens for *Helicobacter pylori* treatment

Standard triple therapy (7-14 d)	PPI - standard dose, *bid* Clarithromycin - 500 mg, *bid* Amoxicillin - 1 g, *bid*
Bismuth quadruple Therapy (10-14 d)	PPI - standard dose, *bid* Bismuth - standard dose, *qid* Tetracycline - 500 mg, *qid* Metronidazole - 500 mg, *tid*
Sequential therapy (5-d dual therapy followed by a 5-d triple therapy)	Dual therapy; PPI - standard dose, *bid* Amoxicillin - 1 g, *bid* Triple therapy; PPI - standard dose, *bid* Clarithromycin - 500 mg, *bid* Metronidazole - 500 mg, *bid*
Concomitant therapy (7-10 d)	PPI - standard dose, *bid* Clarithromycin - 500 mg, *bid* Amoxicillin - 1 g, *bid* Metronidazole - 500 mg, *bid*
Hybrid therapy (7-d dual therapy followed by a 7-d quadruple therapy)	Dual therapy; PPI - standard dose, *bid* Amoxicillin - 1 g, *bid* Triple therapy; PPI - standard dose, *bid* Amoxicillin - 1 g, *bid* Clarithromycin - 500 mg, *bid* Metronidazole - 500 mg, *bid*
Levofloxacin-based triple therapy (10-d)	PPI - standard dose, *bid* Levofloxacin - 500 mg, *qd* Amoxicillin - 1 g, *bid*
Rifabutin-based triple therapy (7-14 d)	PPI - standard dose, *bid* Amoxicillin - 1 g, *bid* Rifabutin - 150 mg, *qd*
Culture-guided therapy (10-d)	PPI - standard dose, *bid* Bismuth - standard dose, *qid* Two antibiotics selected by antimicrobial sensitivity tests

PPI: Proton pump inhibitor; *bid*: Twice daily; *qd*: Once daily; *qid*: Four times daily; *tid*: Three times daily.

microaerophilic spiral bacillus discovered in 1983, affects nearly 50% of the world's population. While the prevalence is as high as 90% in developing countries, it is below 40% in developed countries, besides Japan[1]. Many studies have revealed a strong relation between the organism infection and gastric disorders, especially functional dyspepsia, peptic ulcer disease, gastric carcinoma and mucosa associated lymphoid tissue-lymphoma[2]. Moreover, extra-digestive diseases are also associated with *H. pylori*; idiopathic thrombocytopenic purpura and idiopathic iron deficiency anemia[3]. Therefore, eradication of *H. pylori* is an important issue, which still remains unsolved. Today, there is still not a single optimal antibiotic treatment for eradication. Herein, we focused on many articles published over the past years on *H. pylori* eradication regimens and their efficacy.

FIRST-LINE THERAPY

In the 90's, Bazzoli *et al*[4] first proposed the clarithromycin based standard triple therapy -clarithromycin, proton pump inhibitor (PPI) plus amoxicillin or metronidazole given for 7-14 d - which then became the gold standard in the treatment of *H. pylori* (Table 1). While the high eradication success (> 80%), optimal safety profile and relative simplicity made this regimen one of the standard of care treatments for first-line eradication of *H. pylori,* the raise in clarithromycin resistance in the 2000's caused a significant decline in the efficacy of this standard regimen[5]. In their study, Lee *et al*[6] reported the factors causing treatment failure as; age ⩾ 50 years, female gender, body mass index < 25 kg/m^2, amoxicillin, and/or clarithromycin resistance by univariate analysis. On the other hand, clarithromycin resistance was the only worthy parameter found by multivariate analysis. Clarithromycin works by interrupting the bacterial protein synthesis and resistance is caused by a mutation in the organism, which prevents the binding of the antibiotic to the ribosome of *H. pylori*[7]. The use of clarithromycin for respiratory and gastrointestinal infections causes the increased resistance rates[8]. High bacterial load, strain types, high gastric acidity and low compliance are the other contributors to eradication failure[9]. New evidence suggests that treatment failure may be due to the ability of the bacterium to control T-cell responses[10]. The clarithromycin resistance rate is variable in different parts of the world; the most recent report from European Helicobacter Study Group stated the primary resistance rate for clarithromycin as 17.5%[11]. The threshold of 15%-20% prevalence is used to classify low or high clarithromycin resistance[12]. That determines the approach to *H. pylori* eradication.

First-line treatment may be split into two groups; treatment in areas with low clarithromycin resistance and in areas with high clarithromycin resistance. The most frequently used regimen in areas with low clarithromycin resistance is standard triple therapy while bismuth-containing quadruple therapy is also an alternative. The duration of therapy is suggested as 14 d by meta-analyses with eradication rates 5% higher than those with 7 d[13]. A Cochrane systemic review looked at 75 eligible studies and found that eradication rate was 83.5% for PPI, amoxicillin and clarithromycin; for PPI, clarithromycin and metronidazole the rate was 68.6% and for PPI, amoxicillin and metronidazole it was found to be 82%; each therapy lasting 14 d[14].

Increased dose of PPI, as strong suppression of acid secretion is essential for the stability and biological activity of antibiotics, or the increased length of treatment are factors improving the efficacy rates. A meta-analysis showed a greater beneficial effect with a double dose of esomeprazole while another meta-analysis showed lower cure rates in hosts who are extensive PPI metabolizers (depending on their cytochrome P450 status which PPI function relies on)[13]. Taking this into account, a study found out that administration of a PPI four times daily with amoxicillin or metronidazole in clarithromycin resistance, may result with eradication rate of 98%[15]. In a study done by Altintas *et al*[16] comparing the efficacy of different proton pump inhibitors - omeprazole, lansoprazole and pantoprazole - in combination with amoxicillin and clarithromycin in the first line eradication of *H. pylori*, there wasn't any difference between the three groups[16]. Another meta-analysis done by Vergara

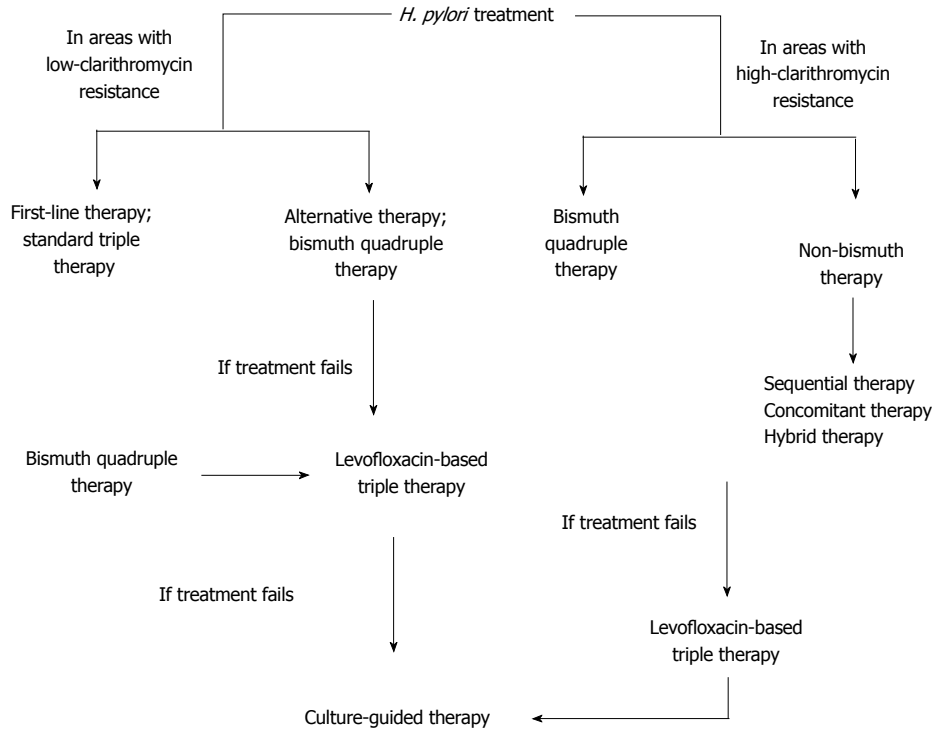

Figure 1 ***Helicobacter pylori*** **treatment algorithm.**

et al[17] including fourteen studies found no difference between proton-pump inhibitors when used in standard triple therapy for *H. pylori* eradication. The *in vitro* antibacterial activity of proton pump inhibitors vary but still the eradication rates are similar which suggests that acid inhibition is the main antibacterial mechanism of proton-pump inhibitors *in vivo*[17]. On the other hand, although the eradication rate of triple therapy with clarithromycin fell to 65%, it remained as high as 84% with metronidazole[18]. It is mostly due to the fact that metronidazole resistance may be overcome by increasing the dose and prolonging treatment duration[19]. In areas with high clarithromycin resistance (*i.e.*, Spain, Turkey, Alaska, China and Japan) bismuth quadruple, sequential, concomitant and hybrid therapies may be used[20].

BISMUTH QUADRUPLE THERAPY

Bismuth quadruple therapy is recommended as a first-line therapy in areas with high clarithromycin resistance, as an alternative first-line therapy in areas with low clarithromycin resistance or as an empirical treatment when clarithromycin therapy fails (Figure 1)[21]. It involves combination of a PPI, bismuth subsalicylate, metronidazole and tetracycline for 10 to 14 d (Table 1). This treatment is ideal as a second-line therapy, as recommended by The Maastricht Ⅳ Consensus Report[22] and the Second Asia-Pacific Consensus Guidelines[23]. Since it doesn't contain clarithromycin, compliance with the regimen is high and also metronidazole resistance *in vitro* does not affect the outcome significantly[19]. Salazar *et al*[24] reported an eradication rate greater than 95% with 14-d bismuth-quadruple therapy. A study from China, looking at the efficacy of bismuth quadruple therapy with lansoprazole as PPI and tetracycline/amoxicillin with metronidazole or furazolidone yielded eradication rates of 87.9%-95.2%; best outcome being the combination of lansoprazole, bismuth, amoxicillin and furazolidone[25].

Among the meta-analyses comparing standard triple therapy with bismuth quadruple therapy as first-line treatment, a study done by Venerito *et al*[26] showed eradication rate of 77.6% with bismuth quadruple therapy whereas it was 68.9% with clarithromycin-based standard therapy. A meta-analysis done by Luther *et al*[27] concluded that quadruple and triple therapies yielded similar eradication rates when applied as primary therapy for *H. pylori* infection and revealed similar side effects. In regions with high clarithromycin resistance, it is suggested as first-line therapy and achieved eradication rate of 82% compared to standard triple therapy[12]. Marin *et al*[28] reported eradication rates of 76%, 77% and 82% for 7, 10 and 14 d, respectively, with bismuth quadruple therapy when they applied bismuth quadruple therapy as rescue therapy. Also, Liang *et al*[25] declared eradication rates of > 90% in patients who did not respond to previous therapies, including those with metronidazole resistance.

As bismuth is concentrated in *H. pylori* and the organism doesn't develop resistance to it, applying bismuth quadruple therapy is advantageous over non-bismuth therapy[7]. The main limitations of this therapy are non-availability of bismuth salts or tetracycline in some countries as well as potential toxicity of bismuth. Nevertheless, no differences in terms of tolerability

were found between non-bismuth and bismuth containing therapies in a study among 4763 patients, except dark stools being more common in bismuth-containing group[29]. A single capsule formulation has been developed (Pylera) to overcome the complexity of quadruple therapy and showed good efficacy[20]. If the bismuth quadruple therapy is not available, sequential, concomitant or hybrid therapies may be administered.

SEQUENTIAL THERAPY

Sequential therapy, proposed by a group of Italian researchers, is a novel treatment intending to administer the antimicrobials in sequence. It is a 10-d treatment consisting of 5 d of PPI therapy with amoxicillin followed by a further 5 d of PPI with clarithromycin and metronidazole (Table 1). The main goal of sequential therapy, which has shown to have success rate of 90%-94% in several studies, is to overcome clarithromycin resistance[20]. It has been deemed that administration of amoxicillin deteriorates the bacterial cell wall, which ends up transferring clarithromycin out of the bacteria by preventing the development of efflux channels[5]. A study from China showed that sequential therapy achieves significantly higher eradication rates than triple therapy in patients with clarithromycin-resistant strains; while neither treatment was good enough to reach an eradication rate higher than 55% when clarithromycin and metronidazole resistance exists[30]. Similar results were obtained in a study from Korea where a high prevalence of clarithromycin resistant *H. pylori* is seen; eradication rates of 79% and 62% with sequential and triple therapy, respectively[31]. One study from Turkey, where clarithromycin resistance is high, reported success rate of 78% with sequential therapy *vs* 53% with standard triple therapy[32]. However, a recent meta-analysis evaluating *H. pylori* eradication rates in children showed that although sequential therapy is superior to 7-d triple therapy, it is not significantly better than 10- or 14-d triple therapy[13]. Three meta-analyses that took place in Asia in 2014 comparing efficacy of sequential therapy with standard triple therapy, favored sequential therapy over standard therapy[33-35]. Also, in a meta-analysis done by Zullo *et al*[36] success rate of sequential regimen was found higher (approximately 10%) compared to the triple therapy. Beside the mentioned study and meta-analyses, in Latin America countries, studies showed that standard triple therapy for 14 d was superior to sequential therapy (82% *vs* 76.5% respectively). This conflicting data might be due to variations in the prevalence of antibiotic resistance, which are lower in Latin America countries[37].

Sequential therapy is also recommended as first-line therapy like bismuth quadruple therapy in areas with high-clarithromycin resistance, *i.e.*, Italy and China with 90%-92% success rates[20]. In areas where bismuth drugs are not available, it may be necessary to prescribe sequential therapy[22]. A study done by Liu *et al*[38] reported that 10-d sequential and modified bismuth quadruple therapies are both highly effective as empirical first-line therapies in Chinese patients.

CONCOMITANT THERAPY

Concomitant therapy is proposed in order to reduce the complexity of sequential therapy which involves simultaneous administration of three antibiotics and a PPI for 10 d (Table 1). This treatment is used in areas where high-clarithromycin resistance is present and bismuth-based quadruple therapy is not available (Figure 1). When compared with standard triple therapy in meta-analyses of randomized trials concomitant therapy was found to be superior to standard triple therapy with 90% eradication rate[39]. In the presence of dual-resistance (100% clarithromycin and 91% metronidazole resistance), eradication rate was only 55% with concomitant therapy[5]. However, in a recent controlled trial done in Greece where clarithromycin resistance was 25% and metronidazole resistance was 40%, eradication rate was 90% under concomitant therapy[40]. Considering the sequential therapy, the eradication rates were found similar in a prospective randomized clinical trial in Spain; 91% with concomitant therapy and 86% with sequential therapy[41]. In addition, Gatta *et al*[42] found no superiority between sequential and concomitant therapies. If the concomitant therapy fails, empirical therapy becomes difficult due to exposure to both metronidazole and clarithromycin; as a result, a levofloxacin-containing or rifabutin-containing regimen may be necessary[7].

HYBRID THERAPY

Hybrid (dual-concomitant) therapy, which consists of two steps; 7 d of PPI and amoxicillin followed by a PPI and clarithromycin, amoxicillin and metronidazole, intends to overcome resistance with the benefits of four drugs of the concomitant therapy (Table 1). This therapy was first described by Hsu *et al*[43] and eradication rate of 99% by per-protocol and 97.4% by intention-to-treat analysis were obtained in 117 treated patients. Studies done involving Spanish and Italian people showed similar eradication rates (approximately 90%) for both hybrid and concomitant therapies[42]. Comparing hybrid and sequential therapies, 89.5% and 76.7% success rates were reported in a study with similar severe adverse effects[13]. More studies are needed to understand the efficacy of hybrid therapy.

LEVOFLOXACIN/RIFABUTIN BASED QUADRAPLE THERAPY

Levofloxacin-based therapy is recommended whenever bismuth quadruple therapy fails, in areas with both low and high clarithromycin resistance. It is a 10-d treatment with amoxicillin, levofloxacin and PPI (Table 1). Although eradication rate is around 90% when used

instead of clarithromycin in either triple or sequential therapies, the obstacle to use levofloxacin as a first-line treatment is the increasing frequency of quinolone resistance; which is currently 40% in America, 20% in Europe and 10% in Asia[5,44]. The meta-analysis done among patients who failed eradication with standard triple therapy showed better eradication rates with levofloxacin triple therapy than bismuth quadruple therapy, 81% and 70% respectively[45]. Including levofloxacin instead of clarithromycin in sequential therapy showed higher eradication rates in a study done by Gatta *et al*[42] Moxifloxacin and sitafloxacin may also be used but there is no evidence supporting advantage over levofloxacin. It is better to reserve fluoroquinolones for use in rescue regimens when the therapy with clarithromycin or metronidazole treatment fails.

Another salvage therapy is with rifabutin (Table 1). The advantage is the low frequency of rifabutin resistance. In a study done in Korea, where high prevalence of levofloxacin resistance is seen, amongst patients who had failed two initial regimens rifabutin triple therapy had better eradication rates than levofloxacin triple therapy, 71% and 57% respectively[46]. 50% success rate was achieved by rifabutin-based therapy in patients who had failed clarithromycin-, metronidazole- and levofloxacin-based therapy[47].

THIRD-LINE THERAPY

The Maastricht Ⅳ Consensus Report recommends antimicrobial susceptibility testing when the second-line treatment fails[22]. Although it will provide the best choice of antibiotics that can be used, the sensitivity of culture has been reported as < 60%[18]. In a study done by Cammarota *et al*[48], 90% of eradication rate was obtained among patients treated with a culture-guided third-line regimen. Culture-guided therapy is a 10-d quadruple therapy comprising a PPI, bismuth and two antibiotics selected by antimicrobial sensitivity tests (Table 1).

ADJUVANT THERAPIES

There are multiple approaches identified to overcome the side effects and increase the efficacy of treatment in *H. pylori* infections. Nowadays, use of probiotics is an emerging treatment option. They improve the eradication rates and side effects of the therapies used in *H. pylori* treatment by reducing *H. pylori* adhesion or colonization[49]. While a meta-analysis evaluating probiotics found increase in eradication rates with both Lactobacillus and Bifidobacterium, no significant improvement in side effects was seen[50]. A meta-analysis of nine studies on probiotic use as an adjuvant therapy found raise in eradication rates by 17%[51]. When Saccharomyces boulardii was added to *H. pylori* eradication regimens, decrease in side effects, especially in diarrhea, was observed as well as higher eradication rates[50,52]. Since the safety profile of probiotics is known, it is reasonable to suggest people under *H. pylori* therapy to eat yogurt.

Pre-treatment with n-acetlycysteine as a mucolytic agent is another approach to destroy the biofilm of *H. pylori* and overcome the antibiotic resistance[53]. In a randomized controlled trial done amongst patients with a history of at least four eradication failures, the eradication rates were found higher in the group who received n-acetylcysteine before a culture-guided regimen[54].

Simvastatin was used in a randomized controlled trial where the proposed mechanism of action was its anti-inflammatory effect other than its cholesterol lowering effect and the eradication rates were found to be increased but no improvement was noted in side effects[55].

The studies on vaccine against *H. pylori* still continue. A recent vaccine based on Cag A - Vac A - neutrophil-activating proteins was developed but although recognized by the host's cellular and humoral immune systems, limited immunogenicity was observed[56]. Altman *et al*[57] modulated *H. pylori* lipopolysaccharides chemically to enhance immunogenicity which enhanced antibody responses and a modest reduction in gastric *H. pylori* load when administered prophylactically. Still, there is no vaccine in use.

CONCLUSION

H. pylori is associated with multiple diseases and 100% eradication is still not possible. Even after a successful eradication, reinfection or recurrence can occur. The efficacy of standard triple therapy is decreasing whilst the bismuth quadruple and sequential regimen has been proven to achieve higher cure rates. Even though there are multiple guidelines (all therapies are summarized in Table 1) about the treatment regimens, increasing antibiotic resistance as well as different frequencies in resistance in different areas of the world suggests the optimal approach in the treatment of patients with *H. pylori* infections to be individualized therapy, which is a highly active and well-tolerated regimen. Use of probiotics, pre-treatment with n-acetylcysteine and statins may help as adjuvant therapies.

REFERENCES

1 **Tonkic A**, Tonkic M, Lehours P, Mégraud F. Epidemiology and diagnosis of Helicobacter pylori infection. *Helicobacter* 2012; **17** Suppl 1: 1-8 [PMID: 22958148]

2 **Zhao B**, Zhao J, Cheng WF, Shi WJ, Liu W, Pan XL, Zhang GX. Efficacy of Helicobacter pylori eradication therapy on functional dyspepsia: a meta-analysis of randomized controlled studies with 12-month follow-up. *J Clin Gastroenterol* 2014; **48**: 241-247 [PMID: 24002127 DOI: 10.1097/MCG.0b013e31829f2e25]

3 **Stasi R**, Sarpatwari A, Segal JB, Osborn J, Evangelista ML, Cooper N, Provan D, Newland A, Amadori S, Bussel JB. Effects of eradication of Helicobacter pylori infection in patients with immune thrombocytopenic purpura: a systematic review. *Blood* 2009; **113**: 1231-1240 [PMID: 18945961 DOI: 10.1182/blood-2008-07-167155]

4 **Bazzoli F**, Pozzato P. Therapy of H. pylori infection. *J Physiol Pharmacol* 1997; **48** Suppl 4: 39-46 [PMID: 9440054]

5 **Papastergiou V**, Georgopoulos SD, Karatapanis S. Treatment of Helicobacter pylori infection: Past, present and future. *World J Gastrointest Pathophysiol* 2014; **5**: 392-399 [PMID: 25400982 DOI: 10.4291/wjgp.v5.i4.392]

6 **Lee JY**, Kim N, Kim MS, Choi YJ, Lee JW, Yoon H, Shin CM, Park YS, Lee DH, Jung HC. Factors affecting first-line triple therapy of Helicobacter pylori including CYP2C19 genotype and antibiotic resistance. *Dig Dis Sci* 2014; **59**: 1235-1243 [PMID: 24599773 DOI: 10.1007/s10620-014-3093-7]

7 **Vakil N**, Vaira D. Treatment for H. pylori infection: new challenges with antimicrobial resistance. *J Clin Gastroenterol* 2013; **47**: 383-388 [PMID: 23388847 DOI: 10.1097/MCG.0b013e318277577b]

8 **Wu W**, Yang Y, Sun G. Recent Insights into Antibiotic Resistance in Helicobacter pylori Eradication. *Gastroenterol Res Pract* 2012; **2012**: 723183 [PMID: 22829809 DOI: 10.1155/2012/723183]

9 **Graham DY**, Fischbach L. Helicobacter pylori treatment in the era of increasing antibiotic resistance. *Gut* 2010; **59**: 1143-1153 [PMID: 20525969 DOI: 10.1136/gut.2009.192757]

10 **Smith SM**. Role of Toll-like receptors in Helicobacter pylori infection and immunity. *World J Gastrointest Pathophysiol* 2014; **5**: 133-146 [PMID: 25133016 DOI: 10.4291/wjgp.v5.i3.133]

11 **Megraud F**, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, Andersen LP, Goossens H, Glupczynski Y. Helicobacter pylori resistance to antibiotics in Europe and its relationship to antibiotic consumption. *Gut* 2013; **62**: 34-42 [PMID: 22580412 DOI: 10.1136/gutjnl-2012-302254]

12 **Cizginer S**, Ordulu Z, Kadayifci A. Approach to Helicobacter pylori infection in geriatric population. *World J Gastrointest Pharmacol Ther* 2014; **5**: 139-147 [PMID: 25133042 DOI: 10.4292/wjgpt.v5.i3.139]

13 **Garza-González E**, Perez-Perez GI, Maldonado-Garza HJ, Bosques-Padilla FJ. A review of Helicobacter pylori diagnosis, treatment, and methods to detect eradication. *World J Gastroenterol* 2014; **20**: 1438-1449 [PMID: 24587620 DOI: 10.3748/wjg.v20.i6.1438]

14 **Yuan Y**, Ford AC, Khan KJ, Gisbert JP, Forman D, Leontiadis GI, Tse F, Calvet X, Fallone C, Fischbach L, Oderda G, Bazzoli F, Moayyedi P. Optimum duration of regimens for Helicobacter pylori eradication. *Cochrane Database Syst Rev* 2013; **12**: CD008337 [PMID: 24338763 DOI: 10.1002/14651858.CD008337]

15 **Malfertheiner P**, Selgrad M. Helicobacter pylori. *Curr Opin Gastroenterol* 2014; **30**: 589-595 [PMID: 25268839 DOI: 10.1097/MOG.0000000000000128]

16 **Altintas E**, Sezgin O, Ulu O, Aydin O, Camdeviren H. Maastricht II treatment scheme and efficacy of different proton pump inhibitors in eradicating Helicobacter pylori. *World J Gastroenterol* 2004; **10**: 1656-1658 [PMID: 15162544]

17 **Vergara M**, Vallve M, Gisbert JP, Calvet X. Meta-analysis: comparative efficacy of different proton-pump inhibitors in triple therapy for Helicobacter pylori eradication. *Aliment Pharmacol Ther* 2003; **18**: 647-654 [PMID: 12969092 DOI: 10.1046/j.1365-2036.2003.01746]

18 **Sasaki H**, Nagahara A, Hojo M, Asaoka D, Matsumoto K, Osada T, Watanabe S. Ten-year trend of the cumulative Helicobacter pylori eradication rate for the 'Japanese eradication strategy'. *Digestion* 2013; **88**: 272-278 [PMID: 24356705]

19 **Graham DY**, Qureshi WA. Antibiotic-resistant H. pylori infection and its treatment. *Curr Pharm Des* 2000; **6**: 1537-1544 [PMID: 10974150]

20 **Urgesi R**, Cianci R, Riccioni ME. Update on triple therapy for eradication of Helicobacter pylori: current status of the art. *Clin Exp Gastroenterol* 2012; **5**: 151-157 [PMID: 23028235 DOI: 10.2147/CEG.S25416]

21 **Furuta T**, Sugimoto M, Shirai N, Matsushita F, Nakajima H, Kumagai J, Senoo K, Kodaira C, Nishino M, Yamade M, Ikuma M, Watanabe H, Umemura K, Ishizaki T, Hishida A. Effect of MDR1 C3435T polymorphism on cure rates of Helicobacter pylori infection by triple therapy with lansoprazole, amoxicillin and clarithromycin in relation to CYP 2C19 genotypes and 23S rRNA genotypes of H. pylori. *Aliment Pharmacol Ther* 2007; **26**: 693-703 [PMID: 17697203 DOI: 10.1111/j.1365-2036.2007.03408]

22 **Malfertheiner P**, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM, Kuipers EJ. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. *Gut* 2012; **61**: 646-664 [PMID: 22491499 DOI: 10.1136/gutjnl-2012-302084]

23 **Fock KM**, Katelaris P, Sugano K, Ang TL, Hunt R, Talley NJ, Lam SK, Xiao SD, Tan HJ, Wu CY, Jung HC, Hoang BH, Kachintorn U, Goh KL, Chiba T, Rani AA. Second Asia-Pacific Consensus Guidelines for Helicobacter pylori infection. *J Gastroenterol Hepatol* 2009; **24**: 1587-1600 [PMID: 19788600 DOI: 10.1111/j.1440-1746.2009.05982]

24 **Salazar CO**, Cardenas VM, Reddy RK, Dominguez DC, Snyder LK, Graham DY. Greater than 95% success with 14-day bismuth quadruple anti- Helicobacter pylori therapy: a pilot study in US Hispanics. *Helicobacter* 2012; **17**: 382-390 [PMID: 22967122 DOI: 10.1111/j.1523-5378.2012.00962]

25 **Liang X**, Xu X, Zheng Q, Zhang W, Sun Q, Liu W, Xiao S, Lu H. Efficacy of bismuth-containing quadruple therapies for clarithromycin-, metronidazole-, and fluoroquinolone-resistant Helicobacter pylori infections in a prospective study. *Clin Gastroenterol Hepatol* 2013; **11**: 802-807.e1 [PMID: 23376004 DOI: 10.1016/j.cgh.2013.01.008]

26 **Venerito M**, Krieger T, Ecker T, Leandro G, Malfertheiner P. Meta-analysis of bismuth quadruple therapy versus clarithromycin triple therapy for empiric primary treatment of Helicobacter pylori infection. *Digestion* 2013; **88**: 33-45 [PMID: 23880479 DOI: 10.1159/000350719]

27 **Luther J**, Higgins PD, Schoenfeld PS, Moayyedi P, Vakil N, Chey WD. Empiric quadruple vs. triple therapy for primary treatment of Helicobacter pylori infection: Systematic review and meta-analysis of efficacy and tolerability. *Am J Gastroenterol* 2010; **105**: 65-73 [PMID: 19755966 DOI: 10.1038/ajg.2009.508]

28 **Marin AC**, McNicholl AG, Gisbert JP. A review of rescue regimens after clarithromycin-containing triple therapy failure (for Helicobacter pylori eradication). *Expert Opin Pharmacother* 2013; **14**: 843-861 [PMID: 23537368 DOI: 10.1517/14656566.2013.782286]

29 **Ford AC**, Malfertheiner P, Giguere M, Santana J, Khan M, Moayyedi P. Adverse events with bismuth salts for Helicobacter pylori eradication: systematic review and meta-analysis. *World J Gastroenterol* 2008; **14**: 7361-7370 [PMID: 19109870 DOI: 10.3748/wjg.14.7361]

30 **Zhou L**, Zhang J, Chen M, Hou X, Li Z, Song Z, He L, Lin S. A comparative study of sequential therapy and standard triple therapy for Helicobacter pylori infection: a randomized multicenter trial. *Am J Gastroenterol* 2014; **109**: 535-541 [PMID: 24642580 DOI: 10.1038/ajg.2014.26]

31 **Park HG**, Jung MK, Jung JT, Kwon JG, Kim EY, Seo HE, Lee JH, Yang CH, Kim ES, Cho KB, Park KS, Lee SH, Kim KO, Jeon SW. Randomised clinical trial: a comparative study of 10-day sequential therapy with 7-day standard triple therapy for Helicobacter pylori infection in naïve patients. *Aliment Pharmacol Ther* 2012; **35**: 56-65 [PMID: 22066530 DOI: 10.1111/j.1365-2036.2011.04902]

32 **Nadir I**, Yonem O, Ozin Y, Kilic ZM, Sezgin O. Comparison of two different treatment protocols in Helicobacter pylori eradication. *South Med J* 2011; **104**: 102-105 [PMID: 21206418 DOI: 10.1097/SMJ.0b013e318200c209]

33 **Yoon H**, Lee DH, Kim N, Park YS, Shin CM, Kang KK, Oh DH, Jang DK, Chung JW. Meta-analysis: is sequential therapy superior to standard triple therapy for Helicobacter pylori infection in Asian adults? *J Gastroenterol Hepatol* 2013; **28**: 1801-1809 [PMID: 24118110 DOI: 10.1111/jgh.12397]

34 **Kim JS**, Ji JS, Choi H, Kim JH. Sequential therapy or triple therapy for Helicobacter pylori infection in Asians: systematic review and meta-analysis. *Clin Res Hepatol Gastroenterol* 2014; **38**: 118-125 [PMID: 24238722 DOI: 10.1016/j.clinre.2013.10.001]

35 **Chung JW**, Ha M, Yun SC, Kim JH, Lee JJ, Kim YJ, Kim KO, Kwon KA, Park DK, Lee DH. Meta-analysis: Sequential therapy is

superior to conventional therapy for Helicobacter pylori infection in Korea. *Korean J Gastroenterol* 2013; **62**: 267-271 [PMID: 24262591 DOI: 10.4166/kjg.2013.62.5.267]

36 **Zullo A**, Hassan C, Ridola L, De Francesco V, Vaira D. Standard triple and sequential therapies for Helicobacter pylori eradication: an update. *Eur J Intern Med* 2013; **24**: 16-19 [PMID: 22877993 DOI: 10.1016/j.ejim.2012.07.006]

37 **Liou JM**, Chen CC, Chen MJ, Chen CC, Chang CY, Fang YJ, Lee JY, Hsu SJ, Luo JC, Chang WH, Hsu YC, Tseng CH, Tseng PH, Wang HP, Yang UC, Shun CT, Lin JT, Lee YC, Wu MS. Sequential versus triple therapy for the first-line treatment of Helicobacter pylori: a multicentre, open-label, randomised trial. *Lancet* 2013; **381**: 205-213 [PMID: 23158886 DOI: 10.1016/S0140-6736(12)61579-7]

38 **Liu KS**, Hung IF, Seto WK, Tong T, Hsu AS, Lam FY, But DY, Wong SY, Leung WK. Ten day sequential versus 10 day modified bismuth quadruple therapy as empirical firstline and secondline treatment for Helicobacter pylori in Chinese patients: an open label, randomised, crossover trial. *Gut* 2014; **63**: 1410-1415 [PMID: 24295850 DOI: 10.1136/gutjnl-2013-306120]

39 **Essa AS**, Kramer JR, Graham DY, Treiber G. Meta-analysis: four-drug, three-antibiotic, non-bismuth-containing "concomitant therapy" versus triple therapy for Helicobacter pylori eradication. *Helicobacter* 2009; **14**: 109-118 [PMID: 19298338 DOI: 10.1111/j.1523-5378.2009.00671]

40 **McColl KE**. Clinical practice. Helicobacter pylori infection. *N Engl J Med* 2010; **362**: 1597-1604 [PMID: 20427808 DOI: 10.1056/NEJMcp1001110]

41 **McNicholl AG**, Marin AC, Molina-Infante J, Castro M, Barrio J, Ducons J, Calvet X, de la Coba C, Montoro M, Bory F, Perez-Aisa A, Forné M, Gisbert JP. Randomised clinical trial comparing sequential and concomitant therapies for Helicobacter pylori eradication in routine clinical practice. *Gut* 2014; **63**: 244-249 [PMID: 23665990]

42 **Gatta L**, Vakil N, Vaira D, Scarpignato C. Global eradication rates for Helicobacter pylori infection: systematic review and meta-analysis of sequential therapy. *BMJ* 2013; **347**: f4587 [PMID: 23926315 DOI: 10.1136/bmj.f4587]

43 **Hsu PI**, Wu DC, Wu JY, Graham DY. Modified sequential Helicobacter pylori therapy: proton pump inhibitor and amoxicillin for 14 days with clarithromycin and metronidazole added as a quadruple (hybrid) therapy for the final 7 days. *Helicobacter* 2011; **16**: 139-145. [PMID: 21435092 DOI: 10.1111/j.1523-5378.2011.00828]

44 **De Francesco V**, Giorgio F, Hassan C, Manes G, Vannella L, Panella C, Ierardi E, Zullo A. Worldwide H. pylori antibiotic resistance: a systematic review. *J Gastrointestin Liver Dis* 2010; **19**: 409-414 [PMID: 21188333]

45 **Saad RJ**, Schoenfeld P, Kim HM, Chey WD. Levofloxacin-based triple therapy versus bismuth-based quadruple therapy for persistent Helicobacter pylori infection: a meta-analysis. *Am J Gastroenterol* 2006; **101**: 488-496 [PMID: 16542284 DOI: 10.1111/j.1572-0241.2006.00637.x]

46 **Jeong MH**, Chung JW, Lee SJ, Ha M, Jeong SH, Na S, Na BS, Park SK, Kim YJ, Kwon KA, Ko KI, Jo Y, Hahm KB, Jung HY. Comparison of rifabutin- and levofloxacin-based third-line rescue therapies for Helicobacter pylori. *Korean J Gastroenterol* 2012; **59**: 401-406 [PMID: 22735872]

47 **Gisbert JP**, Castro-Fernandez M, Perez-Aisa A, Cosme A, Molina-Infante J, Rodrigo L, Modolell I, Cabriada JL, Gisbert JL, Lamas E, Marcos E, Calvet X. Fourth-line rescue therapy with rifabutin in patients with three Helicobacter pylori eradication failures. *Aliment Pharmacol Ther* 2012; **35**: 941-947 [PMID: 22372560 DOI: 10.1111/j.1365-2036.2012.05053.x]

48 **Cammarota G**, Martino A, Pirozzi G, Cianci R, Branca G, Nista EC, Cazzato A, Cannizzaro O, Miele L, Grieco A, Gasbarrini A, Gasbarrini G. High efficacy of 1-week doxycycline- and amoxicillin-based quadruple regimen in a culture-guided, third-line treatment approach for Helicobacter pylori infection. *Aliment Pharmacol Ther* 2004; **19**: 789-795 [PMID: 15043520 DOI: 10.1111/j.1365-2036.2004.01910.x]

49 **Ruggiero P**. Use of probiotics in the fight against Helicobacter pylori. *World J Gastrointest Pathophysiol* 2014; **5**: 384-391 [PMID: 25400981 DOI: 10.4291/wjgp.v5.i4.384]

50 **Testerman TL**, Morris J. Beyond the stomach: an updated view of Helicobacter pylori pathogenesis, diagnosis, and treatment. *World J Gastroenterol* 2014; **20**: 12781-12808 [PMID: 25278678 DOI: 10.3748/wjg.v20.i36.12781]

51 **Zheng X**, Lyu L, Mei Z. Lactobacillus-containing probiotic supplementation increases Helicobacter pylori eradication rate: evidence from a meta-analysis. *Rev Esp Enferm Dig* 2013; **105**: 445-453 [PMID: 24274441 DOI: 10.4321/S1130-0108-2013000800002]

52 **Song MJ**, Park DI, Park JH, Kim HJ, Cho YK, Sohn CI, Jeon WK, Kim BI. The effect of probiotics and mucoprotective agents on PPI-based triple therapy for eradication of Helicobacter pylori. *Helicobacter* 2010; **15**: 206-213 [PMID: 20557362 DOI: 10.1111/j.1523-5378.2010.00751.x]

53 **Malfertheiner P**, Selgrad M, Bornschein J. Helicobacter pylori: clinical management. *Curr Opin Gastroenterol* 2012; **28**: 608-614 [PMID: 23010682 DOI: 10.1097/MOG.0b013e32835918a7]

54 **Cammarota G**, Branca G, Ardito F, Sanguinetti M, Ianiro G, Cianci R, Torelli R, Masala G, Gasbarrini A, Fadda G, Landolfi R, Gasbarrini G. Biofilm demolition and antibiotic treatment to eradicate resistant Helicobacter pylori: a clinical trial. *Clin Gastroenterol Hepatol* 2010; **8**: 817-820.e3 [PMID: 20478402 DOI: 10.1016/j.cgh.2010.05.006]

55 **Malfertheiner P**, Venerito M, Selgrad M. Helicobacter pylori infection: selected aspects in clinical management. *Curr Opin Gastroenterol* 2013; **29**: 669-675 [PMID: 24100726 DOI: 10.1097/MOG.0b013e328365d443]

56 **Ruggiero P**. Helicobacter pylori infection: what's new. *Curr Opin Infect Dis* 2012; **25**: 337-344 [PMID: 22555448 DOI: 10.1097/QCO.0b013e3283531f7c]

57 **Altman E**, Chandan V, Harrison BA, Veloso-Pita R, Li J, KuoLee R, Chen W, Vérez-Bencomo V; Regional Helicobacter pylori Study Group. Design and immunological properties of Helicobacter pylori glycoconjugates based on a truncated lipopolysaccharide lacking Lewis antigen and comprising an α-1,6-glucan chain. *Vaccine* 2012; **30**: 7332-7341 [PMID: 22534169 DOI: 10.1016/j.vaccine.2012.04.035]

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