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EDITORIAL

Effects of physical activity in Parkinson's disease: A new tool for rehabilitation

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Abstract

Parkinson's disease (PD) is a common neurodegenerative disease characterized by bradykinesia, tremor, rigidity, and postural instability. Motor disorders are composite and combined, adversely affecting the patient's health. Tremor and rigidity are correlated with worsening manual dexterity as well as postural changes such as akinesia and camptocormia. Moreover, gait alteration as well as postural instability, with consequent impairment in balance, increase the risk of falls. It is well known that these symptoms respond poorly to pharmacologic therapy in PD patients. Physical therapy is the most effective non-pharmacological aid to PD patients. Available data in the literature indicate that any rehabilitation protocol has to focus on: cognitive movement strategies, cueing strategies, and improved physical capacity and balance. Different training programs for PD patients have been designed and evaluated but only specific training strategies, tailored and individualized for each patient, may produce improvements in gait speed and stride length, decrease motor and balance symptoms and improve quality of life. Furthermore, aerobic training may improve muscle trophism, strength and mobility. It seems reasonable to state that tailored physical activity is a valid tool to be included in the therapeutic program of PD patients, considering that this approach may ameliorate the symptoms as well as the overall physical incapacity, reduce the risk of falls and injuries, and ultimately improve quality of life.

Key words: Parkinson's disease; Motor disorders; Postural instability; Physical exercise; Training

Core tip: A review of the literature underlines the importance of tailored physical activity in patients with Parkinson disease. Several studies demonstrated the key role that specific training strategies may have on motor disorders and postural instability affecting patients with Parkinson disease. Since it has been clearly demonstrated that these symptoms respond poorly to pharmacologic therapy, it seems necessary to combine the traditional treatment of Parkinson disease with a specific exercise training strategy in order to reduce motor disorders as well as postural instability, with the aim of improving quality of life of the patients affected by this neurologic disease.

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PARKINSON'S DISEASE

Parkinson's disease (PD) is the second most common neurodegenerative disorder, after Alzheimer's disease. PD affects 1% of the population over 60 years of age, and the risk increases proportionally with age.

The primary symptoms of PD are bradykinesia, trem-

or, rigidity, and postural instability. Bradykinesia refers to the slowness of the patient's movements[1] and affects every single patient[2]; it is evident especially when evaluating gait, but it affects every voluntary movement. Patients also manifest a global reduction in spontaneous movements (*e.g.*, gestures, winking) and deliberate movements (*e.g.*, arm swing), defined as akinesia[1]. PD's tremor affects 75% of the diagnosed subjects[3]. This "resting tremor" differs from essential tremor since it manifests when the body part affected is not involved in voluntary movements. Rigidity affects 90%-99% of PD patients[4] and it is caused by muscular hypertonia; the typical posture of PD patients, camptocormia, is in fact due to excessive activation of flexor muscles in the trunk and limbs[4]. Postural instability is "the impairment in balance that compromises the ability to maintain or change posture such as standing and walking"[5]. This condition does not usually affect the early stages of the illness, and is one of the most difficult symptoms to be treated[6].

For many reasons, it is essential to describe the impaired Parkinsonian gait. In chronological order, patients experience difficulties when starting to walk, a condition called "start hesitation"[7]; walking speed and stride length are abnormally reduced; lower limb joints excursion is reduced due to rigidity; timing of steps is extremely irregular and asymmetric; and arm swing is decreased or absent. Moreover, 2 typical features are also present: festinating gait or festination is characterized by the patient's sudden acceleration as an attempt to keep their center of gravity between their feet in order to compensate for their flexed posture[8]. Freezing of gait (FoG) is defined as a sudden stop in the patient's gait, often with legs trembling in place and the sensation of being "glued" to the floor. This symptom manifests more frequently while turning, when the path changes or gets more narrow, in a diagonal direction, when dealing with obstacles and other stressful situations or just before reaching the destination[8-11].

Subjects affected by PD also experience several non-motor symptoms, such as autonomic dysfunctions (dysphagia, constipation, urinary incontinence, sexual dysfunction, orthostatic hypotension) cognitive impairment, dementia, depression (which affects 30%-40% of patients)[12], anxiety, sleep disorders and decreased olfactory sense.

PD motor disorders are very complex and interconnected, and their adverse consequences for both patient's health and quality of life are very well known. Tremor and rigidity significantly reduce the quality of life since they worsen manual dexterity, thus affecting simple everyday activities such as cutting food pill-taking and posture[13]. Moreover, gait impairments (especially FoG and stride variability) as well as postural instability (and the consequent reduced balance) represents harmful conditions of PD since they increase the risk of falls[11,14,15]. According to statistics, almost 70% of patients experience falls at least once per year[16] and 25% of subjects in the first 10 years of disease suffer a hip fracture[11], an injury correlated with high morbidity and mortality in PD patients[17]. Furthermore, falls can trigger a vicious circle that aggravates even more the patient's condition: fear of other falls and injuries reduce the subjects' mobilization, which in turn causes sarcopenia, decreased fitness, osteoporosis, loss of independence, social isolation, and reduced participation in simple daily activities[11,18-20]. In extreme cases of these impairments there is a condition which is sometimes referred as "malignant parkinsonism", in which rapid disease progression, premature home nursing, depression and cognitive decline, increase the mortality risks[11].

There is no cure for PD and pharmacological treatment (of which levodopa is the long-term gold standard) still lacks significant effects on the previously described harmful symptoms. Indeed, postural instability, balance problems and gait disorders such as FoG and stride variability respond poorly to medication[9-11,21-24]. Even worse, many authors describe a "paradoxal effect" of medication[11,21,25] since two-thirds of falls occur when patients are under the effect of medication ("on" phase)[21]. This condition may be explained by the fact that dopaminergic treatment improves gait speed and general mobility (thus stimulating the patients to move), but not balance and postural instability[21,25]. Finally, some adverse effects of long-term pharmacological treatment, such as levodopa-induced dyskinesia and orthostatic hypotension, are very well known causes of falls[18,25,26].

Therefore, it is evident that the study of new approaches aimed to improve the motor aspects of PD patients is necessary. Nowadays, besides optimal medication, it is evident that physical therapy is the most effective non-pharmacological aid for patients with PD. It is not the aim of this paper to describe in detail the pathophysiology underlying each symptom, but putative mechanisms for each exercise protocol will be discussed in detail in the ensuing paragraphs.

EXERCISE GUIDELINES FOR PD

The most relevant guidelines for physical exercise in PD have been designed by Morris[27] and Keus *et al*[28] (Table 1).

External cues represent effective interventions aimed to improve motor performance, especially gait. Cognitive movement strategies refer to those mental techniques taught to patients in order to improve their everyday motor tasks. The key intervention is to teach the patient to subdivide complex and automated motor sequences in series of single, simple movements that must be performed in the correct, fixed order. This strategy is aimed to render motor performance as a conscious activity, thus avoiding dual-tasking[27,28] as well as bypassing the defective basal ganglia[28]. Balance training is another crucial point of the model, since it can prevent falls. Furthermore, improving physical capacity with aerobic training, strength and flexibility exercises may reduce symptoms as well as improve the patient's general well-being and quality of life.

In this setting, the individualization of training represents a crucial approach. Indeed, PD symptoms change, often with fluctuations, with the progression of the dis-

Table 1 Essential points in Parkinson's disease exercise therapy

	Effects
Cueing strategies	Improve motor performance (especially gait)
Cognitive movement strategies	Improve everyday motor tasks (walking, standing up, sitting down, dressing, *etc.*), and quality of life
Balance training	Prevent risk of falls, improve postural stability
Aerobic training	Improve physical capacity
Strength and flexibility	Improve general well-being and quality of life

ease. Therefore, physical therapists, in conjunction with the caregivers and neurologists, should adapt, modify and tailor the exercise program to the patient's specific needs. Regular assessments[28] should be performed in order to analyze the effects of both medications and physical therapy, and consequently adapt them to the patient's actual condition.

To achieve feasible improvements, a physical therapy protocol should be at least 8-10 wk long. Ideally, patients should train 3 d per week, 60-75 min per session. Moreover, it is also recommended to perform stretching exercises daily in order to reduce rigidity. Obviously, choosing an enjoyable and stimulating exercise protocol, thus promoting adherence and long-term compliance is of major importance when considering both the patient's prognosis and quality of life.

TRAINING STRATEGIES

Different training programs for patients with PD have been designed and evaluated.

Treadmill

Treadmill training is probably the most examined form of exercise for patients with PD.

Single-pulse Transcranial Magnetic Stimulation studies have shown abnormalities of the Cortical Silent Period (CSP duration) and other corticomotor excitability measures in patients with PD, reflecting greater corticomotor excitability in these patients. It is known that CSP is mainly mediated by gamma-aminobutyric acid (GABA)-B receptors, and abnormalities of GABAergic transmission are key points of the pathophysiology of movement disorders involving the basal ganglia. In addition, voluntary exercise may increase brain-derived neurotrophic factor (BDNF) levels thus enhancing neuronal function by promoting synaptogenesis and neurogenesis. Indeed, BDNF modulates the level of functional inhibition in an activity-dependent manner by regulating the number of GABAergic interneurons. While the role of BDNF in modulating GABA-mediated inhibitory transmission is not fully understood, conceivably the lengthening of CSP, related to high intensity exercise, may be related to an exercise-induced increase in BDNF[29].

The rationale behind the use of a treadmill in PD patients, proposes that the device works as an external cue[9,30-33] which bypasses the defective basal ganglia. In this setting patients do not need to pay attention to triggering[31,34,35], selecting and maintaining a motor sequence[35], and they can focus only on the motor action of walking[35]. Furthermore, as stated by Cakit *et al*[21], "a guiding principle in neurologic rehabilitation is that a skill will be improved if it is practiced"; therefore, walking on a treadmill can improve Parkinsonian gait since, as described by several authors[33,36,37], it can also generate motor learning. The need for improving gait and walking in people affected by PD is related to the achievement of performance improvement. In addition, gait training is necessary to reduce gait disorders (such as FoG, gait variability and festination) which directly cause reduced mobility, falls and injuries that can severely worsen the patient's health and quality of life. As described by Frenkel-Toledo *et al*[31], the rhythm imposed by the machine regularizes the patient's gait, with steps becoming longer and less variable. Considering that stride variability represents the main risk indicator of falls in the elderly[38], this improvement acquires even more significance. Indeed, different studies[21,36,39] show that treadmill training directly decreases risk and actual falls in subjects affected by PD.

As proven by 2 studies, even a single session of treadmill training generates improvements in the gait of PD patients. In the study of Pohl *et al*[32], patients tried 3 different interventions for gait treatment: Speed-Dependent Treadmill Training (SDTT, in which the patient walks 10 s at his/her maximum safe speed, and if that speed was sustainable it would have been increased after a short rest), Limited Progressive Treadmill Training (in which the belt speed was never increased over walking speed reported at baseline) and Conventional Gait Therapy (CGT, based on Proprioceptive Neuromuscular Fasciculation concepts). After just a 30-min training session, both of the treadmill protocols showed significant improvements in gait speed and stride length; also, double stance duration was decreased. Similarly, Bello *et al*[40] described improvements in gait speed and stride length after a single, 20-min treadmill session.

Several studies focused on the effects of a longer-term protocol in Parkinson's patients. Miyai *et al*[41] demonstrated that, in comparison with traditional physical therapy, a 4-wk body weight-supported treadmill training (BWSTT) program was effective on symptoms]as shown by the unified parkinson's disease rating scale (UPDRS)] as well as on gait speed. In another study of Miyai *et al*[37], patients received a 45-min BWSTT training 3 times a week for 1 mo, with belt speed progressively increased over the training period. Results showed higher gait speed and decreased number of steps over a 10 m distance, and effects lasted, respectively, at the 1- and 4-mo-follow up. Herman *et al*[36] demonstrated that an intensive treadmill protocol, with patients training 30 min, 4 times per week over a 6-wk period (with belt speed progressively increased), could generate improvements not only in gait parameters (speed, swing time variability), but also in balance, motor symptoms (as showed by the values of UPDRS-Ⅲ) and quality of life (assessed with Parkinson's Disease Questionnaire-39). Four studies evalu-

Table 2 Results of treadmill training

Pohl *et al*[32]	Single session 10 s at maximum safe speed	After 30 min training	Improvement in gait speed and stride length
Bello *et al*[40]	Single 20 min session		Improvement in gait speed and stride length
Miyai *et al*[41]	Body weight supported treadmill training	45-min session, 3 times/wk, for 4 wk	Decrease of symptoms (UPDRS scale) and improvements in gait speed
Miyai *et al*[37]	Body weight supported treadmill training	45-min session, 3 times/wk, for 4 wk with progressive increasing of belt speed	Improvement in gait speed, decreasing of steps number over a 10 m distance. The effects lasted over 1 and 4 mo of follow up
Herman *et al*[36]	Intensive treadmill protocol	30 min session, 4 times/wk, for 6 wk with progressive increasing of belt speed	Improvement in gait parameters (speed, swing time variability), balance, motor symptoms (UPDRS scale) and in quality of life
Cakit *et al*[21]	Speed dependent treadmill training	30 ± 5 min session, 8 wk	Improvement in tolerated speed and distance walked, in balance and reduction in fear of falls
Fisher *et al*[29]	Body weight supported treadmill training	3.0 metabolic equivalents session, 3 times/wk for 8 wk	Improvement in gait speed, step and stride length, hip and ankle joint excursion, and decrease cortico-motor excitability
Protas *et al*[39]	Walking in all 4 directions and step training	1 h session, 3 times/wk for 8 wk	Reduction of falls, improvement in gait speed and stride length, improvement in dynamic balance
Rose *et al*[42]	Skipping, sprinting, walking, running and jumping on a lower body positive-pressure antigravity treadmill + spatial cues	1 h session, 3 times/wk for 8 wk	Improvement in gait and functional capacity, better quality of life, improvement in motor symptoms (UPDRS scale)

UPDRS: Unified Parkinson's Disease Rating Scale.

ated the treadmill training protocol carried out over 8 wk. Cakit *et al*[21] trained their patients with a SDTT protocol and described improvements in tolerated speed and distance walked, improved balance and reduced fear of falls. Fisher *et al*[29] demonstrated that high-intensity BWSTT 3 times a week over an 8-wk period improved: gait speed, step and stride length, hip and ankle joint excursion, body weight distribution, and, as pointed by the authors, "importantly" in CSP length, which in fact translates into decreased corticomotor excitability. Protas *et al*[39] designed a specific treadmill protocol, in which patients walked in all 4 directions at a speed higher than normal gait; "step training" was also included, as the treadmill belt was suddenly switched off during the exercise. Results showed a substantial reduction in falls, increased gait speed and stride length as well as improved dynamic balance. In the latest study[42], different tasks were performed by patients, such as skipping, sprinting, walking, running, jumping, and also spatial cues where used, with the patient working on a lower body positive-pressure antigravity treadmill. The study by Rose *et al*[42] showed significant and promising results for this brand new, high intensity protocol: improvements affected the score for Movement Disorders Society- UPDR Scale, better quality of life (32% improvement at Parkinson's Disease Questionnaire-39), and increased gait and functional capacity [assessed with the 6-minute walk test (6MWT)].

Finally, it is mandatory to note that treadmill training may generate positive effects also on the central nervous system, namely, dopamine availability and corticomotor excitability, 2 central elements which are impaired by PD and cause motor disorders. Different interventions of treadmill training are shown in Table 2.

Cueing strategies

It is known that the normal footstep pattern is not lost in PD, rather there is a problem in activating the correct stepping response for a given context. Several studies demonstrated that the interaction between the basal ganglia and supplementary motor area (SMA) is disrupted during movement performance. The SMA normally prepares for a forthcoming predictable movement with a steady increase in neuronal activity during the premovement period. Once the external signal to move occurs, the neuronal activity in the SMA abruptly ceases. The basal ganglia discharge with brief bursts of phasic activity at the end of submovements performed in a sequence. This activity represents an internal cue which triggers the rapid drop in SMA neuronal activity. If the basal ganglia cue was absent or disturbed, as in PD, then it is possible that the SMA preparatory activity would be disturbed, leading to an abnormally executed movement[43].

As a consequence of the dopaminergic neuronal defection, the basal ganglia do not provide the correct cues for motor sequences to cortical motor areas (primary motor cortex, premotor cortex and supplementary motor area). This defect justifies the patient's inability to prepare and maintain the execution of complex and well-learned movements, *e.g.*, walking[9,22,34,43-45]. Specific gait impairments like FoG and festination are probably caused by an internal lack of rhythmic cues[9,14,46].

Conceivably, physical therapy should aim to compensate the physiologic defections at the base of the motor impairments[14,32,47]. In this setting, adapted exercise should be planned with the aim of reaching the cortical motor areas and bypassing the affected basal ganglia, in order to improve motor performance. External cues seem to be an effective method[14,27,28], and many authors demonstrated their efficacy. There are 3 types of cues which have been mainly studied: visual (transverse stripes placed on the walk path), auditory (music or simple beeps and sounds) and somatosensory (vibrations). Visual cues seems to facilitate Parkinsonian gait since they focus patient's concentration during the act of walking, thus making it a vol-

Table 3 Results of cueing training

Thaut *et al*[49]	Rhythmic auditory cues	Walking, stop-and-go, stair stepping listening to music	30 min/d for 3 wk	Improvement in gait speed, stride length and cadence
McIntosh *et al*[50]	Rhythmic auditory cues	Walking and stop-and-go listening to music	Single session	Improvement in gait speed, stride length and cadence
del Olmo *et al*[34]	Rhythmic auditory cues	Walking in different condition (with or without metronome cadence)	1 h/d, for 5 times/wk for 4 wk	Improvement in gait temporal stability
Azulay *et al*[48]	Dynamic and static visual cues	Walking on a 12-m walkway with parallel transversal white stripes with normal/ stroboscopic lights	Single session	Increased velocity and stride length in the normal lights condition, suggesting the role of a specific visuo-motor pathway elicited by the moving cues
Rochester *et al*[35]	Auditory + visual cues	Little itinerary performed at home	Single session	Auditory cues improved performance (stride length) in the functional task, and a tendency for increased walking speed was noticed with both types of cue
Nieuwboer *et al*[23]	Visual, auditory or somato-sensory cues	Home-based cueing training program	30 mine session, 3 d/wk for 3 wk	Improvement in posture, gait speed, step length, reduction of FoG episodes and increased confidence in gait tasks
Frazzitta *et al*[9]	Treadmill + external cues	Progressive treadmill training with auditory (musical beats) and visual cues	20 min every day for 4 wk	Improvement in UPDRSIII, 6MWT, gait speed, stride cycle, FoG questionnaire

FoG: Freezing of Gait; 6MWT: 6-Minute walk test; UPDRS: Unified Parkinson's Disease Rating Scale.

untary task[11,48]. Auditory cues provide an external rhythm that bypasses the affected basal ganglia, thus improving gait performance, timing and cadence[9,7,33,49]. No information is given for the putative mechanism of somatosensory cues efficacy but the explanation may rationally be very similar to the auditory cues.

McIntosh and colleagues studied the effect of rhythmic auditory cues on the gait of PD patients. Their first study[49] was a 3-wk home-based gait training program: patients trained 30 min/d, with walking, stop-and-go and stair stepping performed while patients listened to music in a headset (music tempo was progressively increased during the experiment). There were significant improvements regarding gait speed, stride length and cadence. In the second protocol[50], auditory cues again facilitated subjects' gait, with significant improvements in gait speed (patients were able to walk at a speed higher than their maximal one), stride length and cadence. del Olmo *et al*[34] also assessed the effect of auditory cues on PD gait. Fifteen patients underwent gait training (which also included manual dual-tasking) with rhythmic sounds, 1 h per day, 5 d a month: auditory cues increased gait temporal stability, especially in those patients with greater impairment at baseline (a discovery that gains even more significance given that the level of gait impairment is proportional to PD severity).

Azulay *et al*[48] analyzed the effect of both dynamic and static visual cues on PD gait. Participants in the study session walked on a 12-m walkway with parallel transversal white stripes (visual cue) spaced at 45 cm intervals; patients walked first with normal lights on, and then with stroboscopic lights (used to suppress the perception of movement of the stripes). While performance significantly decreased due to the absence of dynamic cues (stroboscopic lights), patients increased velocity and stride length in the normal lights, suggesting the role of a specific visual-motor pathway elicited by the moving cues.

Most of the studies available in the literature compared the different type of cues. Rochester *et al*[35] examined the effect of both visual and auditory cues on a gait dual task performed at home: patients simply had to stand up, go to their kitchen, put 2 cups on a tray, walk back, leave the tray on a near table and sit down again. Auditory cues improved performance in the functional task (stride length), and a tendency for increased walking speed was noticed with both kinds of cue. The most complete study on external cues for PD is the RESCUE trial designed by Nieuwboer *et al*[23]. One hundred and fifty-three patients participated in this home-based cueing training program, in which patients had to perform gait tasks while receiving visual, auditory or somatosensory cues; training sessions lasted 30 min, 3 d per week, for 3 wk. The program generated many significant improvements in: posture and gait, gait speed, step length, reduction of FoG episodes (assessed through the FoG Questionnaire), and increased confidence in gait tasks (assessed with the Falls Efficacy Scale); importantly, 67% of patients preferred auditory cues, while the remaining 33% favored somatosensory cues.

Frazzitta *et al*[9] designed an experimental study, in which 40 patients who suffered FoG were divided into 2 groups: the first underwent progressive treadmill training with auditory (musical beats) and visual cues, while the second group followed a traditional protocol combining visual and auditory cues. Improvements were found in both groups for all the measured parameters (UPDRS-Ⅲ, 6MWT, gait speed, stride cycle, FoG questionnaire), showing the positive effect of both cues on motor performance. Table 3 summarizes the cueing strategies.

Improving physical capacity: Resistance training

Due to both central and peripheral causes, PD patients suffer muscle weakness (sarcopenia). Weakness in the lower limbs particularly affects basic daily tasks such as

standing up from a chair and walking. Moreover, sarcopenia is considered a secondary cause of bradykinesia[51]. Muscle weakness is also strongly related to impaired balance, since it reduces the ability to respond to postural and balance modifications[51]. When considering this observation, its association with the risk of falls is straightforward. Moreover, the consequences of balance loss (falls, injuries, immobilization) may adversely contribute to the maintenance of bone mass density in the hip[52], increasing the risk of hip fracture.

In PD, the nigral dopaminergic deficit results in an increase in tonic inhibition of the thalamus and reduction in the excitatory drive to the motor cortex leading to disruption of cortical activation of the muscle. Conceivably, this disorder may result in impaired motor unit recruitment and could contribute both to bradykinesia and muscle weakness.

When considering these impairments, resistance training has been proposed as an efficient intervention aimed at reducing muscle weakness, bradykinesia, balance problems, as well as improving bone parameters, physical functioning, ADLs and the quality of life[52-54].

Several observations suggest that resistance training may facilitate functional plasticity in the cortex and muscle activation patterns. For this reason, in PD patients, this kind of training could be therapeutic to modify the activity in the cortex and basal ganglia, as well as the connectivity between and within these structures. Indeed, several studies showed that resistance training resulted in an increase in electromyographic activation, possibly explained by improved motor unit recruitment, increased firing rate, and better synchronization[54].

The first resistance training intervention for PD was evaluated by Scandalis *et al*[55] in 2001. Their protocol included exercises for quadriceps, hamstring, calves and abdominal muscles. Training sessions took place twice a week for 8 wk. The authors described improvements in strength, gait speed and stride length.

Several studies assessed the effects of progressive resistance exercise (PRE), which uses a high resistance load progressively increasing during the period of training. In the study of Schilling *et al*[56], patients performed resistance training for lower limb muscles twice a week for 8 wk: as expected, leg strength was significantly improved. Hirsch *et al*[57] evaluated the efficacy of PRE for balance parameters. Patients were assigned to 2 different groups: while group 2 simply performed balance exercises, group 1 trained balance plus high-intensity PRE for knee and ankle muscles. Sessions took place 3 times per week on non consecutive days for 10 wk. Both groups improved balance, strength and reduced falls, but the group treated with PRE and balance training performed better and their results were greater. PRE, as shown by the study of Hass *et al*[53], may also improve typical walking impairment of PD such as gait initiation. Their 10-wk PRE program, which focused mainly on lower limb muscles, generated improvements both in postural adjustment and spatiotemporal parameters during gait initiation, and consequently muscle strength. O'Brien *et al*[58] focused more on the patient's perception of a PRE protocol. After 20 sessions (performed twice weekly), the researchers interviewed the participants. Patients reported physical and psychological benefits from the PRE program and expressed positive feedback as well as the intention to attend future programs.

Another very efficient strengthening method is eccentric training. The advantage of this type of conditioning lies in the fact that eccentric contraction can generate high forces and perform more work (and thus increase strength quicker) while requiring less energy when compared with concentric contraction, thus reducing fatigue. Dibble and colleagues rightly speculated on this assumption, and after having assessed the safety and feasibility of high-intensity eccentric training for PD patients, they evaluated its benefits. The protocol was the same for their 2 studies[59,60]: 2 groups (experimental and control) underwent the same exercise program, which included calisthenics, treadmill, balance training and conditioning 3 times per week for 12 wk; the only difference regarded lower muscle conditioning, for which the experimental group performed high-intensity eccentric exercise, while the control group underwent traditional strength exercises. Convincingly, in both studies, the experimental group results showed significant improvement in all tested parameters, namely: muscle hypertrophy, strength, mobility, bradykinesia, quality of life and UPDRS score (Table 4).

Balance training

Degeneration of the basal ganglia involves several physiological systems essential for balance control. Dysfunction of the basal ganglia influences the ability of central nervous system to translate sensory information (somatosensory, visual and vestibular) into a single reference frame, which is important for assessment of limb and body position in relation to the environment. Deficient motor regulation in PD manifests as poor inter-segmental coordination, difficulties adopting postural synergies and delayed adjustment in motor commands when moving from one task to another. To be specific, balance training needs to target functions, or impairments, of balance control associated with PD symptoms[61].

As already discussed, postural instability is probably the symptom with the lower response to pharmacotherapy[10,11,24]. A reduced ability to adapt to balance changes and perturbations automatically increases the risk of falling as well as the consequent injuries such as hip fracture[11]. The need for an integrative therapy that would ameliorate the PD patient's response is evident. With this aim, exercise interventions focused specifically on balance have been developed and evaluated.

Tai Chi, a Chinese martial arts discipline, has been proposed as a useful exercise program for PD patients since it encompasses techniques such as weight shifting, slow and controlled movement, trunk rotations, different stances, multidirectional stepping, and maintenance of postures that directly target PD balance and gait[62,63]. Many authors evaluated the effect of a Tai Chi program in PD patients (Table 5).

Table 4 Effects of resistance training

Scandalis *et al*[55]	Exercises for quadriceps, hamstring, calves and also abdominal muscles	2 times/wk for 8 wk	Improved strength, gait speed and stride length
Schilling *et al*[56]	PRE for lower limbs muscles	2 times/wk for 8 wk	Improved leg strength
Hirsch *et al*[57]	Balance exercise plus high-intensity PRE for knee and ankle muscles	3 times/wk for 10 wk	Improved balance, strength and reduced falls
Hass *et al*[53]	PRE program, focused mainly on lower limbs muscles	2 times/wk for 10 wk	Improvement in both postural adjustment and spatiotemporal parameters during gait initiation (protective effect on falls), and improved muscle strength
O'Brien *et al*[58]	PRE	2 times/wk for 10 wk	Physical and psychological benefits
Dibble *et al*[59,60]	High Intensity eccentric training exercise program for lower muscles which included calisthenics, treadmill, balance training and conditioning	3 times/wk for 12 wk	Improvement in muscle hypertrophy, strength, mobility, bradykinesia, Quality of life and UPDRS score

PRE: Progressive resistance exercise; UPDRS: Unified Parkinson's Disease Rating Scale.

Table 5 Effects of balance training

Li *et al*[62]	Tai Chi *vs* resistance training and stretching	60 min sessions 2 times/wk for 24 wk	Tai Chi group improved their postural stability significantly more than both the other groups; stride length and velocity, strength, timed up-and-go test, functional reaching and UPDRS-Ⅲ score were significantly higher in the Tai Chi group when compared with stretching; Tai Chi improved stride length, reduced rate of falls at follow up and, as shown by the posturography, there was a reduction of deviations of movement, which the authors suggest to be a reduction of dyskinesia
Hackney *et al*[63]	Tai Chi program	60 min session for 10-13 wk (total 20 session)	Improved global and motor symptoms (UPDRS and UPDRS-Ⅲ), balance, tandem stance, one leg stance, backward walking, and gait endurance (6MWT)
Schmitz-Hubsch *et al*[24]	Qi Gong program	90 min weekly training for 2 mo	Intervention showed a "stabilizing effect on PD symptoms": specifically, postural instability improved, as well as UPDRS-Ⅲ score. Also, autonomic dysfunction (constipation and pain) decreased, and during physiotherapy sleep disturbances and daytime sleepiness diminished

6MWT: 6-Minute Walk Test; UPDRS: Unified Parkinson's Disease Rating Scale.

Li *et al*[62] compared the effect of a Tai Chi program with resistance training and stretching (used as control). Subjects in each group trained twice a week for 6 mo. The Tai Chi training program was particularly designed for balance and gait training. Results showed that the Tai Chi group improved their postural stability significantly more than either of the other groups. Stride length and velocity, strength, timed up-and-go test (which evaluates static and dynamic balance and gait), functional reaching and UPDRS-Ⅲ score were significantly higher in the Tai Chi group when compared to stretching. Furthermore, 2 findings are of particular significance: the rate of falls at follow-up was lower in Tai Chi group, and, as shown by posturography, there was a reduction in deviations of movement as a result of reduced dyskinesia. Hackney and Earhart[63] evaluated the effect of 20 lessons (over 13 wk) of Tai Chi. Patients improved their global and motor symptomatology (UPDRS and UPDRS-Ⅲ), balance, tandem stance, one leg stance, backward walking, and gait endurance (6MWT). In addition, "patients reported enjoyment in the protocol and physical and psychological improvements".

Schmitz-Hübsch *et al*[24] determined the effect of another Chinese exercise therapy, Qi Gong (which includes posture, breathing techniques and attention strategies). Their 2-mo intervention showed a "stabilizing effect on PD symptoms": specifically, postural instability improved, as well as UPDRS-Ⅲ score. Moreover, autonomic dysfunction (constipation and pain) decreased, and during physiotherapy sleep disturbances and daytime sleepiness diminished.

Finally, worthy of mention is the innovative, balance-specific program designed and assessed by Esculier *et al*[64]. The researchers submitted PD patients to a home-based program using Nintendo Wii Fit with balance board. The device focuses on balance tasks and visual feedback of movements is constantly provided, together with auditory and proprioceptive cues. Additionally, the console and the games seemed to be very enjoyable and motivating for the patient. In this study, patients trained for 40 min, 3 d a week, for 6 wk. Results were meaningful, with improvements in static and dynamic balance, gait, functional strength of the lower limbs, one-leg stance time and reduced fear of falling.

Dance

Due to its nature, dance appears to be one of the most effective exercise protocols for PD patients. Indeed, as discussed by Dr. Earhart, all the recommended key areas[28] for physical therapy in PD are met[65]. Music serves as an

Table 6 Results of dance training

Hackney *et al*[66,69]	Tango	60 min session, 2 d/wk for 10 wk (total 20 sessions)	Decreased UPDRS score, improved balance, reduced fear of falling. Trends of improvement for FoG and at Timed Up and Go test
Hackney *et al*[67]	Tango *vs* Foxtrot	60 min session, 2 d/wk for 13 wk (total 20 session)	Both types of dance improved gait speed, balance, backward stride length, cardiovascular function and symptoms (UPDRS); only Tango generated improvements for FoG
Duncan *et al*[70]	Tango in patients "off medication"	60 min session, 2 d/wk for 12 mo	Bradykinesia and motor symptoms severity (assessed with MDS-UPDRS-Ⅲ) were reduced; gait speed, balance, dual task walking speed and upper extremity function all improved; rigidity, FoG and gait endurance remained stable, but in the control group they progressively worsened, ("braking" effect on PD progression)

PD: Parkinson's disease; UPDRS: Unified Parkinson's Disease Rating Scale.

external cue, thus facilitating motor performance; specific movement strategies are taught; balance is trained, especially in its dynamic form. Although not directly addressed, dance may improve strength and flexibility. Finally, when trained at the right intensity, dance promotes cardiovascular functioning as an optimal form of aerobic exercise. This discipline may be considered an ideal choice among all adapted physiotherapy programs, since it addresses specific Parkinsonian impairments such as walking backward, turning and multitasking. Furthermore, the Tango appears to be the most Parkinson-specific discipline, since the basic step used is walking; frequent stops and starts are common (thus challenging the patient's start hesitation); directional changes and turning are included, and dancing at different rhythms and speeds addresses bradykinesia. Furthermore, some techniques like stepping or tapping the partner's feet, crossing feet, and shifting the body weight from one leg to another, are very similar to strategies used in rehabilitation of FoG[65-68].

Researchers have evaluated 2 dance discipline so far: waltz/foxtrot and, of course, Tango. In 2 studies, Hackney *et al*[66,67] reported that 20 Tango sessions diminished symptoms, improved balance, and reduced fear of falling. Moreover, trends of improvement in FoG and the Timed Up and Go test (which measures static and dynamic balance) were found. Significantly, in both studies, half of the patients decided to participate in additional Tango sessions; an unequivocal sign of the patients' enjoyment of the protocol. Hackney and Earhart[68,69] also tried to evaluate the difference between partnered and non-partnered dance, since "the partner's importance and influence remains equivocal". Again, 20 Tango sessions generated improvements in gait, balance and functional mobility. No difference were found between the 2 groups, but the authors suggested that, for safety reasons, a partner may be useful for patients in the later stages of PD since the partner acts as a balance support. Duncan and Earhart[70] reported a 12-mo study, in which 52 patients assigned to a Tango group trained twice a week for 1 h. In this study, patients were tested only off medication "to ultimately determine whether exercise may be disease modifying". Significantly, patients benefitted greatly from the long-term protocol: motor symptom severity (assessed with MDS-UPDRS-Ⅲ) was reduced, as well as bradykinesia. Gait speed, balance, dual task walking speed and upper extremity function all improved. Rigidity, FoG and gait endurance remained stable, but in the control group they progressively worsened. These results confirm how exercise can have a "braking" effect on PD progression (Table 6).

CONCLUSION

With the exception of tremors, tailored physical activity has shown to improve all the prominent motor symptoms of PD patients including those harmful disturbances such as FoG, stride variability and balance impairments. Furthermore, it has been clearly demonstrated that each of the different types of physical activity resulted in a better quality of life. It is therefore reasonable to state that tailored physical activity could be considered as a valid intervention to be included in the therapeutic program of PD patients.

Each training protocol has specific technical characteristics targeting different PD deficiencies. Studies applying treadmill training described improvements in patient gait in quick FoG, festinating gait and balance loss. Moreover, in several studies researchers reported improvements in UPDRS, functional capacity and quality of life.

The use of external cues to bypass the affected basal ganglia also showed promising results. Most of the spatiotemporal walking parameters, such as gait speed, stride variability, cadence, and step length improved after the use of external cues. Nieuwboer *et al*[23] also reported that external cues reduced FoG events and improved patient confidence when considering the risk of falls.

Dancing is an alternative program which seems promising and efficient for the treatment of PD symptoms. Specific Parkinsonian patterns such as bradykinesia, dynamic balance, backward walking, turning and multitasking are directly targeted with dance. In addition, the Argentinian Tango can be labeled as a "Parkinson-specific discipline". The effect of the dance relies on its social and enjoyable nature, which stimulates patient compliance for longer periods, thus potentially enhancing the positive effects of the program and expanding its beneficial effect to the emotional and psychological sphere.

The beneficial effects of resistance training are not limited to muscle hypertrophy and improved strength. Indeed, significant improvements have been described when considering balance, bradykinesia, gait, mobility

and quality of life. Balance-specific protocols, mainly Tai Chi, resulted in significant improvements when considering gait, balance and posture, finally leading to a reduced risk of falls.

As discussed by most of the authors cited, the main limit of adapted physical therapy in PD patients relies in the lack of a standardized therapeutic protocol for common use. Even if future investigations should address this issue, we strongly support the adoption of an individualized approach. Indeed, PD is a very complex disease, with different and fluctuating symptoms which affect the patients almost uniquely. Consequently, a physical therapy protocol should not be standardized, but tailored and individualized to the patient's personal condition in order to target his/her precise motor impairments.

Consequently, it seems crucial to educate the patient early about the benefit of an active lifestyle, including regular participation in an specific physiotherapy program, in order to promote independence, physical functionality and quality of life. Patients should choose an enjoyable program in order to promote adherence. Based on the available experience, training sessions should last 60-75 min at least 3 times per week, especially in the earlier stages. It seems to be useful to perform stretching exercises daily in order to reduce rigidity and improve joint and muscular capability.

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REVIEW

Closer look at white-coat hypertension

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Abstract

This review aims to clarify novel concepts regarding the clinical and laboratory aspects of white-coat hypertension (WCHT). Recent studies on the clinical and biological implications of WCHT were compared with existing knowledge. Studies were included if the WCHT patients were defined according to the 2013 European Society of Hypertension guidelines, *i.e.*, an office blood pressure (BP) of $\geqslant$ 140/90 mmHg, a home BP of $\leqslant$ 135/85 mmHg, and a mean 24-h ambulatory BP of $\leqslant$ 130/80 mmHg. WCHT studies published since 2000 were selected, although a few studies performed before 2000 were used for comparative purposes. True WCHT was defined as normal ABPM and home BP readings, and partial WCHT was defined as an abnormality in one of these two readings. The reported prevalence of WCHT was 15%-45%. The incidence of WCHT tended to be higher in females and in non-smokers. Compared with normotensive (NT) patients, WCHT was associated with a higher left ventricular mass index, higher lipid levels, impaired fasting glucose, and decreased arterial compliance. The circadian rhythm in WCHT patients was more variable than in NT patient's, with a higher pulse pressure and non-dipping characteristics. Compared with sustained hypertension patients, WCHT patients have a better 10-year prognosis; compared with NT patients, WCHT patients have a similar stroke risk, but receive more frequent drug treatment. There are conflicting results regarding WCHT and markers of endothelial damage, oxidative stress and inflammation, and the data imply that WCHT patients may have a worse prognosis. Nitric oxide levels are lower, and oxidative stress parameters are higher in WCHT patients than in NT patients, whereas the antioxidant capacity is lower in WCHT patients than in NT patients. Clinicians should be aware of the risk factors associated with WCHT and patients should be closely monitored especially to identify target organ damage and metabolic syndrome.

Key words: White-coat hypertension; Ambulatory blood pressure; Target organ damage; Glucose dysregulation

Core tip: There is contradictory information regarding the clinical presentations and prognosis of white-coat hypertension (WCHT). This review aims to summarize recent research and compare it to existing knowledge about WCHT. Published studies on the prevalence of WCHT, the associated target organ damage and cardiovascular markers, and WCHT patient prognosis were reviewed. WCHT may be a marker of future obesity and metabolic syndrome, is related to glucose dysregulation and left ventricular hypertrophy and may progress to sustained hypertension. Clinicians should be aware of the risk factors associated with WCHT, and patients should be closely monitored, especially to identify target organ damage and metabolic syndrome.

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DEFINITION OF WHITE-COAT HYPERTENSION

According to the 2013 guidelines published by the Euro-

pean Society of Hypertension and the European Society of Cardiology for the management of hypertension white-coat hypertension (WCHT) is defined for patients who are not taking medication as an office blood pressure (BP) of ⩾ 140/90 on at least three occasions in the presence of a health care worker (particularly a physician),with normal 24-h (⩽ 125-130/80 mmHg) and day ambulatory BP monitoring (ABPM) (⩽ 130-135/85 mmHg) or a normal home BP (average of several readings, ⩽ 130-135/85 mmHg)[1]. There were no changes in the definition of WCHT in the JNC8[2]. The American Heart Association (AHA) recommends home BP monitoring for patients with pre-hypertension (120-139/80-89 mmHg) and for patients diagnosed with hypertension (systolic BP ⩾ 140 mmHg or diastolic BP ⩾ 90 mmHg)[3].

It is recommended that at least two 24-h ABPM measurements be recorded to confirm WCHT[4]. WCHT can be divided into two subgroups: partial WCHT, where either the ABPM or the home BP readings are elevated; and true WCHT, in which both the ambulatory and home BP values are normal[5].

WCHT should be differentiated from the white coat effect, which is a rise in BP in response to the presence of a medical practitioner and may be observed in all types of patients, from normotensive (NT) persons to patients with sustained hypertension (SHT) in the presence or absence of therapy[6].

The prevalence of WCHT, its associations with other clinical conditions and vascular biomarkers, the risk of target organ damage and patient prognosis in comparison with other forms of hypertension will be discussed in this review.

Studies were included if the WCHT patients were defined according to the 2013 European Society of Hypertension guidelines. WCHT studies published since 2000 were selected, although a few studies performed before 2000 were used for comparative purposes.

THE PREVALENCE OF WHITE-COAT HYPERTENSION

The Finn-Home study was performed on 1540 untreated participants with an age range of 44 to 75 years. Two hundred thirty-three patients had WCHT. The prevalence of WCHT was 15.1%. The home BP levels of the WCHT patients were higher than those of the NT individuals. The WCHT group was older and had a higher proportion of men; WCHT patients had more metabolic risk factors than NT individuals[7].

The PAMELA study was a population study performed in Monza, Italy. BP was measured in the office and, twice daily, by subjects at home. The investigators assessed the cardiovascular and all-cause mortality over 16 years (1992-2008) in 2051 patients with an age range of 25-74 years. The prevalence of WCHT among untreated hypertensive patients was estimated at 15%-45%, and WCHT was associated with non-smoking, female gender and increasing age. The prevalence of partial WCHT was 58%, whereas the prevalence of true WCHT was 42%[5].

Pickering *et al*[8] reported that the prevalence of WCHT was 21% in a 1988 study of 292 patients. WCHT patients tended to be female, be younger, and have a lower body weight.

PREVALENCE OF WHITE-COAT HYPERTENSION IN PATIENTS WITH CHRONIC RENAL DISEASE

The prevalence of WCHT was 15% among 355 long-term hemodialysis patients. When a pre-dialysis BP threshold of 140/90 mmHg was used to classify patients into BP categories, the prevalence of WCHT was 26%[9].

In a study by Bangash *et al*[10], the prevalence of WCHT was 18.3% in 980 patients with chronic renal disease (CRD). The threshold for identifying hypertension based on clinic and ambulatory BP measurements strongly influenced the risk of being diagnosed with masked hypertension (MHT) rather than WCHT. In studies of CRD patients with overt proteinuria, the lower threshold for WCHT (< 140/90 mmHg) was responsible for the increased prevalence of WCHT.

PREVALENCE OF WHITE-COAT HYPERTENSION IN TYPE 2 DIABETES MELLITUS PATIENTS

In a Chinese study of 473 patients, the prevalence of WCHT was 7.36% in the overall population, 6.13% in male patients and 8.88% in female patients ($P < 0.05$). Age, etiology of type 2 diabetes mellitus (DM) and male gender were dependent factors, whereas female gender, smoking and alcohol consumption were independent risk factors for WCHT in patients with type 2 DM[11]. These findings are in accordance with previous studies, and the characteristics of WCHT in diabetic patients are similar to those in the general population.

WHITE-COAT HYPERTENSION AND METABOLIC SYNDROME

According to the 2009 Joint Statement on Metabolic Syndrome, metabolic syndrome is defined as the presence of abnormalities in three of the following five characteristics: waist circumference, triglyceride levels, high-density lipoprotein levels, BP and fasting blood glucose[12].

Helvaci *et al*[13,14] analyzed 955 patients (566 females and 389 males) and suggested that WCHT is not a predisposing factor for hypertension (HT) or atherosclerosis but rather it is an alarm signal. There was an increasing prevalence of obesity, impaired glucose tolerance (IGT) or DM, and coronary heart disease (CHD) between the WCHT and HT groups compared with the NT group. According to this study, the prevalence of dyslipidemia

was the highest in the WCHT group (41.6%, $P < 0.05$), followed by 35.5% in the SHT group and 19.6% in the NTgroup.

In another study by Björklund *et al*[15], 602 male patients aged 50 years and older who had WCHT were followed for 20 years. Their baseline body mass index (BMI) values were similar to those of the individuals in the NT group (23.0 kg/m^2 *vs* 23.8 kg/m^2) at age 70. However, metabolic abnormalities (insulin sensitivity, elevated blood glucose, and increased serum insulin) and elevated heart rate (HR) developed over time in patients with WCHT and SHT. A lower BMI and a more favorable dietary fat composition predicted the development of WCHT as opposed to SHT.

The PAMELA study demonstrated that patients with WCHT had similar baseline BMI values to patients with SHT(27 ± 4.3 kg/m^2 *vs* 27.4 ± 5.2 kg/m^2) but had higher fasting glucose levels compared with NT individuals (93.2 ± 20.9 mg/dL *vs* 85.5 ± 12.5 mg/dL, $P < 0.05$). At the end of the 10-year follow-up period, patients in the WCHT group were more likely to develop diabetes than those in the NT group (OR = 2.9)[16].

A study conducted in Turkey by Afsar[17] revealed that the progression from sustained NT to SHT involves an increase in serum uric acid levels, impaired fasting glucose levels, and increases in BMI and waist circumference. The presence of metabolic syndrome was highest in people with SHT and lowest in those with sustained NT ($P < 0.0001$). This study concluded that the changes in these parameters were not as substantial in the WCHT group as they were when comparing the MHT and SHT groups to the NT group.

Several other studies have shown that the prevalence of impaired fasting glucose levels and abnormal glucose tolerance test results is higher in WCHT patients than in NT individuals. These findings suggest that WCHT is associated with glucose dysregulation and an increased risk for diabetes[18,19].

Therefore, WCHT is an initial sign of deteriorating health. It commonly accompanies hyperlipidemia, elevated fasting glucose levels and a tendency toward being overweight.

WHITE-COAT HYPERTENSION IN ELDERLY PATIENTS

Two studies have analyzed WCHT in elderly people. In the first study, which was published by Hekman *et al*[20], elderly women (age range, 60-83 years; mean age, 69 ± 7 years) with WCHT had a higher SBP than NT elderly women between the hours of 8 am and 12 pm (133 ± 8.0 mmHg *vs* 123 ± 9.0 mmHg, $P < 0.001$). The BP variability was higher in the WCHT group only during the wakeful period (between 7 am and 11 pm, $P = 0.02$).

Age and BMI positively correlated with mean SBP at night. In the elderly women with WCHT, a higher SBP was associated with increasing age and BMI ($P = 0.015$ and $P = 0.055$, respectively). Elderly women with WCHT were more likely to smoke ($P = 0.014$) and snore ($P = 0.046$)[20].

In the second WCHT study on the elderly, Franklin SS. and colleagues analyzed 1168 untreated subjects with a mean age of 48.8 ± 16.6 years and with isolated systolic hypertension (ISH); 28.6% of the study participants had WCHT. The cardiovascular risk in untreated WCHT patients with ISH was similar to that in NT individuals ($P = 0.63$). Compared with the untreated NT individuals, individuals with WCHT undergoing treatment for ISH as well as treated NT individuals were at a higher cardiovascular risk ($P < 0.01$)[21]. The results suggested that age, BMI and the need for treatment increased the cardiovascular risk in elderly patients with WCHT.

CIRCADIAN RHYTHMS IN PATIENTS WITH WHITE-COAT HYPERTENSION

BP, HR, cardiac output and serum catecholamine levels increase during the day and decrease during the night. These changes enable an organism to adapt to the need for higher activity levels while awake. The decrease in BP at night is defined as "dipping," and patients who fail to show this pattern are called "non-dippers"[22].

A study by Koroboki *et al*[22] determined that WCHT patients have the same circadian pattern as NT, MHT and untreated HT patients; however, the daytime and nighttime pulse pressures were higher in WCHT patients than in NT individuals, with nighttime pulse pressures reaching those in MHT patients. Circadian BP and HR profiles in MHT and WCHT patients have been compared with those in NT patients and in treated and untreated SHT patients using ambulatory BP measurements.

Pierdomenico *et al*[23] studied 12 NT, 12 WCHT and 12 SHT patients in 2000. The subjects underwent ABPM. Power spectral analyses of the R-R intervals were performed to obtain the low and high frequency components, concomitant with 24-h urine testing for epinephrine and norepinephrine. This study demonstrated that patients with WCHT and SHT have similar circadian patterns based on ABPM; however, the other findings indicated sympathetic overactivity throughout the day in SHT patients but not in WCHT patients, suggesting that the two conditions may have different pathophysiological backgrounds[23].

Vyssoulis *et al*[24] classified WCHT patients according to the presence of accompanying metabolic syndrome traits and thus divided the patients into two groups based on the presence ($n = 522$) or absence ($n = 1778$) of metabolic syndrome. Patients with WCHT and a greater number of metabolic syndrome traits had non-dipping characteristics, along with elevated nighttime SBP levels that are indicative of an increased cardiovascular risk[24].

WHITE-COAT HYPERTENSION AND TARGET ORGAN DAMAGE

In 2003, Karter *et al*[25] studied 50 NT, 90 WCHT and

101 SHT subjects and reported that WCHT patients had a higher BMI and a greater left ventricular mass index (LVMI) than NT individuals ($P < 0.001$). Urinary albumin excretion was similar in WCHT patients and in those with SHT. No difference in renal function between WCHT and HT patients was noted by Pierdomenico *et al*[26].

In the PAMELA study, Cardillo, Weber, Mancia and Mulè reported that LVMI was increased in WCHT patients compared with NT patients ($P < 0.01$), whereas Pierdomenico and Hoeghelm found no difference in LVMI[5,26-31].

Arterial compliance was lower in WCHT patients than in the NT group in studies by Karter's ($P < 0.001$) and Gomez[25,32]. Turfaner *et al*[33] analyzed 47 dipper and 43 non-dipper WCHT patients and determined, that non-dipping in WCHT patients was related to decreased arterial compliance and that the global risk load for target organ damage was higher in non-dipper WCHT subjects.

The carotid artery intimal media thickness (IMT), which is used to measure the progression of atherosclerosis in WCHT patients, has been reported to be similar in NT and WCHT individuals (see studies by Pierdomenico, Karter, Roman and Gariepy[25,34-36]).

In contrast, the HARVEST study by Puato *et al*[37] compared the baseline and follow-up IMT values in 35 WCHT, 20 NT and 39 SHT patients over five years. The baseline ($P = 0.004$) and follow-up ($P < 0.01$) IMT values were significantly higher and increased faster in WCHT patients than in NT controls. There was no significant difference between patients with WCHT and those with SHT ($P = 0.27$). This increase in IMT was associated with triglyceride levels, age and mean arterial pressure at ABPM, as determined by multivariate regression analysis[37].

In a cross-sectional survey that included 2915 Japanese patients aged ⩾ 40 years, the carotid IMT was significantly thicker in WCHT patients than in NT patients (0.73 mm *vs* 0.67 mm, $P = 0.001$)[38].

WHITE-COAT HYPERTENSION AND BIOVASCULAR MARKERS

Studies on endothelial damage and angiogenesis, which indicate an increased risk for a poor prognosis in WCHT patients, have suggested that WCHT is associated with significantly higher endothelin-1 and vascular endothelial growth factor levels[39].

There is controversy regarding the amount of Nitric Oxide (NO) in WCHT patients. Karter observed higher NO levels in WCHT patients compared with NT patients ($P < 0.001$). Karter *et al*[39], Pierdomenico *et al*[40] and Güven *et al*[41] showed no significant differences in NO levels in WCHT and NT patients. Pierdomenico *et al*[40] and Guven *et al*[41] demonstrated that NO levels were higher in WCHT patients than in SHT patients ($P < 0.05$). The difference was more significant in the Karter study ($P < 0.001$). In the Karter study, the threshold for clinical WCHT was defined as a DBP > 85 mmHg; in other studies, the threshold was defined as a BP ⩾ 140/90 mmHg, which may result in a difference in endothelial dysfunction parameters.

Homocysteine and asymmetric dimethylarginine levels were examined in two different studies with contradictory results. Pierdomenico found no significant differences in homocysteine levels among WCHT patients and NT patients, whereas Curgunlu *et al*[42,43] showed significantly higher homocysteine levels in WCHT patients ($P < 0.001$). Homocysteine levels were significantly higher in SHT patients than in WCHT patients, with P values of 0.0003 and $P < 0.001$ in the studies performed by Pierdomenico *et al*[44] and Curgunlu *et al*[42,43], respectively. Regarding inflammation, only one of three WCHT studies on C-reactive protein found a significant difference between the WCHT and NT groups[41,45,46].

The reports on oxidative stress markers in WCHT are conflicting. Among the oxidative stress parameters, paraoxonase (PON-1) levels were significantly lower ($P < 0.001$) and malondialdehyde (MDA) levels were higher ($P < 0.026$) in WCHT patients compared with NT patients, whereas ox-LDL was not significantly different between the NT, WCHT and SHT groups[47].

A study by Caner *et al*[48] on other oxidative stress parameters, such as protein carbonyl(PCO) and antioxidant capacity, showed that PCO was higher ($P < 0.001$) and that antioxidant markers(plasma thiol, plasma CuZn-SOD and erythrocyte glutathione) were lower ($P < 0.01$)in WCHT patients compared with the NT group. Plasminogen activator 1(PAI-1) and von Willebrand factor levels were not different between the WCHT and NT groups[45].

PROGNOSIS AND STROKE RISK IN PATIENTS WITH WHITE-COAT HYPERTENSION

Verdecchia *et al*[49] reported that the cumulative hazard for stroke in WCHT patients (based on ABPM) tended to increase after 6 years of follow-up and exceeded that of ambulatory hypertensive patients after 9 years of follow-up.

In Japan, 1332 subjects (872 females and 460 males, age ⩾ 40 years) who were representative of the Japanese population were followed for 10 years to monitor stroke risk as part of the OHASAMA study. There was no significant difference in outcome between WCHT and NT patients (daytime BP < 135/85 mmHg based on ambulatory BP)[50].

Pierdomenico *et al*[51] compared the cardiac and cerebrovascular risks in SHT and WCHT individuals, which were reported to be RR = 4.16, 95%CI: 1.48-11.6, $P = 0.007$, and RR = 4.12, 95%CI: 1.62-10.5, $P = 0.003$, respectively. There was no significant difference between the NT and WCHT individuals in this study, which followed 1732 subjects (1333 SHT, 399 WCHT, and 305 NT) for 6 years.

In another study, Pierdomenico *et al*[52] compared the cardiovascular risk in NT and WCHT patients and found no statistically significant differences, regardless of the

NT population type or the follow-up duration. They noted that the WCHT patients were more likely to be receiving drug treatment when compared with the NT patients.

The PAMELA study reported that in partial WCHT patients with either ABPM or home BP monitoring abnormalities, the incidence of fatal events was markedly increased, with a 60% higher fully adjusted risk of cardiovascular and all-cause mortality compared with NT controls; however, the risks of cardiovascular and all-cause mortality were not significantly different from those in NT subjects with true WCHT[5,29].

TREATMENT

Subjects with WCHT frequently have dysmetabolic risk factors and asymptomatic organ damage, which increase the cardiovascular risk. In these higher-risk individuals with WCHT, drug treatment may be considered in addition to appropriate lifestyle changes. Both lifestyle changes and drug treatment may also be considered when normal ambulatory BP values are accompanied by abnormal home BP values (or vice versa) because this condition is also characterized by increased cardiovascular risk. In the absence of additional cardiovascular risk factors, intervention may be limited to lifestyle changes but should include meticulous follow-up (including periodic out-of-office BP monitoring) because the out-of-office BP is often higher in WCHT patients than in truly NT individuals, and people with WCHT have a greater risk of developing organ damage or progressing to diabetes and SHT[1,2].

CONCLUSION

WCHT is a sign of deteriorating health. It is often accompanied by hyperlipidemia, elevated fasting glucose levels and a tendency toward being overweight. Based on the clinical and laboratory features, WCHT can be placed on a spectrum of BP disorders that extends between NT and SHT. Commonly, WCHT progresses to SHT, obesity and metabolic syndrome. In the elderly, the cardiovascular risk associated with WCHT increases with age, BMI and the need for treatment[53].

Patients with WCHT should be assessed for the presence of target organ damage and for the development of cardiovascular risk factors. These assessments should include an oral glucose tolerance test. Patients should be educated regarding increased cardiovascular and diabetes risks, with a special emphasis on maintaining or losing weight. Patients should limit salt intake and should not consume processed food. Patients with WCHT should be monitored for conversion to SHT (ABPM every six months or yearly and/or regular home monitoring).

In the future, with the increased use of ABPM or home monitoring, patients with WCHT will be identified more often and more easily; it may become possible to protect these patients from developing target organ damage[54].

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REVIEW

Dental movement acceleration: Literature review by an alternative scientific evidence method

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Abstract

The aim of this study was to analyze the majority of publications using effective methods to speed up orthodontic treatment and determine which publications carry high evidence-based value. The literature published in Pubmed from 1984 to 2013 was reviewed, in addition to well-known reports that were not classified under this database. To facilitate evidence-based decision making, guidelines such as the Consolidation Standards of Reporting Trials, Preferred Reporting items for systematic Reviews and Meta-analyses, and Transparent Reporting of Evaluations with Non-randomized Designs check list were used. The studies were initially divided into three groups: local application of cell mediators, physical stimuli, and techniques that took advantage of the regional acceleration phenomena. The articles were classified according to their level of evidence using an alternative method for orthodontic scientific article classification. 1a: Systematic Reviews (SR) of randomized clinical trials (RCTs), 1b: Individual RCT, 2a: SR of cohort studies, 2b: Individual cohort study, controlled clinical trials and low quality RCT, 3a: SR of case-control studies, 3b: Individual case-control study, low quality cohort study and short time following split mouth designs. 4: Case-series, low quality case-control study and non-systematic review, and 5: Expert opinion. The highest level of evidence for each group was: (1) local application of cell mediators: the highest level of evidence corresponds to a 3B level in Prostaglandins and Vitamin D; (2) physical stimuli: vibratory forces and low level laser irradiation have evidence level 2b, Electrical current is classified as 3b evidence-based level, Pulsed Electromagnetic Field is placed on the 4th level on the evidence scale; and (3) regional acceleration phenomena related techniques: for corticotomy the majority of the reports belong to level 4. Piezocision, dentoalveolar distraction, alveocentesis, monocortical tooth dislocation and ligament distraction technique, only had case series or single report cases (4th level of evidence). Surgery first and periodontal distraction have 1 study at level 2b and corticision one report at level 5. Multiple orthodontic acceleration reports on humans were identified by an alternative evidence level scale, which is a simple and accurate way of determining which techniques are better and have a higher rate of effectiveness. The highest level of evidence for a specific procedure to accelerate orthodontic dental movement up to October 2013 was surgery first followed by low level laser application, corticotomy and periodontal distraction located on level 2, recommendation grade b from this proposed scientific evidence-based scale.

Key words: Orthodontic movement; Evidence-based dentistry; Dental movement acceleration

Core tip: Orthodontic systematic reviews of randomized clinical trials, meta analysis and meta analysis network are difficult to develop due to a lack of high quality randomized clinical trials related to orthodontic therapies. The correct classification of the scientific literature fol-

lowing the evidence-based hierarchy facilitates the answers to specific clinical questions, and thus its application in every scientific subject. The resources available to speed up orthodontic movement had been widely examined. Due to a lack of evidence-based strength, the latter method cannot be taken into account in clinical protocols, thus we are left with the main already clinically proven methods: local injection of cellular mediators, physical stimuli, and surgically assisted orthodontics.

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INTRODUCTION

Any clinical inquiries in orthodontics should only be responded to after a thorough and critical analysis of the available scientific literature on the subject in question. Orthodontic patients deserve the highest level of care that is only possible through the strict use of the best available current information[1].

The best method for optimal information analysis involves stratified levels of evidence and grades of recommendations, regardless of the current classification. Evidence Based Dentistry is the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of each patient[2-5].

The Oxford University evidence-based classification system includes every study carried out in humans using a very complete system (Table 1). The OCEBM levels have the advantage of offering levels of evidence for therapy, prognosis, diagnosis, differential diagnosis, and economic analysis all in a single table. In 2011, this system was simplified and modified, but left out the inclusion or possible adaptation of orthodontic scientific studies (Table 2).

In Table 3 an alternative scientific method to classificate scientific articles related to orthodontic therapies is proposed.

Orthodontic systematic reviews of randomized clinical trials (RCTs), meta-analysis and meta-analysis network are difficult to develop due to a lack of high quality randomized clinical trials related to orthodontic therapies. In the future, orthodontics ideally should develop and include prospective meta-analyses, thus avoiding the classic limitations of previous randomized clinical trials. The correct classification of the scientific literature following the evidence-based hierarchy facilitates the answers to specific clinical questions, and thus its application in every scientific subject.

The resources available to speed up orthodontic movement have been widely investigated in humans and animals. Due to a lack of evidence-based strength the latter cannot be taken into account in clinical protocols, thus we are left with the main already clinically proven methods: local injection of cellular mediators, physical stimuli, and surgically assisted orthodontics.

The main objective of this literature review was to analyze successful publications and the methods used to speed up orthodontic treatment and determine which publications carry a high evidence-based value.

LITERATURE SEARCH

The following clinical question was asked: Is there a way to move a tooth faster than conventional orthodontics? In order to begin the related literature search, the available methods to accelerate dental movement in adults were researched to determine which of these methods showed the highest level of scientific evidence.

Literature published in Pubmed from 1984 to October 2013 was reviewed, in addition to well-known reports that were not classified under this database.

To facilitate evidence-based decision making, guidelines such as the Consolidation Standards of Reporting Trials, Preferred Reporting Items for Systematic Reviews and Meta-analyses, and Transparent Reporting of Evaluations with Non-randomized Designs check list were used[6-8].

INCLUSION CRITERIA

Studies in any language and controlled or randomized clinical studies in humans.

EXCLUSION CRITERIA

In vitro or animal studies, reports that included non-effective methods to speed up dental movement and reports on the acceleration of dental movement that did not evaluate time in their research.

The studies were initially divided into three groups: local application of cell mediators, physical stimuli, and techniques that took advantage of the regional acceleration phenomena.

The articles were classified according to their level of evidence as shown in Table 3.

LOCAL APPLICATION OF CELL MEDIATORS

Local application of prostaglandins

The highest level of evidence corresponds to a 3B level from 3 publications: Yamasaki *et al*[9] developed a study which was divided into three phases. The first phase was on premolars which were to be extracted, on one side, they used sub mucosal injections of prostaglandin E1 (PGE1) and on the other side a vehicle substance was injected. The rate of movement of the teeth towards the buccal area was approximately 2-fold at the site of PGE1 injection. A similar result was obtained in the second phase where PGE1 injections were administered in the canine retraction areas for a period of 3 wk. The third

Table 1 The Oxford University evidence based classification applies and includes all studies performed on humans using a very complete system

Level	Therapy/ prevention, aetiology/harm	Prognosis	Diagnosis	Differential diagnosis/ symptom prevalence study	Economic and decision analyses
1a	SR (with homogeneity) of RCTs	SR (with homogeneity) of inception cohort studies; CDR" validated in different populations	SR (with homogeneity) of Level 1 diagnostic studies; CDR" with 1b studies from different clinical centres	SR (with homogeneity) of prospective cohort studies	SR (with homogeneity) of Level 1 economic studies
1b	Individual RCT (with narrow confidence interval")	Individual inception cohort study with > 80% follow-up; CDR" validated in a single population	Validating cohort study with good reference standards; or CDR" tested within one clinical centre	Prospective cohort study with good follow-up	Analysis based on clinically sensible costs or alternatives; systematic review(s) of the evidence; and including multi-way sensitivity analyses
1c	All or none	All or none case-series	Absolute SpPins and SnNouts	All or none case-series	Absolute better-value or worse-value analyses
2a	SR (with homogeneity) of cohort studies	SR (with homogeneity) of either retrospective cohort studies or untreated control groups in RCTs	SR (with homogeneity) of Level > 2 diagnostic studies	SR (with homogeneity) of 2b and better studies	SR (with homogeneity) of level > 2 economic studies
2b	Individual cohort study (including low quality RCT; *e.g.*, < 80% follow-up)	Retrospective cohort study or follow-up of untreated control patients in an RCT; Derivation of CDR" or validated on split-sample only	Exploratory cohort study with good reference standards; CDR" after derivation, or validated only on split-sample or databases	Retrospective cohort study, or poor follow-up	Analysis based on clinically sensible costs or alternatives; limited review(s) of the evidence, or single studies; and including multi-way sensitivity analyses
2c	"Outcomes" Research; ecological studies	"Outcomes" Research		Ecological studies	Audit or outcomes research
3a	SR (with homogeneity) of case-control studies		SR (with homogeneity) of 3b and better studies	SR (with homogeneity) of 3b and better studies	SR (with homogeneity) of 3b and better studies
3b	Individual case-control study		Non-consecutive study; or without consistently applied reference standards	Non-consecutive cohort study, or very limited population	Analysis based on limited alternatives or costs, poor quality estimates of data, but including sensitivity analyses incorporating clinically sensible variations
4	Case-series (and poor quality cohort and case-control studies)	Case-series (and poor quality prognostic cohort studies)	Case-control study, poor or non-independent reference standard	Case-series or superseded reference standards	Analysis with no sensitivity analysis
5	Expert opinion without explicit critical appraisal, or based on physiology, bench research or "first principles"	Expert opinion without explicit critical appraisal, or based on physiology, bench research or "first principles"	Expert opinion without explicit critical appraisal, or based on physiology, bench research or "first principles"	Expert opinion without explicit critical appraisal, or based on physiology, bench research or "first principles"	Expert opinion without explicit critical appraisal, or based on economic theory or "first principles"

Produced by Bob Phillips, Chris Ball, Dave Sackett, Doug Badenoch, Sharon Straus, Brian Haynes, Martin Dawes since November 1998. Updated by Jeremy Howick March 2009. RCT: Randomized clinical trials; SR: Systematic Review.

phase involved routine canine retraction and PGE1 was applied only on one side, which resulted in 1.6-fold faster movement on the treated side. The researchers did not find any adverse macroscopic effects either in the gum tissue or the alveolar bone. Only mild pain related to the dental movement was observed.

A second preliminary study was performed in 5 patients by Spielmann *et al*[10], with the common objective of assessing the effect of PGE1 on tooth movement. This differed from the previous study in that force was applied to the upper right and left premolars which were to be extracted later during the course of routine orthodontic treatment, and a reciprocal force was used. The method consisted of the local administration of anesthesia 0.1 mL of 0.01% (w/v) PGE1 solution in saline which was injected under the palatal mucoperiosteum to the test tooth and 0.1 mL saline palatal to the contralateral control tooth. Injections were repeated at weekly intervals.

On average the experimental teeth moved 3 times faster than the control teeth without any pathological changes.

Patil *et al*[11] in 2005, performed a clinical assay on 14 patients who were injected for three days with a dose of 1 g of PGE1 (3 g in total), using lidocaine as a vehicle substance in the distal buccal area of canines retracted with Niti open coils. The left side only received a vehicle substance as a control. The patients were monitored for 60 d and the authors concluded that following a minimal dose of PGE1 an increase in the rate of movement was

Table 2 Oxford centre for evidence-based medicine 2011 levels of evidence

Question	Step 1 (Level 1[1])	Step 2 (Level 2[1])	Step 3 (Level 3[1])	Step 4 (Level 4[1])	Step 5 (Level 5)
How common is the problem?	Local and current random sample surveys (or censuses)	Systematic review of surveys that allow matching to local circumstances[2]	Local non-random sample[2]	Case-series[2]	N/A
Is this diagnostic or monitoring test accurate? (Diagnosis)	Systematic review of cross sectional studies with consistently applied reference standard and blinding	Individual cross sectional studies with consistently applied reference standard and blinding	Non-consecutive studies, or studies without consistently applied reference standards[2]	Case-control studies, or "poor or non-independent reference standard[2]	Mechanism-based reasoning
What will happen if we do not add a therapy? (Prognosis)	Systematic review of inception cohort studies	Inception cohort studies	Cohort study or control arm of randomized trial[1]	Case-series or case-control studies, or poor quality prognostic cohort study[2]	N/A
What are the COMMON harms? (Treatment Harms)	Systematic review of randomized trials or n-of-1 trials	Randomized trial or observational study with dramatic effect	Non-randomized controlled cohort/follow-up study[2]	Case-series, case-control studies, or historically controlled studies[2]	Mechanism-based reasoning
What are the RARE harms? (Treatment Harms)	Systematic review of randomized trials, systematic review of nested case-control studies, nof-1 trial with the patient you are raising the question about, or observational study with dramatic effect	Individual randomized trial or (exceptionally) observational study with dramatic effect	Non-randomized controlled cohort/follow-up study (post-marketing surveillance) provided there are sufficient numbers to rule out a common harm. (For long-term harms the duration of follow-up must be sufficient)[2]	Case-series, case-control, or historically controlled studies[2]	Mechanism-based reasoning
Is this (early detection) test worthwhile?	Systematic review of randomized trials or n-of-1 trial	Randomized trial or (exceptionally) observational study with dramatic effect			
Is this (early detection) test worthwhile? (Screening)	Systematic review of randomized trials	Randomized trial	Non-randomized controlled cohort/follow-up study[2]	Case-series, case-control, or historically controlled studies[2]	Mechanism-based reasoning

[1]Level may be graded down on the basis of study quality, imprecision, indirectness (study PICO does not match questions PICO), because of inconsistency between studies, or because the absolute effect size is very small. Level may be graded up if there is a large or very large effect size; [2]As always, a systematic review is generally better than an individual study. OCEBM Levels of Evidence Working Group. "The Oxford 2011 Levels of Evidence". Oxford Centre for Evidence-Based Medicine. http://www.cebm.net/index.aspx?o=5653.

evident compared to the control group.

Vitamin D

The highest level of evidence 3B corresponds to an original Spanish article by Blanco *et al*[12]. The main objective of this study was to determine if a systemic dose of calcitrol supplement (0.25 μg), accelerates canine retraction movement over 60 d as compared to a control group.

Twenty patients (20 ± 5 years) whose canines were retracted using a stainless steel loop by applying a 75 g force when necessary every 15 d were included in this study. The subjects were randomly assigned to two groups: 10 received an oral dose of calcitrol 0.25 μg daily for 60 d and were monitored 10 times; the remaining 10 subjects acted as controls. An increased rate of movement was found in the experimental group ($P = 0.00028$). The researchers concluded that the average speed of movement was faster in the experimental group than in the control group.

PHYSICAL STIMULI

Vibratory forces

These are placed on the 4th level of the evidence scale, and the publications include a case series published by Kau in 2009[13] and 2010[14]. The main objective in the first publication was to report data resulting from use of the Acceledent System. In 2010, the clinical effects of the cyclical force generated by the device (Acceledent) on teeth and the average treatment time were reported. In addition, the levels of patient compliance and satisfaction were assessed. The sample size was the same for the two studies, 14 patients, 11 during leveling and alignment and 3 with space closure. The results of both of these studies were within the range of 0.526 mm of movement per week using Acceledent type I for 20 min daily during 6 consecutive months. Good patient compliance and satisfaction were observed.

Pulsed electromagnetic field

This is classified 3b evidence based on the study by Showkatbakhsh *et al*[15] in 2010, who showed that a pulsed electromagnetic field was capable of accelerating orthodontic tooth movement. The canines on one side in 10 patients who required canine retraction were exposed to a pulsed electromagnetic field (PEMF); the canines on the contralateral side in the same patients were not exposed to the PEMF. A circuit and a watch battery were used to

Table 3 Levels of evidence for therapies in orthodontics

Level	Therapy
	Prospective Meta analysis
	Meta analysis
1a	SR (with homogeneity) of RCTs
1b	Individual RCT
2a	SR (with homogeneity) of cohort studies
2b	Individual cohort study, CCTs and low quality RCT
3a	SR (with homogeneity) of case-control studies
3b	Individual Case-Control study, low quality cohort study and short time split mouth design
4	Case-series, low quality case-control study and non systematic review
5	Expert opinion

RCTs: Randomized clinical trials; SR: Systematic review; CCTs: Controlled clinical trials.

generate the PEMF (1 Hz). The generator was embedded in a removable device. Foil was used to prevent PEMF exposure in the control group. Showkatbakhsh *et al*[15] reported that the accumulative distance moved was significantly larger in the experimental group (5.0 ± 1.3 mm *vs* 3.5 6 ± 1.6 mm, $P \geqslant 0.001$) after 5 ± 0.6 mo.

Electrical current

Kim *et al*[16] demonstrated that an electrical current was capable of accelerating orthodontic tooth movement. Moreover, as only females (7) were included in this study, we do not know the effects of the electrical current in males. The electric appliance was set in the maxilla to provide a direct electric current of 20 microns. The maxillary canine on one side represented the experimental side, and the maxillary canine on the other side represented the control. The experimental canine received orthodontic force and an electric current. The control side received orthodontic force only. An electric current was applied to the experimental canines for 5 h daily, the authors showed that the accumulative distance moved was significantly larger in the experimental group after 1 mo (2.42 ± 0.26 mm *vs* 1.89 ± 0.27 mm). The electrical current was delivered to the mucosa of canines through a fixed electrical appliance assembly (20 MA, 5 h per day). This report is classified as 3b evidence based level.

Low level laser irradiation

Evidence based level 3b: Cruz *et al*[17] were the first to publish research on the effects of low level laser irradiation (LLLI) on the average speed of dental movement. The sample consisted of 11 patients who received a 150 g maxillary canine retraction force bilaterally for 2 mo, one side was irradiated and the other side was used as a control. Irradiation standards were wavelength 780 nm, power 20 mW, energy flow 2 J, energy density 5 J/cm^2, and total dose 8 J. The authors registered a 34% increase in the speed of dental movement on the experimental side compared to the control side[17].

Limpanichkul *et al*[18] used a different set of standards during laser application: 860 nm, 100 mW, 25 J/cm^2, 18.4 J around the experimental tooth (buccal mucosa, distal and palatal) 4 times over a month for a total dose of 294.4 J. The results did not show significant statistical differences between the experimental and control sides, concluding that the dose used (5 J/cm^2) was too low to achieve an increase in the rate of dental movement. To assess the effects of the laser (Ga-Al-As) during the retraction phase in canines, Youssef *et al*[19] irradiated the cervical, middle and the apical surface of the tooth on its buccal and palatal sides with 809 nm and 100 mW for 40 s; the total dose to the right upper and lower canines was 8 J (2 × 40 s at 100 mW), the left side was used as a control. The laser was applied using intervals of 0, 3, 7 and 14 d. The retraction coil was activated on day 21 for both sides. The study results showed a significant increase in movement rate for the irradiated canines when compared to the control[20].

Sousa *et al*[20] evaluated the effect of LLLI on the speed of orthodontic dental movement in 26 canines with retraction NiTi coil springs (150 g). 13 were irradiated (780 nm, 20 mW, 10 s, 5 J/ cm^2), and the other 13 were used as controls. The groups were followed for 4 mo with a total of 9 laser irradiations during that time. The authors concluded that the laser group, using the parameters described, showed an increased rate of orthodontic dental movement, and this could lead to a reduction in treatment time[21].

Evidence level 2b: Dominguez *et al*[21] in 2010 in a prospective cohort study, started at 5 mm crowding non-extraction and finished with a sample of 45 patients between 20 and 30 years old. The experimental group was irradiated at each appointment 1 mm away from the mucosa on the buccal and palatal sides, following the long axis of the tooth for 22 s on each surface. The control group did not receive laser irradiation.

The measurement unit used was days of treatment, the dosage and parameters of irradiation were: 830 nm, 100 mW, energy density 80 J/cm^2, an active laser point of 0.028 cm^2 and the energy was 2.2 J. These parameters allowed a reduction of 30% in the LLLI treated group during the total treatment time.

REGIONAL ACCELERATION PHENOMENA RELATED TECHNIQUES

Regional acceleration phenomena (RAP) healing is a complex physiologic process with dominant features involving accelerated bone turnover and decreases in regional bone densities. Following surgical wounding of cortical bone, RAP potentiates tissue reorganization and healing by a transient burst of localized hard and soft tissue remodeling[22-24].

Corticotomy

The majority of reports on corticotomy belong to level 4 in the scale of evidence.

In 1959, Kole[25] using the crowns of the teeth as

handles, believed that he was able to move the blocks of bone independently of each other as they were only connected by the less-dense medullary bone. He reported that combining orthodontics with corticotomy led to active tooth movement in adult orthodontic cases in 6 to 12 wk. The technique was known as "bony block". The interproximal corticotomy cuts were extended through the entire thickness of the cortical layer, just barely penetrating into the medullary bone. These vertical cuts were connected beyond the apices of the teeth with a horizontal osteotomy cut extending through the entire thickness of the alveolus, essentially creating blocks of bone in which one or more teeth were embedded.

Gantes *et al*[26] showed in 5 patients, that the corticotomy procedure caused minimal changes in the periodontal attachment apparatus. The surgical procedure included intracrevicular incisions and an elevation of buccal and lingual mucoperiosteal flaps. Buccal and lingual vertical grooves penetrating the cortical bone were then made between the roots. These grooves were extended from just below the interproximal alveolar bone margin to beyond the apex levels of the teeth. Buccal and lingual horizontal grooves joined the apical extensions of the vertical grooves. The orthodontic appliance was activated immediately upon wound closure.

In 1991, Suya *et al*[27] reported surgical orthodontic treatment of 395 adult Japanese patients with an improved surgical procedure that he referred to as "corticotomy-facilitated orthodontics."

The authors who have major quantities of scientific reports are the Wilcko[28-33] brothers starting in 2000 up to 2009 and these techniques are now known as Periodontally Accelerated Orthodontic and Osteogenic Techniques. Their reports show high success in acquiring accelerated dental movement which they attribute to an osteoclastic phase or catabolic phase from the regional acceleration phenomena. The Wilcko brothers introduced a technique combining alveolar corticotomies and bone grafting to prevent the risk of dehiscence and fenestration, while increasing the scope of orthodontic corrections. In this conventional approach, cortical incisions circumscribing the roots are made on both the buccal and palatal side following full thickness mucoperiosteal flaps. The bone graft is then placed facing the teeth to be moved and the flaps are then repositioned and sutured at the papilla.

This highly effective technique was also proven to be useful for the intrusion of overerupted molars as reported by Hwang *et al*[34] and Oliveira *et al*[35,36] and for incisive retraction by Germec *et al*[37].

In the study by Akay *et al*[38] all individuals received combined subapical corticotomy and a skeletal anchorage procedure, and intrusion forces of 200 to 300 g were applied to the attachments of each molar and both premolars for 12 to 15 wk. Their results indicated that the use of combined treatment with corticotomy and skeletal anchorage provided safe and noncompliant intrusion of posterior teeth in a short period and may be regarded as an alternative method for skeletal open bite correction in adults who reject orthognathic surgery.

Choo *et al*[39] performed a study to assess the results of surgical accelerated orthodontics in protrusive adults. 24 adults with maxillary or bimaxillary protrusion were treated with speedy surgical orthodontics, including maxillary perisegmental corticotomy followed by orthopedic en-mass retraction against C-palatal miniplate anchorage.

The authors found that the average total treatment time was 20 mo (range, 11-42 mo) and concluded that surgically accelerated orthodontics could be an excellent treatment alternative for adult patients with severe maxillary or bimaxillary protrusion.

In 2012, Bhat *et al*[40] knowing that significant acceleration in orthodontic tooth movement had been extensively reported studied a combination of selective alveolar decortication and bone grafting surgery. The latter was responsible for the increased scope of tooth movement and long-term improvement in the periodontium. A study was carried out in six patients diagnosed with class I malocclusion and bimaxillary protrusion. A modified corticotomy procedure was performed. Active orthodontic treatment began within 1 wk after surgery and the patients were followed up. The mean treatment time for these patients was 17.4 mo, and distalization of the canines was mostly completed within 8.5 mo.

Corticotomy studies level 3b: Fischer[41] evaluated the effectiveness of corticotomy comparing six consecutive patients presenting with bilaterally impacted canines. One canine was surgically exposed using a conventional surgical technique, while the contralateral canine was exposed using a corticotomy-assisted technique. The results showed a reduction in treatment time of 28%-33% for the corticotomy-assisted canines.

Aboul-Ela *et al*[42] evaluated 13 patients requiring the therapeutic extraction of the maxillary first premolars, with subsequent retraction of the maxillary canines. By using miniscrews as anchorage, canine retraction was initiated *via* closed nickel-titanium coil springs applying 150 g of force per side. Corticotomy-facilitated orthodontics was randomly assigned to one side of the maxillary arch of the canine-premolar region, and the other side served as the control. The average daily rate of canine retraction was significantly higher on the corticotomy side than the control side by 2-fold during the first 2 mo after corticotomy surgery. This rate of tooth movement declined to only 1.6-fold higher in the third month and to 1.06-fold by the end of the fourth month.

A study was conducted by Lee *et al*[43] on 65 Korean adult female patients with bimaxillary dentoalveolar protrusion to compare the orthodontic treatment outcomes of anterior segmental osteotomy and corticotomy-assisted orthodontic treatment. It was concluded that orthodontic treatment and corticotomy-assisted orthodontic treatment were indicated for patients with severe incisor proclination with normal basal bone position, although corticotomy-assisted orthodontic treatment had the advantage of shorter treatment duration. Anterior segmen-

tal osteotomy is recommended for bimaxillary dentoalveolar protrusion patients with gummy smile, basal bone prognathism, relatively normal incisor inclination, and relatively underdeveloped chin position.

Corticotomy study level 2b: Shoreibah *et al*[44] conducted a study to evaluate the effect of corticotomy-facilitated orthodontics (CFO) in adults using a further modified technique *vs* traditional therapy in orthodontic tooth movement. The sample included twenty orthodontic patients with moderate crowding of the lower anterior teeth which were randomly divided and treated with either a modified technique of corticotomy-facilitated orthodontic tooth movement (Group Ⅰ) or conventional orthodontic therapy (Group Ⅱ). The authors showed that there was a statistically significant difference between the two groups regarding treatment duration: 17.5 ± 2.8 wk in the CFO group and 49 ± 12.3 wk in the conventional orthodontic therapy group.

Piezocision

To overcome the disadvantages of other corticotomy techniques, Dibart *et al*[45] introduced a minimally invasive, flapless procedure combining piezo surgical cortical micro-incisions with selective tunneling that allows for bone or soft-tissue grafting. Due to their small size and precision, piezoelectric cutting inserts realize precise osteotomies without the risk of osteonecrosis[46]. The authors removed the lingual flap by performing only vestibular incisions, but the elevation of a flap prior to the corticotomy was maintained, thus only relatively reducing surgical time and postoperative discomfort.

Combined with proper treatment planning and a good understanding of the biological events involved, this novel technique can locally manipulate alveolar bone metabolism in order to obtain rapid and stable orthodontic results. Piezocision allows for rapid correction of severe malocclusions without the drawbacks of traumatic conventional corticotomy procedures. Previous reports and those published in 2011 are case series or single case reports (4th level of evidence) which conclude that piezocision is an effective therapy to reduce treatment time when compared to treatments such as Invisalign[47,48].

According to Uribe *et al*[49] corticotomies can potentially reduce the treatment time dramatically in patients who require a significant amount of molar protraction. The authors reported a single case (level 4), of a patient with agenesis of the lower second premolars, after the extraction of primary second molars, mucoperiostal flaps were elevated and interproximal vertical corticotomies were performed on the labial aspect of the mandibular molars with a piezo surgical microsaw. The vertical groove corticotomies were performed mesial to the first and second molars bilaterally and extended just below the crestal bone to the apex. Dried-freeze demineralized bone allograft was packed on the buccal surface covering the grooves and exposed labial cortical bone surface, including a dehiscence on the first molar. The edentulous zone was closed in ten months.

Dentoalveolar distraction

Dentoalveolar distraction (DAD) was performed by making monocortical perforations on alveolar bones around the canines, followed by distracting the canine using distractors.

The scientific literature shows the following case series and a single case report (evidence level 4).

According to Kişnişci *et al*[50] the concept of distraction osteogenesis for rapid orthodontic tooth movement is promising and feasible for clinical practice.

They reported a case series of eleven patients whose first premolars were extracted, and the buccal bone was carefully removed. After wound closure, a special orthopedic device was mounted and cemented to the first molar and canine teeth. Distraction started the same day at the rate of 0.4 mm twice a day and continued until adequate movement of the canine teeth was achieved.

According to Işeri *et al*[51] the dentoalveolar distraction technique is an innovative method that reduces overall orthodontic treatment time by nearly 50%. The authors conducted a study that consisted of 20 maxillary canines in 10 subjects, the first premolars were extracted, the dentoalveolar distraction surgical procedure was performed, and a custom-made intraoral, rigid, tooth-borne distraction device was put in place. The canines were moved rapidly into the extraction sites in 8 to 14 d, at a rate of 0.8 mm per day and full retraction of the canines was achieved in a mean time of 10.05 (± 2.01) d. The same results with the same sample characteristics were published by Akhare *et al*[52] in 2011.

Kurt *et al*[53] reported a 15-year-old skeletal and dental class Ⅱ female patient, with an overjet of 9 mm who was treated by DAD osteogenesis. A custom-made, rigid, tooth-borne intraoral distraction device was used for rapid canine retraction. Osteotomies surrounding the canines were performed to achieve rapid movement of the canines within the dentoalveolar segment, in compliance with distraction osteogenesis principles. The amount of canine retraction was 7.5 mm in 12 d at a rate of 0.625 mm per day.

Kisnisci *et al*[54] reported Dentoalveolar Transport Osteodistraction to distalized canines in 73 alveolar cleft cases. Overall management of selected cases with wider defects may also be optimized and simplified through the transport distraction of a tooth-bone segment. The osteotomy involves designing a partial-thickness bony segment of the transportation of a canine tooth to close the gap resulting from the extraction of the first premolar without a discontinuity defect.

Periodontal distraction

Periodontal distraction was performed by making vertical grooves on the mesial side of the first premolar extraction sockets followed by the same distraction techniq ue as used in DAD. Liou *et al*[55] performed the procedure in fifteen consecutive orthodontic patients, in which twenty-six canine distractions, including 15 upper and 11

lower canines, were carried out with custom made, tooth-borne, intraoral distraction devices. Right after the first premolar extraction, the interseptal bone distal to the canine was undermined with a bone bur, grooving vertically inside the extraction socket, along the buccal and lingual sides, and extending obliquely toward the base of the interseptal bone to weaken its resistance. The interseptal bone was not cut through mesiodistally toward the canine. The intraoral distraction device was delivered for canine distraction right after the first premolar extraction. It was activated 0.5 to 1 mm/d. The authors concluded that the periodontal ligament can be distracted just like the midpalatal suture in rapid palatal expansion. By using this concept, canines can be distracted distally 6.5 mm in 3 wk without significant complications.

Other case series (Level 4) reports are as follows: Gürgan *et al*[56] in 2005, during a 12 mo follow-up period, but without a control group, analyzed 36 maxillary canines until full retraction of the canines was achieved in 10.36 ± 1.93 d (range 8-14 d) at a rate of 0.8 mm/d using a custom-made intraoral rigid tooth-borne distraction device. The periodontal follow-up results allowed them to conclude that dentoalveolar distraction is an innovative technique with no unfavourable long-term effects on the gingival tissues of rapidly retracted canine teeth.

Sukurica *et al*[57] in a six month follow-up study, evaluated twenty canine retraction movements in eight patients. The distraction procedure was completed in 12 to 28 d (mean 14.65 ± 3.49 d). The distal displacement of the canines ranged from 3 to 8 mm (mean 5.35 ± 1.22 mm).

Kumar *et al*[58] concluded that canines can be rapidly retracted by periodontal ligament distraction without complications. The analysis was carried out in 16 upper canines in eight patients who required first premolar extractions. The upper first premolars were extracted and the interseptal bone distal to each canine was thinned and undermined surgically. Custom-built distractors were placed and activated immediately to distract the canines into the extraction spaces. The canines were retracted to proximal contact with the second premolars in 20.33 ± 1.87 d.

In a larger study of 43 canine teeth in 18 (seven male and 11 female) patients who required first premolar extractions conducted by Sayin *et al*[59], the canine retraction was carried out with teeth using semi-rigid, individual tooth-borne distractors. The maxillary canines were distalized an average of 5.76 mm with 11.47 degrees distal tipping. The mean distal movement of the mandibular canines was 3.5 mm with 7.16 degrees distal tipping.

In a split mouth randomized clinical trial without blinded outcome assessment (level 2b) involving 30 patients, Mowafy *et al*[60] evaluated the amount and time of canine retraction concomitant with periodontal ligament distraction using intermittent and continuous forces. For each patient, one side was randomly allocated to receive a screw-based dental distractor, and the other side received a continuous force coil spring distractor. The authors found that the average time needed for canine retraction was 5.3 ± 1.3 wk.

Mtdld technique

Case series: Evidence level 4: Vercellotti *et al*[61], developed a surgical-orthodontic technique [The monocortical tooth dislocation and ligament distraction (MTDLD) technique] to maximize the rapidity of dental movement and prevent damage to the periodontal tissues. During the procedure they performed a microsurgical corticotomy around each tooth, buccal monocortical tooth dislocation and palatal ligament distraction movement and the immediate application of biomechanical force. The report included 8 patients with malocclusion who underwent the procedure and the authors concluded that compared to traditional orthodontic therapy, the average treatment time with the MTDLD technique in the mandible and maxilla was reduced by 60% and 70%, respectively.

In 2011, Bertossi *et al*[62] performed piezosurgical bone cuts to 10 patients affected by different dental malformations to determine the effects of a shorter treatment time. This method (MTDLD technique) is simple, and performing osteotomic lines laterally and apically to the tooth radix on the bone has proved useful in reducing the treatment time. In addition, the technique is very easy to use and has a low incidence of complications. In 5 patients with dental ankylosis, dental repositioning was achieved within 18 to 25 d and in another 5 preoperative patients affected by maxillary hypoplasia and transverse maxillary diameter reduction, in 68 to 150 d.

In 2010, Kharkar *et al*[63] conducted a non-randomized pilot study. The aim of this study was to assess and evaluate the best approach to reduce the overall orthodontic treatment time by means of distraction osteogenesis. The sample consisted of six patients, comprising two groups, who were compared using two different surgical techniques: dento-alveolar distraction and periodontal distraction to bring about rapid canine retraction using a designed intra-oral distractor. Dento-alveolar distraction was superior to periodontal distraction in the time required for retraction, canine tipping, anchorage loss and amount of external root resorption. As a controlled clinical trial with a small sample this was classified as evidence level 3b.

Comparing acceleration techniques and amount of dental movement, Long *et al*[64] in 2013 conducted a systematic review with an evidence level 3 that included cases and control studies and concluded that corticotomy its an effective and safe method to accelerate dental movement in orthodontics. Alveolar or periodontal distractions are promising methods to promote orthodontic movement acceleration, but they lack enough convincing evidence to support them.

Alveocentesis (micro-osteoperforations)

Evidence level 4: Nicozisis[65] showed clinical examples of orthodontic treatments using propel, used in rotation, molar uprighting, Quicker Pre-surgical Orthodontics, intrusion and crowding. In addition, other reports which can be used successfully include, but are not limited to, TADs, Invisalign®, Sure Smile,® and conventional braces.

This includes the study by Teixeira *et al*[66] in 2010, and the results of both animal and clinical studies have demonstrated that the PROPEL System using the Alveocentesis technique decreases orthodontic treatment time by 50%-60% or more in combination with any type of orthodontic force.

Surgery first

The performance of surgery without orthodontic preparation (*i.e.*, "surgery first"), followed by regular postoperative dental alignment, was proposed by Nagasaka *et al*[67]. The authors used this approach to correct skeletal class Ⅲ malocclusion with the aid of skeletal anchorage system orthodontics. The total treatment time was noticeably reduced. In addition, preoperative profile worsening due to incisor decompensation was avoided and immediate profile improvement after surgery was greatly appreciated by the patient.

According to Liou *et al*[68], the advantages of the surgery-first approach are as follows: (1) the patient's chief complaint, dental function, and facial esthetics are achieved and improved at the beginning of treatment; (2) the entire treatment period is shortened to 1 to 1.5 years or less depending on the complexity of the orthodontic treatment; and (3) the phenomenon of postoperative accelerated orthodontic tooth movement reduces the difficulty and treatment time of orthodontic management in the surgery-first approach.

Liou *et al*[69] conducted a study in twenty-two adult patients, who received Le Fort I osteotomy of the maxilla and bilateral sagittal split of the mandible for dentofacial deformities. Crevicular fluid levels of serum alkaline phosphatase and C-terminal telopeptide of type I collagen were determined, as well as tooth mobility of the maxillary and mandibular incisors in these patients. The results support the hypothesis that the phenomenon of postoperative accelerated orthodontic tooth movement is due to the increase in osteoclastic activity and metabolic changes in the dentoalveolar caused by orthognathic surgery. The orthognathic surgery triggers 3 to 4 mo of higher osteoclastic activity and metabolic changes in the dentoalveolar postoperatively, which possibly accelerates postoperative orthodontic tooth movement.

Studies on surgery first are mainly case reports and case series (Evidence based level 4).

Uribe *et al*[49], described a 16-year-old female with a concave profile and class Ⅲ malocclusion, who received a surgical maxillary LeFort 1 advancement and completed her whole treatment within eight months. This was followed by a number of successful case reports that showed short treatment time for ortho- surgical cases using the surgery first approach in class two and three patients: Sugawara *et al*[70], Yu *et al*[71], Villegas *et al*[72] (asymmetrical class Ⅲ), Baek *et al*[73], and Oh *et al*[74].

Hernández-Alfaro *et al*[75] reported 2 cases successfully treated with bimaxillary surgery first. In patient 1, the total orthodontic treatment required 250 d. Arch settlement and leveling achieved a Class I relationship, with adequate root parallelism that was stable at follow-up 1 year later. For patient 2, the total orthodontic treatment lasted 185 d, after which an adequate Class I occlusion and an esthetically balanced profile was achieved.

In 2013, Hernández-Alfaro *et al*[76] reported treating forty-five patients with a surgery first approach. Selected cases presented with symmetrical skeletal malocclusions with no need for extractions or surgically assisted rapid palatal expansion. Standard orthognathic osteotomies were followed by buccal interdental corticotomies to amplify the regional acceleratory phenomenon. Miniscrews were placed for postoperative skeletal stabilization. Orthodontic treatment began 2 wk after surgery. Mean duration of orthodontic treatment was 37.8 wk (range, 24 to 52 wk). Orthodontic retention followed in all cases. An average of 22 orthodontic appointments (range, 14 to 29) occurred. The authors concluded that the surgery first approach significantly shortened total treatment time and was favorable in patients and orthodontists. Nevertheless, careful patient selection, precise treatment planning and fluent bidirectional feedback between the surgeon and the orthodontist are mandatory.

Evidence level 2b: Choi *et al*[77] in 2013, performed a prospective study to determine intervention outcomes in 24 standard and 32 surgery-first approaches for patients with skeletal class Ⅲ dentofacial deformity. In the surgery-first approach, a dental model was created and a novel preoperative orthodontic simulation of the standard presurgical orthodontic treatment was performed to determine the final occlusion between the maxilla and mandible. Changes in cephalometric landmarks were compared between the standard and surgery-first groups in the preoperative, immediate postoperative, and postoperative periods. The researchers found that a surgery-first approach without presurgical orthodontic treatment is possible and can give similar results to standard orthognathic surgery.

Corticision

"Corticision" was introduced as a supplemental dentoalveolar surgery in orthodontic therapy to achieve accelerated tooth movement with minimal surgical intervention. In this technique, a reinforced scalpel is used as a thin chisel to separate the interproximal cortices transmucosally without reflecting a flap[78].

In Young-Guk Park's[79] lecture (level 5), he described the procedure in detail: (1) in previously anesthetized subjects the surgical blade is inserted interproximally and parallel to the occlusal plane 5 mm apical from the tip of the papilla. The blade is tapped with a mallet to a depth of approximately 8 mm. The angle of the blade to changed to approximately 45 degrees apically and the blade is tapped to a depth of 10-12 mm. The blade is changed after four to five slices. The goal is to cut the cancellous bone between the roots to 50%-75% of the root length. To remove the blade, the blade and handle are grasped and the scalpel is worked up and down a few times before pulling the blade out. The blade is pulled

rather than the handle to avoid breaking the blade. Test the mobility of the teeth by forcibly trying to move them slightly. Apply orthodontic forces immediately. The patient is seen every two weeks and the teeth are forcibly mobilizing to induce minor trauma to extend the effect; and (2) according to Park, this is a minimally invasion technique to induce accelerated tooth movement by stimulating osteoblasts and bending alveolar bone that has been surgically separated.

According to Bondemark[80], there is no movement acceleration technique that provides strong evidence (at least two studies with high value of evidence: Randomized clinical study or a prospective study with a well-defined control group). Accordingly, it is necessary to have an alternative to classify the limited literature available on this particular subject, and randomized clinical trials on this topic must be developed.

Multiple reports on orthodontic acceleration in humans have been observed using an alternative evidence level scale and this a simple and accurate way of determining which technique is most effective. The highest level of evidence for a specific procedure to accelerate orthodontic dental movement up to October 2013, is for surgery first, followed by low level laser application and corticotomy located on level 2, recommendation grade b from this proposed scientific evidence scale. Nonetheless, there is a necessity for more studies with a higher level of evidence,considering that this teraphies are located on a moderate level of evidence.

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REVIEW

World health dilemmas: Orphan and rare diseases, orphan drugs and orphan patients

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Abstract

According to global annual estimates hunger/malnutrition is the major cause of death (36 of 62 million). Cardiovascular diseases and cancer (5.44 of 13.43 million) are the major causes of death in developed countries, while lower respiratory tract infections, human immunodeficiency virus infection/acquired immunodeficiency syndrome, diarrhoeal disease, malaria and tuberculosis (10.88 of 27.12 million) are the major causes of death in developing countries with more than 70% of deaths occurring in children. The majority of approximately 800 million people with other rare diseases, including 100000 children born with thalassaemia annually receive no treatment. There are major ethical dilemmas in dealing with global health issues such as poverty and the treatment of orphan and rare diseases. Of approximately 50000 drugs about 10% are orphan drugs, with annual sales of the latter approaching 100 billion USD. In comparison, the annual revenue in 2009 from the top 12 pharmaceutical companies in Western countries was 445 billion USD and the top drug, atorvastatin, reached 100 billion USD. In the same year, the total government expenditure for health in the developing countries was 410 billion USD with only 6%-7% having been received as aid from developed countries. Drugs cost the National Health Service in the United Kingdom more than 20 billion USD or 10% of the annual health budget. Uncontrollable drug prices and marketing policies affect global health budgets, clinical practice, patient safety and survival. Fines of 5.3 billion USD were imposed on two pharmaceutical companies in the United States, the regulatory authority in France was replaced and clinicians were charged with bribery in order to overcome recent illegal practises affecting patient care. High expenditure for drug development is mainly related to marketing costs. However, only 2 million USD was spent developing the drug deferiprone (L1) for thalassaemia up to the stage of multicentre clinical trials. The criteria for drug development, price levels and use needs to be readdressed to improve drug safety and minimise costs. New global health policies based on cheaper drugs can help the treatment of many categories of orphan and rare diseases and millions of orphan patients in developing and developed countries.

Key words: World health issues; Global diseases; Orphan drugs; Orphan diseases; Rare diseases; Orphan patients; Thalassaemia; Deferiprone; Deferasirox; Deferoxamine; Iron overload

Core tip: The major world health problems are related to poverty and other monetary health issues, including the supply of orphan drugs for the treatment of rare and orphan diseases. Differences in disease profile, disease burden and monetary health policies influence the mortality and morbidity rates of patients in developed and developing countries. The inexpensive developmental procedure of the iron chelating drug, deferiprone, used in thalassaemia is proposed as a paradigm for orphan and rare drug development. Improve-

ments in worldwide health policies including procedures for inexpensive drug development and alleviation of poverty could reduce the mortality and morbidity rates of patients worldwide.

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INTRODUCTION

The efforts of international organisations such as the World Health Organisation (WHO) and The United Nations Children's Fund, as well as many other national government organisations and non governmental organizations are continuously improving health standards worldwide and global health care is becoming a reality[1]. However, at the same time there are many challenges, ethical dilemmas and major issues related to health policies and strategies that still need to be addressed and resolved globally, including the treatment of patients in developing countries and patients worldwide with rare diseases. Major limiting factors for addressing such problems are the ability to provide successful treatments and the availability of financial resources[2,3].

Despite continuous medical progress in the treatment of diseases in the last few decades, the level of poverty and malnutrition, as well as the lack of health facilities and medicinal products are still considered the major factors leading to the high mortality and morbidity rates observed globally and mostly in developing countries[3]. In the developed countries, the disease profile classification affecting the mortality and morbidity rate is comparatively different with obesity, ageing and environmental pollution being considered as some of the major causes of many illnesses[4].

Monetary issues are very important in relation to the provision of health care and unless patients are self-sufficient financially, relevant decisions and priorities for selecting which disease, which drug and which patient to be treated are not yet fully clarified in each country or even in each hospital. Such dilemmas are more prevalent in developing countries, where financial resources are very limited. For example malnutrition and diarrhoea in infants and treatment of diseases such as malaria and human immunodeficiency virus infection/acquired immunodeficiency syndrome (HIV/AIDS) are priorities for long-term health strategies in the developing countries, whereas cancer, cardiac and neurological diseases are treatment priorities for most developed countries[1,2].

Among the major obstacles affecting the level of global health care is the availability and cost of medicinal drugs. There are many economic, ethical and other issues affecting the supply of drugs for different groups of patients in each country[5-7]. Within this context, regulatory procedure differences, preventative and diagnostic procedures, marketing influences and monetary factors can variably affect the treatment of patients in each country[8-10]. Many such treatments usually involve the use of vaccines, generic and sometimes new patented drugs.

In many cases, the progress of the disease and the outcome of the treatment may be affected because of wrong evaluations and choices regarding the available therapeutic options. Similarly, the risk/benefit assessment for the use of specific drugs is not in many cases clearly defined and in some cases is not necessary. For example, the widespread use of antibiotics for the treatment of the common flu is inappropriate because the common flu is caused by a viral infection. Other related issues are the toxicity of drugs, where the risks outweigh the benefits and can sometimes cause severe damage or can even be fatal.

Incentives for the development of new drugs, especially for diseases where effective treatments are not currently available, are considered a major challenge for academic and pharmaceutical industry researchers. In general, the development of new drugs is market driven and has little to do with rare diseases in the developed countries or serious tropical diseases in the developing countries. However, monetary incentives have been introduced in recent years in the United States, Japan and Europen Union (EU) countries, for the development of "orphan drugs" for "orphan diseases", where a small number of patients in these countries are affected by a rare condition in comparison to the general population. The motive in most cases for orphan drug development is the lucrative profit from monopolies of patented drugs and not humanitarian concerns for the well-being of the small group of patients with rare or orphan diseases in developed countries or the lack of treatment for patients in the developing countries (orphan patients)[11].

The expansion of the pharmaceutical industry in the developing countries and the local production of generic drugs are major advances in the treatment of local patients and the overall health levels in these countries[7]. However, despite the encouraging progress and the production of medicinal drugs in developing countries, many patients especially those with chronic conditions cannot afford the cost of even the locally developed generic drugs or newly available technologies[12].

A major obstacle in the introduction of new drugs for the treatment of any diseases is the anticipated high cost of drug development[13]. A further obstacle in the supply of new drugs in the developing countries is the high cost due to the drug monopolies implemented by world trade laws. It appears that such laws mostly benefit multinational pharmaceutical companies which are based in the developed countries[14].

Despite the fact that in many cases there are substantial improvements in the treatment of diseases due to the introduction of new patented drugs, there are also many other cases where the opposite result is observed. The latter may be caused by many factors including wrong decision making by those responsible for the approval and supply of the drugs due to misinformation regard-

ing drug safety and efficacy. Within this context there are many grey areas in the development and use of such new drugs, which in the long-term may worsen the treatment of affected patients and ultimately decrease the progress in the treatment of related diseases.

The design of new patented drugs, which is a major research task for pharmaceutical companies and academic institutions, usually involves many stages including the synthesis of many analogues, *in vitro* and *in vivo* screening studies and clinical trials. For example, in the case of the iron chelating drug, deferiprone (L1), which was invented and developed in academic institutions, more than one hundred analogues and other similar compounds have been designed and tested for possible application in the treatment of iron overload in thalassaemia and other diseases[15-18].

Despite the fact that thousands of new compounds are designed, tested and patented for possible application as new pharmaceuticals, only a very small number of these compounds reach the stage of investigational new drug (IND) or new medical entity and selected for further development.

Excessive costs for toxicological, carcinogenicity and other screening, clinical trials and post-marketing surveillance, as well as other costs such as patent fees make it impossible for individuals or academic institutions to proceed to the full development of a new drug. It is estimated that the cost for the introduction of a new drug from the stage of design to post-marketing monitoring is about 0.5 billion USD[19]. However, most of this expenditure is not related to the scientific evaluation and development of the drug, but due to its marketing. This procedure adds substantially to the price of a drug and to the overall public health spending, while at the same time it reduces the prospect of drug availability in developing countries[20]. In contrast, the development of academically based orphan drugs such as L1 can cost less than 5% of the above amount.

Present practices suggest that irrespective of how effective and safe new INDs might be, these are not likely to be developed, unless they are under patent monopoly and can potentially make huge profits following their registration and marketing. This issue is indicative of the current role of multinational pharmaceutical companies in societies, where profit is the major target for drug development and not the treatment of diseases. Pharmaceutical companies involved in the production of generic drugs are also trying to maximise profits. In the latter case, the profit levels are lower due to competition with other companies because of the absence of monopolies.

A paradigm which could affect future strategies in drug design, development and use are the iron chelating drugs. These drugs are primarily used for the iron removal treatment in thalassaemia and other transfusional iron loaded diseases such as myelodysplasia and sickle cell disease. In chemical terms, chelation can be considered a chemical reaction involving the formation of bonds between a metal ion and a chelator (Greek: χηλή-claw of a crab) resulting in a metal-chelator complex. A chelator can be a natural or synthetic chemical compound, or in this case a drug molecule capable of forming a heterocyclic ring with a metal ion as the closing member *i.e.*, like a crab holding the metal ion in its claw. In transfusional iron loading conditions the aim of the administration of iron chelating drugs is the binding and removal of excess toxic iron from the body[15-17].

HEALTH ISSUES AFFECTING GLOBAL MORBIDITY AND MORTALITY

Global health is related to a dynamic state of interactions, involving many factors and many players such as the WHO, local and governmental health authorities. Some of these factors include the availability of financial resources, the severity, transmission and extent of infectious, communicable and other diseases and the differences between ages and gender. In an ever expanding world population, major issues such as health provision and resource allocation have a major impact on human survival, morbidity, mortality and quality of life. Within this context, human activities such as monetary policies, wars, accidents and injuries, food production and distribution, health education, provision of medicines and health care as well as environmental pollution, infectious, chronic and genetic diseases, all interact and influence health outcomes in each country and worldwide[21].

Health models and schemes have been designed where diseases and patients have been included in different categories. At the same time, different strategies have been developed for addressing local and international health problems with one of the major limiting factors being the availability of financial resources. In all cases, financial resources for health care are limited and many classifications have been designed for outlining the importance, impact, morbidity, and mortality of each disease on a national and international scale (Table 1)[22-28].

In an attempt to prioritise the impact and severity of different diseases, including effects on the survival rate and quality of life of patients, several parameters have been introduced such as quality-adjusted life years (QALY), disability-adjusted life year (DALY) and years lived with disability (YLDs) which are mostly used in the developed countries for comparison among diseases and individual patient cases. QALY is a term referring to a calculated score for the comparison of different healthcare interventions which takes into account an average life expectancy and the quality of life or both. For example one year of perfect health is equal to 1 QALY, death is 0 QALY and a year of less than perfect health is scored between 0 and 1. A parameter related to health resource allocation is the cost/QALY, which is different for each intervention.

A DALY is another term used for measuring the amount of health lost due to a disease or injury. It is calculated as the present value of the future years of disability-free life that are lost as a result of premature death or disability occurring in a particular year. YLDs is another

Table 1 Main causes of death in developed and developing countries excluding malnutrition

People in developed countries (2002)	Millions	People in Developing countries (2002)	Millions	Globally (2011)	Millions
Heart attack	3.08	LRTI	2.81	Heart attack	7.00
Stroke	1.78	HIV/AIDS	2.55	Stroke	6.20
LTB cancer	0.61	Heart attacks	2.53	LRTI	3.20
LRTI	0.45	Infections at birth	1.78	COPD	3.00
COPD	0.42	Diarrhoeal disease	1.53	Diarrhoeal disease	1.90
Colon, rectal cancer	0.35	Stroke	1.45	HIV/AIDS	1.60
Diabetes	0.24	Malaria	1.25	Diabetes	1.50
Self inflicted injuries	0.23	Tuberculosis	0.96	LTB Cancer	1.40
Hypertensive heart disease	0.23	COPD	0.76	Road injuries	1.30
All causes	13.43	All causes	27.12		
Estimates 2006[1]		Malnutrition	36.00	All causes	62.00

[1]The total number of deaths globally in 2006 was estimated to be about 62 million, of which 32 million were related to hunger and malnutrition. COPD: Chronic obstructive pulmonary disease; LRTI: Lower respiratory tract infections; LTB cancer: Lung, tracheal and bronchial cancer. Adapted from ref[22-28].

term also used for health resource allocation of different interventions.

The main cause for the highest rate of mortality globally is hunger and malnutrition, which is found almost exclusively in the developing countries. It is estimated that one in twelve people worldwide is malnourished and that 58% of the total number of deaths is related to hunger or diseases due to deficiencies in micronutrients (Table 1). Despite the fact that world food production is adequate for feeding the entire human population, several causes such as insufficient food production, supply and distribution in the developing countries, as well as excess food use and waste in developed countries are the main reasons for the observed rate of malnutrition and human mortality today. In contrast, the high incidence of obesity, physical inactivity and smoking are some of the main causes for the high mortality and morbidity rates observed in relation to the most common diseases in the developed countries such as cardiovascular diseases, cancer and diabetes (Table 1)[22-28].

The cost/QALY for feeding the malnourished population in the developing countries is considered the lowest cost intervention globally. However, the adopted global food and health policies for solving this problem are insufficient and controversial. Similar controversial and ethical issues apply in the spectrum of diseases as well as related strategic policies aimed at increasing the survival and quality of life of people in developed and developing countries.

In relation to morbidity, the global outlook of diseases has general characteristics and individual variations between developed and developing countries. It is estimated that 13% of the global burden of disease is related to global mental health, surpassing both cardiovascular disease and cancer[29]. Depression, Alzheimer's disease and other dementias, epilepsy, schizophrenia, migraine, insomnia, multiple sclerosis, Parkinson's disease, alcohol dependence and other mental, neurological and substance-abuse disorders are included in this category of diseases[29]. Examples of the impact of diseases related to mental health is the annual rate of mortality from suicide which is estimated as 900000 people worldwide (200000 in China, 170000 in India, 140000 in high income countries) and the cost of dementia treatment, which for the United States alone has been estimated at 609 billion USD in 2009[29-31].

With the improvement of health practices and treatments there has been an overall increase in life span worldwide and a related increase in prognosis. Corresponding increases in expenditure have also been observed for many diseases in the developed countries, especially chronic diseases such as cardiovascular and neurological diseases, diabetes and cancer. The global burden of cancer for example, in 2002 was estimated at 10.9 million new cases, with 24.6 million persons alive with cancer (within 5 years of diagnosis) and 6.7 million deaths (61% in developing and 39% in developed countries). The incidence of cancer in men is as follows: lung followed by prostate, stomach, colorectal and liver cancer, and in women is breast followed by cervix uteri, colorectal, lung and stomach cancer. It has been suggested that the major causes of cancer in the United States are smoking (29%-31%), diet (20%-50%), infection (10%-20%), reproductive hormones (10%-20%), alcohol (4%-6%) and occupation (2%-4%)[32]. Cardiovascular disease was estimated to cause more than 17 million deaths worldwide in 2007 and this is projected to increase to 26 million in 2020, with 19 million in the developing and 7 million in the developed countries[25,26]. The major causes of cardiovascular disease are related to physical inactivity, tobacco use, high blood pressure, obesity, unhealthy diet, diabetes mellitus and alcohol use[33].

The estimated global burden of diabetes mellitus was 366 million people in 2012 with a projected increase to 552 million in 2030. Patients with prediabetes are estimated to reach 470 million by 2030 with a parallel increase in associated complications such as nephropathy, neuropathies and vascular complications[34].

In relation to transmitted diseases, a prominent position globally is HIV/AIDS with an estimated prevalence in 2007 of 33.2 million people living with HIV, including about 5% of adults in sub-Sarahan Africa and with an annual incidence of 2.5 million new cases and mortality of 2.1 million. Mortality due to HIV/AIDS has been reported to have decreased in 2011 due to prophylactic measures and new, more effective treatments (Table 1)[35,36].

Infectious diseases are one of the top groups of diseases with the highest morbidity and mortality rate affecting mainly patients in developing countries (Table 1). Neonatal and infant children are more susceptible to

Table 2 The largest health care companies in the world based on annual revenues

Rank	Company	Country	Total annual revenue (USD billions)
1	Johnson and Johnson	United States	61.90
2	Pfizer	United States	50.01
3	Roche	Switzerland	47.35
4	GlaxoSmithKline	United Kingdom	45.83
5	Novartis	Switzerland	44.27
6	Sanofi	France	41.99
7	Astra Zeneca	United Kingdom/Sweden	32.81
8	Abbott Laboratories	United States	30.76
9	Merck and Co.	United States	27.43
10	Bayer HealthCare	Germany	22.30
11	Eli Lilly	United States	21.84
12	Bristol-Myers Squibb	United States	18.81

The companies were ranked by revenue as of March 2010 according to their released 2009 annual reports.

such diseases, especially in poor areas with poor sanitary conditions and lack of clean water. More than 70% of deaths in this category are related to neonatal causes, pneumonia, diarrhea and malaria[28].

Many factors influence the morbidity and mortality rate for each disease with variations in different areas of the world. The major risk factors include malnutrition, sanitation, unsafe sex, tobacco, alcohol and illicit drugs, physical inactivity, obesity, hypertension and environmental pollution[37-39]. The global burden of diseases is in a dynamic state of continuous change, which can be monitored and hopefully will allow predictions and future strategies to be developed including the introduction of preventative measures and prognosis[22-39]. Such strategies can only be implemented if the necessary financial resources become available. Public spending on health, including the cost of drugs and services is under continuous evaluation and any adjustments may help to decrease current morbidity and mortality rates in many countries and also globally.

THE CONCEPTS OF ORPHAN DRUGS AND ORPHAN DISEASES

The attempts for global health coverage continue progressively and new national and international strategies for achieving this goal are steadily increasing with major successes[1-3,40,41]. The development of new drugs is part of this strategy and involves pharmaceutical companies mainly in Western countries where investment is available and revenues from sales could be colossal (Table 2).

A major challenge for global health coverage is also the development of treatment strategies for orphan and rare diseases and the development of orphan drugs. The term "orphan drugs" was introduced by governments of developed countries to help in the production and marketing of medicinal drugs by the pharmaceutical industry for patients suffering from rare conditions living in their own countries. This concept was based on monetary incentives and regulatory relaxations for attracting pharmaceutical companies to orphan drug production since it was assumed that the cost of developing and bringing to the market such a medicinal product cannot be recovered by the expected sales. On ethical grounds this concept is intended to help patients suffering from rare conditions to be entitled to the same quality of treatment as other patients with diseases affecting large numbers of the population.

Orphan drug legislation varies among the developed countries and was introduced at different times, first in the United States in 1983, Singapore in 1991, Japan in 1993, Australia in 1997 and in the EU in 2000. In the EU, an orphan medicinal product is intended for the diagnosis, prevention and treatment of an orphan disease with a prevalence of less than 5 affected per 10000 persons. The term orphan drug can also apply to a seriously debilitating condition even if its prevalence is more than 5 per 10000 persons. In the United States an orphan drug is intended for any rare disease with an incidence of less than 200000 persons. It is estimated that there are about 7000 orphan diseases ranging from genetic diseases such as thalassaemia to rare infections in the West such as malaria, tuberculosis and blinding trachoma. In addition, subsets of commoner diseases such as Crohn's disease of the oesophagus are also classified as orphan.

It is estimated that about 350 orphan drugs for 200 orphan diseases have been developed since 1983. Before the United States act of 1983 fewer than 40 products were developed, whereas between 1983 and 2009, the food and drug administration (FDA) approved 275 orphan drugs for 337 orphan indications and during the 2000s it was estimated that orphan products comprised 22% of all new molecular pharmaceutical entities[42]. Among the incentives for pharmaceutical companies in the United States, are market exclusivity for 7 years, grants of up to 30 million USD per annum, waiving of user fees (approximately 1.2 million USD for every application) paid to the FDA for review of the sponsor's application, tax incentives and easier to gain marketing approval. Similar conditions and relaxations are included in the EU legislation, but market exclusivity is for 10 years. It is estimated that global orphan drug sales have increased about 10% per year between 2005 and 2011, and are now approaching 100 billion USD annually[13].

Research in orphan diseases was until recently carried out mainly by academic institutions, biotech companies and smaller, specialty drug companies. Large pharmaceutical corporations have also lately taken interest, mainly for exploiting the orphan drug legislations by targeting sub-groups of common diseases[42].

Examples of a list of orphan-designated drug products with at least one marketing approval in the United States for a rare disease indication are shown in Table 3[43-45]. The drugs approved are mostly related to various cancers and other conditions with low prevalence in the developed countries. Marketing approval in the United States was also provided for orphan-designated drug products for both common and rare disease indications

Table 3 Examples of orphan-designated drug products with at least one marketing approval in the United States for a rare disease indication

Drug product name	Orphan indications
Alglucerase injection	Replacement therapy in Gaucher's disease
Alitretinoin	Acute promyelocytic leukemia
Alpha1-Proteinase Inhibitor	Cystic fibrosis
Ambrisentan	Idiopathic pulmonary fibrosis
4-Aminosalicylic acid	Crohn's disease
Amifostine	Chemoprotective agent in cancer
Anagrelide	Polycythemia vera
Anti-tac (human)	Prevention of acute graft-*vs*-host disease
Arsenic trioxide	Multiple myeloma, MDS, CML, CLL
Atovaquone	*Toxoplasma gondii* encephalitis
Azacitidine	Acute myeloid leukemia
Beractant	Newborn infants with pneumonia
Bosentan	Idiopathic pulmonary fibrosis
Busulfan	Primary brain malignancies
Calfactant	Acute respiratory distress syndrome
Canakinumab	Juvenile idiopathic arthritis
Capsaicin	Erythromelalgia
Cladribine	Non-Hodgkin's lymphoma, CLL, AML
Clofarabine	Acute myelogenous leukemia
Coagulation factor Ⅶa	Bleeding in Glanzmann thrombasthenia
Cysteamine hydrochloride	Huntington's disease
Cytarabine	Gliomas
Daunorubicin liposomal	Acute myeloid leukemia
Decitabine	Sickle cell anemia, CML, AML
Eculizumab	Dermatomyositis
Epoprostenol	Replacement of heparin in hemodialysis patients
Filgrastim	Myelodysplastic syndrome and AIDS
Fludarabine phosphate	Non-Hodgkins lymphoma
Heme arginate	Myelodysplastic syndromes
Idarubicin	AML in pediatrics, MDS and CML
Ifosfamide	Bone and soft tissue sarcomas
Iloprost solution for infusion	Heparin-associated thrombocytopenia
Indium^{111} pentetreotide	Neuroendocrine tumors
Interferon gamma-1b	Idiopathic pulmonary fibrosis
Lenalidomide	Mantle cell lymphoma and CLL
Levocarnitine	Pediatric cardiomyopathy
Mecasermin	Amyotrophic lateral sclerosis
Mecasermin rinfabate	Burns that require hospitalization
Melphalan	Cutaneous melanoma
Mesna	Inhibition of the urotoxic effects
Miglustat	Neurological manifestations
Mitomycin-C	Refractory glaucoma
Mycophenolate mofetil	Pemphigus vulgaris
Nilotinib	Gastrointestinal stromal tumors
Nitazoxanide	Intestinal amebiasis
Nitisinone	Alkaptonuria
Nitric oxide	Acute respiratory distress syndrome
Pentostatin	Cutaneous T-cell lymphoma and CLL
Porfimer sodium	Cholangiocarcinoma
Pralatrexate	Diffuse large B-cell lymphoma
Primaquine phosphate	*Pneumocystis carinii* pneumonia
Protein C concentrate	Replacement therapy in protein C deficiency
Procarbazine hydrochloride	Malignant glioma
Quinine sulfate	Non *Plasmodium falciparum* malaria
Rapamycin (mTOR) inhibitor	Tuberous sclerosis complex
Rifabutin	*Mycobacterium avium* disease
Riluzole	Huntington's disease
Sermorelin acetate	Induction of ovulation in women
Sodium phenylbutyrate	Sickling disorders
Sodium thiosulfate	Platinum-induced ototoxicity
Somatropin	Induction of ovulation in women with infertility
Succimer	Mercury toxicity and kidney stones
Synthetic human secretin	Diagnostic procedures in pancreatic carcinoma
Synthetic porcine secretin	Diagnostic procedures in pancreatic carcinoma
Temozolomide	Advanced metastatic melanoma.
Tetrabenazine	Moderate/severe tardive dyskinesia
Thalidomide	Graft *vs* host disease in BMT
Topotecan HCl liposomal	Gliomas
Tretinoin	Acute and chronic leukemia
Trimetrexate	Metastatic carcinomas
Vorinostat	Multiple myeloma and mesothelioma

ALL: Acute lymphoblastic leukaemia; AML: Acute myeloid leukaemia; CML: Chronic myeloid leukemia; CLL: Chronic lymphocytic leukaemia; MDS: Myelodysplastic syndrome; BMT: Bone marrow transplantation. Adapted from references[43-45].

as shown in the examples in Table 4[43-45]. In this group of orphan-designated drug products, new formulations and new indications of commonly used drugs (*e.g.*, doxorubicin, bleomycin and cyclosporine) have been included (Table 4)[43-45]. A list of drugs approved for rare diseases in the EU is also shown in Table 5[46]. The list includes many drugs intended in most cases for the treatment of various cancers, infectious diseases and other conditions with low prevalence in the EU (Table 5)[46]. It is estimated that there are about 2000 orphan diseases in the EU and 6500 in the United States[46,47].

The orphan diseases with the highest number of drug designations and most orphan drug approvals are shown in Table 6[48,49]. With the exception of malaria, which is a very rare condition in developed countries, but a major problem in developing countries, all other 13 diseases are rare diseases found in developed countries (Table 6)[48,49]. It is estimated that 11 tropical diseases related to helminth, protozoan and bacterial infections affect about 800 million people in developing countries, excluding malaria, communicable, environmental and genetic diseases (Table 7). Overall, the neglected tropical and other diseases found almost exclusively in developing countries are extremely rare in developed countries and are not a priority for orphan drug development in developed countries (Table 7). It is evident that the orphan drug and orphan disease concepts are mostly based on monetary considerations and not an effort for the treatment or elimination of rare and neglected diseases with high incidence in the developing countries. Patients suffering with neglected tropical and other rare diseases in developing countries can be considered as orphan patients. Similarly, as budgetary limitations on health are expanding in the developed countries the concept of orphan patients is also adopted for patients with rare and other diseases in the developed countries.

DILEMMAS IN MEDICAL ETHICS AND ORPHAN PATIENTS

Present Western philosophies and medical ethics are based upon monetary concerns, which affect local and global health levels. For example, enough food is pro-

Table 4 Examples of orphan-designated drug products with marketing approvals in the United States for both common and rare disease indication

Drug product name	Orphan indications
Adalimumab	Paediatric Crohn's disease
Aldesleukin	Primary immunodeficiency disease
Allopurinol	*Ex vivo* preservation of kidneys for transplants
Aminosidine	Tuberculosis and *Mycobacterium avium*
Azathioprine	Graft-*vs*-host disease
Aztreonam	Improvement of symptoms in bronchiectasis
Bevacizumab	Ovarian, stomach and pancreatic cancer
Bleomycin sulphate	Pancreatic cancer
Cetuximab	Pancreatic cancer
Cisplatin liposomal	Osteogenic sarcoma metastatic to the lung
Colchicine	Behcet's Syndrome
L-Cycloserine	Gaucher's disease
Cyclosporine	Prophylaxis and treatment of GVH disease
Cyclosporine A implant	Prevention of rejection in cornea transplant
Cyclosporine liposomal	Bronchiolitis obliterans
Doxorubicin	Hepatocellular carcinoma
Doxorubicin HCl liposomal	Soft tissue sarcomas
Doxorubicin nanoparticles	Hepatocellular carcinoma
Eflornithine HCl	*Pneumocystis carinii* pneumonia in AIDS
Epoetin alpha	Myelodysplastic syndrome
Erlotinib HCl	Malignant gliomas
Etidronate disodium	Degenerative metabolic bone disease
Everolimus	Gastroenteropancreatic tumors
Histrelin	Acute intermittent and other porphyries
Immuneglobulin	Juvenile rheumatoid arthritis
Infliximab	Chronic sarcoidosis
Interferon alfa-2a	Esophageal carcinoma
Peginterferon alfa-2a	Chronic myelogenous leukemia
Interferon alfa-2b	Ovarian carcinoma, brain tumors
Peginterferon alfa-2b	Chronic delta hepatitis
Metronidazole (topical)	Perioral dermatitis
Metronidazole	Pouchitis
N-acetylcysteine	Acute liver failure
Paclitaxel	Pancreatic cancer
Paclitaxel aqueous gel	Esophageal and brain cancer
Paclitaxel micellar	Ovarian cancer
Paclitaxel protein-bound	Stage ⅡB to Ⅳ melanoma
Sorafenib	Stage ⅡB through stage Ⅳ melanoma
Ribavirin	Haemorrhagic fever with renal syndrome
Rituximab	Immune thrombocytopenic purpura
Thiotepa	Haematopoietic stem cell transplantation
Tranexamic acid	Hereditary angioneurotic edema
Urofollitropin	Initiation and re-initiation of spermatogenesis
Ursodiol	Cystic fibrosis liver disease

GVH: Graft versus host. Adapted from ref[43-45].

duced to feed the whole world population, but large quantities are wasted or destroyed based on existing market policies. This allows millions of people to die from hunger and malnutrition. Similarly, market policies are also partly responsible for the limited success in the effort to prevent or eliminate many diseases and for the lack of basic medicines in developing countries. In general, access to drugs and treatments and health levels for each individual and each country depends on their ability to pay. This happens even in developed countries for example in deciding by clinical boards who can receive a kidney, heart, liver and other transplants or hip replacement or cardiac surgery. Similar dilemmas exist in deciding who can be treated with haemodialysis machines or by new, but very expensive drugs for the treatment of cancer and other serious conditions. Within this context the vast majority of orphan patients are in the developing countries, but there are also many orphan patients in developed countries, where treatments may be available, but patients have no access to them due to limited availability and health resources[22-27].

There are many conflicting interests, ethical and other issues affecting the healthcare of each individual at local and global levels. Healthcare strategies and policies are developed based upon different approaches, influences and philosophies. The ultimate decisions affecting healthcare resource allocation rely on government policies and legislations, which are influenced by political groups, commercial interests, patient groups, and other society groups in general[2].

Government policy in most countries relies on political dogmas between the capitalist approach suggesting that healthcare is another way of spending money and if people cannot afford it that is their bad luck, whereas in the socialist approach it is suggested that the distribution of healthcare is a matter of social justice and all individuals should be treated as equals. Many developed countries are using the utilitarian approach on healthcare resource allocation, which is for the greatest good for the greatest number of people and is measured by QALY. However, there are many dilemmas in resource allocation on healthcare related to QALY measurements, where terms such as good and quality of life have not been fully defined. For example there are age related issues, where treating younger patients will save more years of life, or social worth issues where contribution to the society may be considered as a morally relevant factor, or personal responsibility where individuals such as smokers and obese people are personally responsible for their ill health.

Health economics are increasingly becoming a major part of healthcare and medical education with a major emphasis on better allocation of resources by minimising costs and maximising healthcare output in the primary state control and run sector. This sector is the main healthcare provider in most countries and allocates the funds from taxes. Cost benefit, effectiveness and utility analysis are a major part of healthcare strategies since it is becoming increasingly clear that there are not enough professionals and not enough money to provide a comprehensive state controlled healthcare service in many countries. For example it was estimated in 2010 that medicines alone cost the National Health Service in the United Kingdom more than £13 billion per annum, which accounts for around 10% of the overall health budget[50].

In many developed countries expert independent committees have been instituted to tackle healthcare problems and design healthcare strategies. An example is the United Kingdom national institute for health and clinical excellence (NICE), which uses QALYs to determine which treatments are most suitable for each disease. Accordingly, clinicians use the advice of NICE to decide which treatments to prescribe their patients[50,51]. However, despite the fact that such efforts are necessary the

Table 5 List of drugs approved for rare diseases in Europe

Rare disease category	Drug product name (Indication)
Leukaemias, lymphomas and related diseases	Histamine dihydrochloride and Decitabine (AML) Ofatumumab (CLL) Nilotinib (CML) Mercaptopurine and Clofarabine (ALL) Cladribine (Hairy cell leukaemia) Ponatinib (Philadelphia chromosome positive ALL and CML) Dasatinib (CML and AML) Azacitidine (Myelodysplastic syndromes, CML, AML) Bosutinib (Philadelphia chromosome positive CML) Nelarabine (T-cell ALL and lymphoma) Brentuximab vedotin (Hodgkin lymphoma and anaplastic large cell lymphoma) Ruxolitinib (Primary and other myelofibrosis cases) Plerixafor (Lymphoma and multiple myeloma)
Carcinomas and related diseases	Sorafenib tosylate (Hepatocellular carcinoma, renal cell carcinoma) Mitotane (Adrenal cortical carcinoma) Mifamurtide (Osteosarcoma) Temsirolimus (Renal cell carcinoma and mantle cell lymphoma) Trabectedin (Soft tissue sarcoma, liposarcoma, ovarian cancer) 5-Aminole-vulinic-acid hydrochloride (Malignant glioma) Thalidomide and Lenalidomide (Multiple myeloma)
Chelating drugs and haemoglobinopathy related diseases	Deferoxamine, Deferiprone and Deferasirox (Iron overload in beta thalassaemia) Dexrazoxane (Anthracycline extravasation) Hydroxycarbamide (Sickle Cell Syndrome) Zinc acetate dehydrate (Wilson's disease) Eculizumab (Proxysmal nocturnal haemoglobinuria and haemolytic uraemia)
Pulmonary hypertension	Bosentan monohydrate (Pulmonary arterial and systemic sclerosis) Iloprost (Primary pulmonary hypertension) Ambrisentan and Sildanafil citrate (Pulmonary arterial hypertension)
Cystic fibrosis	Mannitol, Aztreonam and Ivacaftor (Cystic fibrosis) Tobramycin (Pseudomonas aeruginosa in Cystic Fibrosis)
Enzymes used as drugs	Velaglucerase alpha (Type 1 Gaucher disease) Alpha-glucosidase (Pompe disease) Galsulfase (Mucopolysaccharidosis VI) Idursulfase (Hunter syndrome) Proteolytic enzymes enriched in bromelain (Burns)
Drugs used in other rare conditions	Carglumic acid (Hyperammonaemia due to n-acetylglutamate synthase deficiency) Betaine anhydrous (Homocystinuria) Stiripentol (Severe myoclonic epilepsy in infancy) Pirfenidone (Idiopathic Pulmonary Fibrosis) Icatibant acetate (Hereditary angioedema) Amifampridine (Lambert-Eaton myasthenic syndrome) Alipogene tiparvovec (Familial lipoprotein lipase deficiency) Mecasermin (Growth failure in primary insulin-like growth factor 1 deficiency) Rufinamide (Lennox Gastaut syndrome) Sapropterin dihydrochloride (Phenylketonuria and tetrahydropterin deficiency) Romiplostim (Immune thrombocytopenic purpura) Nitisinone (Hereditary tyrosinemia type 1) Ibuprofen (Patent ductus arteriosus) Caffeine citrate (Primary apnea) Hydrocortisone (Adrenal insufficiency) Zicotide (Chronic pain) Teduglutide (Short Bowel Syndrome) Pasireotide (Cushing's disease) Thiotepa (Haematopoietic progenitor cell transplantation) Everolimus (Tuberous sclerosis complex) Tafamidis (Transthyretin amyloidosis) Anargelide hydrochloride (Essential thrombocythaemia) Miglustat (Type 1 Gaucher disease and Niemann-Pick type C disease)

ALL: Acute lymphoblastic leukaemia; AML: Acute myeloid leukaemia; CML: Chronic myeloid leukemia; CLL: Chronic lymphocytic leukaemia. Adapted from ref[46].

task is colossal considering that there are so many medicinal products available including 50000 drugs. These efforts are also limited by many conflicting interests such as conflicting literature, rivalry between pharmaceutical companies, rivalry between academics, differences between regulatory authorities, different priorities by health authorities, different patient group interests and society concerns[5,7,9,52]. The decisions by NICE are based on several factors including the guidance from ministers on the resources available, the clinical needs of patients in relation to other available technologies, the National Health System's priorities, the broad balance between benefits and costs and the potential impact on other National Health System's resources.

The ultimate decision to choose which treatments can be prescribed for the patients is made by the clinician or group of clinicians in charge. Despite the fact that treatment decisions by clinicians are expected to be guided by the Hippocratic oath, which broadly suggests that the treatment regimen to be followed should be according to the doctor's ability and judgment for the benefit of patients, many other factors and influences are involved. For example, the United Kingdom General Medical Council states that doctors should provide effective treatments based on the best available evidence, but also making efficient use of the resources available. On the other hand, thousands of pharmaceutical company representatives are continuously lobbying clinicians and other related groups to influence their decision in choosing appropriate treatments in favour of their products (Figure 1)[5,20].

One of the most important factors influencing healthcare economics and resource allocation is the cost of drugs. Patient requirements for drugs, the role and effects of pharmaceutical companies on drug pricing, efficacy and safety are in most cases being evaluated by expert committees. Within this context, pharmacoeconomics is a new expanding scientific area, influencing decision making of major healthcare resource allocation organisations such as NICE, the National Institute for Health in the United States and similar national organisations in many other countries worldwide.

DRUG DEVELOPMENT AND LARGE PHARMACEUTICAL COMPANIES

The lucrative market of pharmaceuticals and the patent monopolies of new drugs is a major contributory factor

Table 6 Orphan diseases with the most orphan drug approvals

Disease	Drug designation	Drugs approved
AIDS	57	8
Acute myeloid leukaemia	34	5
Ovarian cancer	34	4
Multiple myeloma	32	6
Glioma	29	4
Chronic myelogenous leukaemia	19	4
Acute lymphoblastic leukaemia	17	6
Pneumocystis carinii pneumonia	15	5
Respiratory distress syndrome, infant	14	6
Multiple sclerosis	14	5
Growth hormone deficiency	13	9
Idiopathic pulmonary hypertension	12	4
Kaposi's sarcoma	11	5
Malaria	11	4

Adapted from ref[48,49]. AIDS: Acquired immunodeficiency syndrome.

Table 7 Examples of neglected tropical and other diseases in developing countries

Disease categories	Diseases
Genetic diseases	Thalassaemias, sickle cell disease
Helminth infections	Ascariasis, hookworm, trichuriasis, schistosomiasis, lymphatic, filariasis, onchocerciasis, dracunculiasis
Protozoan infections	Human african trypanosomiasis chagas disease, leishmaniasis
Bacterial infections	Buruli ulcer, leprosy, trachoma
Environmental poisoning	Arsenate toxicity, bantou siderosis, mining industry, nuclear industry
Communicable and other diseases	HIV/AIDS, tuberculosis, malaria

HIV/AIDS: Human immunodeficiency virus infection/acquired immunodeficiency syndrome.

to the national economy and income of the most affluent developed countries. The revenue of the twelve top multinational pharmaceutical companies exceeded 445 billion USD in 2009 (Table 2). It should be noted that in the same year the total government expenditure for health in the developing countries was 410 billion USD[2]. Only, 6-7% of this expenditure was received as foreign aid from developed countries. For example, the global health fund of the United States over 6 years (2009-2014) was 63 billion USD[21]. The biggest selling drug of all time is the statin, Lipitor (atorvastatin), from the United States pharmaceutical company Pfizer with lifetime sales of 100 billion USD, until patent expiration in 2011[53].

Six of the twelve top pharmaceutical companies including the top two are based in the United States, two in Switzerland, two in the United Kingdom (one jointly with Sweden) and one each in France and Germany (Table 2). Rarely such companies are involved in the development of orphan drugs, unless they are familiar with the market potential and the income is similar to the non-orphan drugs. An example of an orphan drug is deferasirox (DFRA), which is marketed by one of the top twelve companies (Novartis) and used in the treatment of iron overload in thalassaemia and intended for many other iron loaded conditions[54].

Despite the fact that the standard regulatory authority procedures and laboratory tests needed for drug approval may differ slightly between organisations such as the United States FDA and EU European medicines agency (EMA), the major aspects of screening are based on similar preclinical and clinical testing. In general these procedures involve preclinical testing and usually four distinct clinical phases carried out over many years.

Drug design and development is usually undertaken by pharmaceutical companies in developed countries, due to suspected high expenditure requirements. The drug discovery period can take on average about 10 years and in general involves the design and screening of a large number of known chemical compounds of different classes from chemical libraries. Computer aided technology is a new method of drug design and development. Such methods involve, among others, the mimicking of existing drugs and introduction of structural modifications which may lead to higher efficacy and lower toxicity. However, such approaches are limited due to the complexity of the biological and physiological systems, which cannot be theoretically fully evaluated. Following the identification of leading groups of compounds, new chemical compounds are synthesised and screened to select the most promising ones for further evaluation and development. The screening process for the identification of a new product is tedious and success is limited. For example in the pharmaceutical company Hoechst during the period between 1972 to 1985, out of the 120000 new compounds synthesised and tested, it has been possible to launch only 15 new products.

Structure/activity correlation and preclinical safety testing can take 2-6 years and clinical safety and efficacy studies can take 6-10 years. In the case of orphan drugs, the preclinical and clinical testing period is shorter and involves fewer procedures. The testing requirements and regulatory approval for orphan drugs appear to be different between the United States, EU and other countries. For example, the iron chelating drug L1 was first approved in India in 1994, in the EU and other countries in 1999 and the United States in 2011[55].

The preclinical testing of new drugs involves *in vitro* and *in vivo* experiments. Chemical, biochemical and cell studies, including mutagenicity studies, are carried out during the *in vitro* testing. In the *in vivo* testing, animal studies in at least three different mammalian species using different doses are assessed to evaluate preliminary information on efficacy, absorption, distribution, metabolism and excretion (ADME), pharmacokinetics and toxicity. Following this initial screening procedure the drug could be selected for further evaluation as an investigational new drug (IND).

The clinical testing can be initiated, provided the preclinical testing is satisfactory. In the clinical testing, the initial studies (Phase I) involve in general the administration of low sub-therapeutic doses of the IND to a small number of (*e.g.*, 10-15) normal volunteers to establish whether the drug is tolerated and to derive

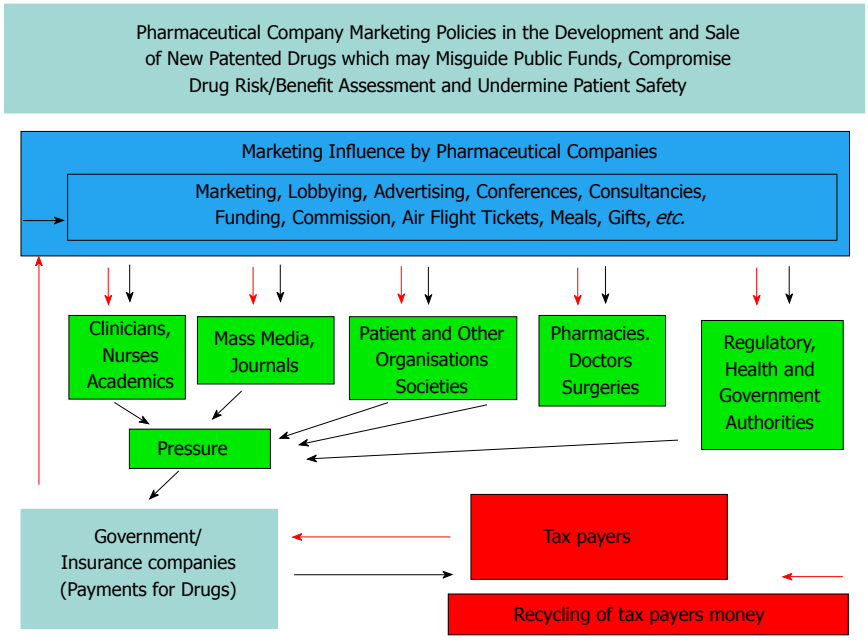

Figure 1 Ethical issues arising from the influence of pharmaceutical companies. A diagram of a theoretical model describing the marketing influence of pharmaceutical companies on various sectors and organisations in relation to new patented drugs and its effect on public spending.

pharmacokinetic, pharmacodynamic and metabolic data. Provided that the preliminary data are satisfactory, further clinical studies involving a larger number of normal volunteers (*e.g.*, 20-100) or sometimes patients are carried out using escalating doses in order to establish a therapeutic dose range, and to assess safety and tolerability. More information on pharmacokinetic and pharmacodynamic data are gathered at the higher dose levels and other parameters are investigated such as the effect of food on drug absorption if the drug is planned to be administered orally.

Phase Ⅱ clinical trials involve a larger number (*e.g.*, 300) of normal volunteers and patients, with the major aim of establishing safety and efficacy ranges. The identification of a therapeutic dose range and further pharmacokinetic studies are also carried out at this phase.

Phase Ⅲ studies involve many more patients (*e.g.*, 1000) over longer periods, usually in randomised controlled multicentre clinical trials, with the main aim of comparing the new drug to the current gold standard drug. The duration of the studies depends on the medical condition and is much longer for chronic conditions. In addition to long-term toxicity monitoring in this phase, other toxicity parameters such as carcinogenicity and drug interaction studies are also carried out. Provided the phase Ⅲ trials are successful and the safety and efficacy results are satisfactory then all the data from chemistry to human studies are submitted and the drug could be registered and approved by the regulatory authorities for marketing. Specific recommendations on the labeling for directions of use and list of adverse effects are included in the marketed product. Following approval by the regulatory authorities, the assessment of the drug may be extended to different patient subgroups and diseases.

Post-marketing surveillance, which is also known as phase Ⅳ trials or pharmacovigilance, can be introduced to detect any rare or long-term adverse effects in a much larger patient population, which was not available during the previous clinical trial phases. Many drugs have been withdrawn or their use restricted due to toxicity at this stage, *e.g.*, rofecoxib[56]. In many cases phase Ⅴ, which is also sometimes referred to as translational research, is now being used to compare the overall effect of the new treatment with other treatments and its impact on public health and the general patient population[20].

It should be noted that there are many variations in the clinical testing procedures and phases for each drug that can affect the length of studies. These include for example the seriousness of a condition, the categories of patients that can be treated, the concomitant use of other drugs and new requirements on safety and efficacy that may be requested by the regulatory authorities. Similarly, other parameters that can also contribute to the length of development and marketing of a drug are whether the drug is needed for urgent treatments or untreated conditions or orphan diseases. In all cases of the introduction of a new drug, a risk/benefit assessment and comparison with the standard treatment should prevail. However, in most cases the major factor for the development and sale of a new drug is financial gain through patent monopoly and intensive marketing.

A major issue in drug development and subsequent use is the level of toxicity. Despite the fact that all drugs have toxic side effects and each individual's susceptibility to toxic side effects is different, no major effort or procedures have been instituted in the drug development or subsequent post-marketing period for studying and reducing or reversing the cause of drug toxicities. The same lack of interest also applies to the design of diagnostic and prophylactic procedures for reducing the incidence of drug toxicities for generic drugs.

Emphasis in both the case of new and generic drugs involve marketing methods for increasing sales, but not improvements for patient safety such as protocols for

minimizing or preventing the toxic side effects or the production of drug antidotes. Within this context, the introduction of patents of new drug formulations, which in most cases have similar efficacy and toxicity to the old formulations, is another area exploited by multinational pharmaceutical companies for making additional huge profits due to the patent monopoly restrictions.

It is estimated that there are about 50000 different drugs available globally, which cause about 8000 different toxic side effects most of which affect patients to a different degree. It is also estimated that approximately 5% of the patients in hospitals are receiving treatments related to the toxic side effects of drugs. There are also many patients affected by toxicity due to impure drugs, idiosyncratic reactions, drug interactions and organ function complications.

The investigation of individual variations in drug response such as pharmacogenomics and metabolomics, as well as the introduction of drug combinations are fast expanding areas in drug development and for the application of personalised medicine.

THALASSAEMIA AND ORPHAN IRON CHELATING DRUGS

Thalassaemia and sickle cell disease are some of over 200 inherited haemoglobinopathies which are included in the category of orphan diseases. Similar to other orphan diseases there is a need for the development of orphan drugs for their treatment. Within this context, iron chelating drugs which are essential for the treatment and long-term survival of thalassaemia patients are classified as orphan drugs. The development of iron chelating drugs requires a basic understanding of iron metabolic processes and methods targeting the effective elimination of iron[17].

Iron is an essential element required by many biological processes and for normal physiological function. There are many iron metabolic disorders affecting millions of people. Iron deficiency is thought to affect a quarter of the world's population, but is not considered to be a severe condition and can in most cases be treated with iron supplements. In contrast, iron overload is considered to be the most common metal toxicity condition worldwide, with severe implications in morbidity and mortality[57]. The most common conditions of iron overload are caused by increased gastrointestinal iron absorption (primary haemochromatosis) or multiple red blood cell transfusions (secondary haemochromatosis) or a combination of these two processes. While in normal individuals there is a balance of iron intake and iron loss, in iron overloaded patients there is a net intake of iron. The rate of net iron intake in patients with primary haemochromatosis is slower (about 2-6 mg/d) than that of transfused patients with secondary haemochromatosis (about 15-30 mg/d)[57].

It is estimated that in general patients with refractory anaemias such as β-thalassaemia are regularly transfused with 1-3 units (1 unit = 200 mg of iron) of red blood cells every 1-4 wk. An excess of 100-125 g of iron, which is equivalent to about 500 units of red blood cells can be stored in the body of β-thalassaemia patients by the time they reach adulthood. Most of the iron accumulated from transfusions and increased iron absorption is not excreted, but is stored as excess, mostly intracellularly in the form of the iron storage proteins ferritin and especially haemosiderin. The organs mostly affected are the liver, heart, spleen and endocrine system. The damage to these and other organs due to iron overload toxicity is detectable when about 50-100 units of red blood cells have been transfused and is so extensive that in many cases it can become irreversible and fatal, unless iron chelation therapy is commenced[58].

Transfusional iron overload in refractory anaemias has the highest mortality and morbidity rate worldwide by comparison to any other form of metal overloading condition. The most seriously affected group of transfused patients are those with β-thalassaemia, but there are also increasing numbers in other transfused categories of patients affected such as sickle cell anaemia and myelodysplasia. In the latter two conditions, iron chelation therapy may not be critical since the overall iron accumulation in most cases is less and accordingly the rate of mortality caused by iron overload toxicity is lower in comparison to β-thalassaemia.

The epidemiological data of regularly transfused patients with different conditions has not yet been fully evaluated. It is estimated, for example, that 100000 children are born with β-thalassaemia and about the same number with sickle cell disease each year[59]. The latter is prominent in the black populations in African countries and their descendants in other continents, especially North America. A smaller number of patients with sickle cell disease can also be found in other countries such as those of the Middle East. β-thalassaemia is found mainly in countries in the Mediterranean area, Middle East and South East Asia. More than 80% of β-thalassaemia patients live in South East Asia and the Middle East and less than 10% worldwide receive adequate transfusions and iron chelation therapy mainly due to the unaffordable cost of treatment. The vast majority of β-thalassaemia patients in developing countries are left to die untreated.

In countries like Cyprus, β-thalassaemia heterozygotes are estimated to be 16% of the population, whereas in India it is 1%-10% of the population depending on the area. The incidence of thalassaemia in Western Europe and North American countries is very low and is related to the flow of immigrants from endemic areas. In Western Europe and North America, β-thalassaemia is considered an "orphan disease" because of the small number of patients in comparison to the rest of the population, who are not carriers of the β-thalassaemia gene[59]. There is a 25% chance that a couple who are heterozygotes, carriers of the β-thalassaemia gene, can give birth to a β-thalassaemia major child. β-Thalassaemia major patients can only survive if they receive regular red blood cell transfusions from normal haemoglobin blood

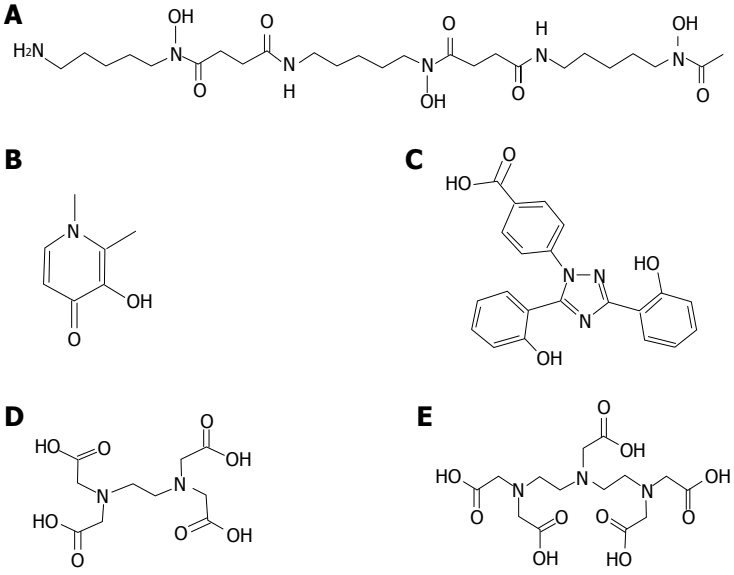

Figure 2 The chemical structure of chelators in clinical use. The chemical structures of the three orphan iron chelating drugs (A-C) which are used for the treatment of iron overload in thalassaemia and two other chelators used in other conditions (D and E): (A) deferoxamine (DF); (B) deferiprone (L1), (C) deferasirox (DFRA), (D) ethylenediaminetetraacetic acid (EDTA) and (E) diethylenetriaminepentaacetic acid (DTPA).

donors in order to replace their ineffective erythrocytes, which contain an abnormal non-functional haemoglobin, unable to transport oxygen to the tissues.

Heart failure as a result of iron overload toxicity from repeated red blood cell transfusions has been until recently the major cause of death in β-thalassaemia patients, which usually occurs before the age of twenty years[58]. This can be minimised or prevented with iron chelation therapy, especially since L1 was introduced[60]. In many developed countries, bone marrow transplantation is used instead of transfusions and iron chelation therapy. This method of treatment is usually applied to a small percentage of mostly very young β-thalassaemia patients and incurs a mortality rate of about 5%-9%[61]. Most global efforts are focused on the prevention of births of β-thalassaemia children using prenatal diagnosis and antenatal procedures[59].

Iron chelating drugs are primarily used for the treatment of iron overload in thalassaemia, which is considered an "orphan disease" in the EU, United States and many other developed and developing countries. The general objective for the design and development of iron chelating drugs for the worldwide treatment of iron overload in thalassaemia and other diseases is that they should be inexpensive, orally effective and non-toxic. However, in addition to thalassaemia and other diseases of transfusional iron overload there has recently been an increased interest in the use of chelating drugs as the main, alternative or adjuvant therapy in many non-iron loaded diseases. The design of iron chelating drugs for clinical applications other than the treatment of iron overload requires different selection criteria and developmental procedures[62].

There are three main iron chelating drugs in clinical use at present, which are used for the treatment of transfusional iron overload, namely deferoxamine (DF), L1 and DFRA (Figure 2). The chelating drugs ethylenediaminetetraacecetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) have been previously used in transfusional iron overload, but were not selective for iron and are currently used for the detoxification of other toxic metals[63]. In particular, EDTA is used in millions of patients in alternative medicine worldwide and DTPA in the detoxification of plutonium and other radionucleotides in the nuclear industry[55,64,65].

There are many controversial, ethical and other issues surrounding the design strategies that led to the discovery and development of the oral chelating drugs L1 and DFRA, their comparison to the injectable drug DF and their current therapeutic use in developed and developing countries.

CONTROVERSIES IN THE USE OF IRON CHELATING DRUGS

The historic development of the iron chelating drugs including efficacy, toxicity, cost and ethical aspects as well as other issues can be followed chronologically through the published medical literature. Within this context, there have been many exchanges questioning the role of pharmaceutical companies and academia in the development of iron chelation therapy.

Both DF and L1 are generic drugs, whereas DFRA is a relatively new patented drug and all three are marketed in many countries worldwide. Deferoxamine has been the mainstay of iron chelation therapy since the 1960's and was marketed by Ciba Geigy (now Novartis). Both DF and L1 are currently marketed by several companies worldwide. In contrast, DFRA is marketed by Novartis worldwide except India, where a local company (Cipla) has registered DFRA based on a local court ruling and is sold in India at a price 60-times cheaper than in the EU and United States (Scrip 2008: S00990226)[66,67].

Deferiprone was invented in 1981 in the United Kingdom and selected as a leading chelating compound out of about 150 related analogues and other compounds[15-18]. It was developed as an academic initiative, which at the first stages was mostly financed by the thalassaemia patient's

organisation in the United Kingdom. Following many preclinical studies, L1 received approval for clinical trials from the local hospital ethical committee and the Department of Health of the United Kingdom in 1986[68,69]. The encouraging clinical trial results in the United Kingdom prompted the expansion of the clinical trials in many European countries, in Canada and in India[70-74]. The multicenter clinical trials were part of an academic initiative involving mainly thalassaemia patients who were unable to receive DF due to toxicity, low compliance or both. Deferiprone was first registered in India in 1994 and then in the EU, Asia and other countries in 1999 and the United States in 2011[55]. The cost of development of L1 in the United Kingdom up to the stage of multicentre clinical trials was less than 2 million USD.

There were many controversies and exchanges regarding L1, amongst academics and between academics and pharmaceutical companies, with one case reaching the mass media involving a Canadian pharmaceutical company (Apotex) and an academic clinician claiming liver toxicity during clinical trials[75-77]. Similarly, embryogenic and other toxicity caused by L1 in non-iron loaded animals was claimed by the company Ciba Geigy (now Novartis), which was then manufacturing DF[78]. In both cases the toxicity was not confirmed by other groups studying L1 in clinical trials with thalassaemia patients and in iron loaded animals[79-81]. These controversies and exchanges highlight the marketing tactics of competing pharmaceutical companies on drugs, which in this case may appear to have been planned to delay the use of L1 until a new owned drug, in this case DFRA was introduced. Similar exchanges were published regarding the possible use of L1 in thalassaemia patients in developing countries, since the high price of DF was prohibitive for the vast majority of patients, who could not afford chelation therapy[81,82].

Deferasirox is a known compound developed by Novartis, which was selected out of a library of more than 1000 compounds and was provisionally approved for clinical use in 2005. The preclinical studies with DFRA were limited and did not address many of the *in vitro* and *in vivo* efficacy and toxicity studies, which were carried out and published during the preclinical development of L1. Initial clinical trials with DFRA in 2003 showed that negative iron balance was not achieved at the then maximum dose of 30 mg/kg per day[83]. Higher doses were not then recommended because of the prospect of renal damage[83]. The maximum dose has now been increased to 40 mg/kg per day for better efficacy, but with increasing prospects of toxicity[84]. Regarding the cost of chelating drugs, it is estimated that in the EU and other developed countries the approximate yearly cost of effective dose protocols for a 50 kg man at 45 mg/kg, 5 d per week on DF is about 5000 euros (excluding needles and pumps, *etc.*) and for L1 at 80 mg/kg per day, 8000 euros. For DFRA at 30 mg/kg per day the cost is about 33000 euros and at 40 mg/kg per day about 60000 euros. The cost of the L1/DFO combination, *e.g.*, the ICOC L1/DFO combination protocol of using L1 during the day (80-100 mg/kg per day) and of subcutaneous DF (40-50 mg/kg at least 3-4 d/wk) is 11000-14000 euros. Deferiprone is sold in India at a price around 4 times cheaper than comparison in Europe and is even sold at a much cheaper price by a local company in Thailand. The same applies for DFRA, where the sale price in India is more than 60 times lower than in Europe and the United States (Scrip 2008: S00990226).

It is estimated that DFRA is costing about 150 euros per patient per year to manufacture and is sold at 60000 euros, with most of the cost claimed as marketing expenses[20]. Both L1 and DFRA can be sold at a price more than 100 times cheaper by non-profit organizations in developing countries, but such initiatives are still lacking, despite the fact that 80% of the thalassaemia patients die untreated in these countries.

Regarding the treatment aspects of transfusional iron overload in thalassaemia, DF is considered to be effective when administered subcutaneously or intravenously at 40-60 mg/kg per day and for oral L1 at 75-100 mg/kg per day, whereas the efficacy of oral DFRA at 20-40 mg/kg per day is still questionable and under investigation[83,85-89]. Iron removal from the heart, which is the main cause of mortality in iron loaded thalassaemia patients, is achieved mainly by L1 and to a lesser extent by DF and DFRA. The latter two chelators appear to be more effective in iron removal from the liver.

In terms of efficacy, the International Committee on Chelation (ICOC) L1/ DF combination protocol is considered to be the most effective because it reduces iron load to normal range body iron store levels[90-93]. The introduction of the ICOC L1/DF combination was an academic initiative based on a model for improving efficacy and reducing toxicity. Many drug combinations are widely used for other diseases in clinical practice without the need for regulatory approval. In contrast, almost all pharmaceutical companies disapprove of such synergistic combinations unless the drugs prescribed are both owned by the same company. Monotherapy with DF, L1 or DFRA are generally less effective than the ICOC L1/ DF combination protocol and only L1 has been shown to reduce and maintain thalassaemia patients' body iron store levels to normal physiological ranges[92,94].

Another major issue in relation to the efficacy of chelation therapy is compliance, which is reduced in patients receiving DF due to the long term 8-24 h daily injections, whereas it is much higher in patients receiving the oral chelators L1 and especially DFRA. In addition to efficacy and compliance, the toxicity properties of the chelating drugs are also a major factor in the overall risk/benefit assessment. Optimal chelation therapy is based on the selection of the appropriate chelating drug(s) and chelation protocol in each condition and for each patient. As shown in many other conditions, each patient appears to have an individual ADME, sensitivity, toxicity and efficacy profile for each chelating drug. Such individual response profiles are currently investigated within the framework of different parameters such as pharmacogenomics, metabolomics and proteomics, which are essential requirements for designing personalised medicine

protocols[85,95].

Different toxic side effects have been reported during the 50 year long-term use of DF and 27 years for L1 in different cohorts of patients. However, in contrast to the other two chelators and despite the fact that toxicity in the medical literature is rarely reported, it is evident from post-marketing reports that DFRA has one of the highest rates of fatal toxicity among new patented drugs, which is associated with renal, hepatic, gastrointestinal and bone marrow failure[96-102]. In FDA and user community toxicity monitoring, 4113 fatalities in patients using DFRA were reported in 2012. Previous FDA individual case based reports of 2474 deaths, suggest that there has been uncontrollable and indiscriminate use of DFRA in many categories of iron loaded and non-iron loaded patients, as well as a lack of toxicity monitoring and absence of prophylactic measures[100]. In a post-marketing report in 2009, DFRA was listed as the second most frequent suspect drug in reported patient deaths following rosiglitazone, with 1320 and 1354 fatalities, respectively[98]. In an EMA meeting the same year, an 11.7% mortality rate (1935 cases out of 16514 patients) was reported and a warning was issued that DFRA's toxicity is likely to increase when the maximum recommended dose increases from 30 to 40 mg/kg per day[99].

A few fatalities have also been reported in the case of DF mainly in relation to mucormycosis, yersiniasis and bone marrow aplasia[103-105]. Similarly, a few cases of fatal agranulocytosis have also been reported with L1, especially in patients who did not adhere to mandatory weekly or fortnightly blood counts, which are used as prophylaxis[106-108].

In addition to these fatal cases, there were many other non-fatal, but serious toxic side effects reported for DFRA which were mainly associated with damage to kidneys, liver, bone marrow and gastrointestinal tract, as well as several other organs such as ocular and auditory abnormalities and skin rashes[109-113].

The toxic side effects in the case of DF were ocular and auditory abnormalities, administration site (injection) related complications of mainly topical or sometimes systemic toxicity and yersiniasis[17]. The toxic side effects of L1 included in addition to agranulocytosis, cases of neutropenia, joint and musculoskeletal pain, gastric intolerance and zinc deficiency, all of which may affect about 5%-10% of patients[17].

The selection of optimal iron chelation therapy protocols in thalassaemia mainly includes the use of DF and L1. The overall risk/benefit assessment suggests that for the vast majority of patients the combination of L1 with DF is the most effective, least toxic protocol and where it is appropriately used it has resulted in a substantial decrease in morbidity and mortality in different thalassaemia patient cohorts[114-116]. This therapeutic approach has changed thalassaemia from a fatal to a chronic disease[116]. Similarly, the ICOC L1/DF combination protocol has resulted in normalisation of the iron stores in thalassaemia patients, which can in most cases be maintained by low doses of L1 monotherapy[90-95]. Despite these encouraging findings, many patients are still treated with DFRA monotherapy which has a better compliance but lower efficacy and a much higher risk/benefit ratio in comparison to DF, L1 and their combination. Only a small proportion of patients can benefit from the use of DFRA, especially those having toxicity complications with DF and L1[85,89-95]. Many patients are also using DF and L1 monotherapy, where iron overload is stabilised, but not eliminated to the extent of achieving normal range body iron store levels.

The variation in iron chelation treatments in different hospitals, countries and overall worldwide, reflects the influence of doctors on drug selection, patient safety and treatment outcome. This variation also reflects the marketing influence of pharmaceutical companies on doctors, health authorities and governments, and highlights the factors influencing the ability of doctors and health authorities to identify and select appropriate therapy protocols for patients[20].

Further developments in the area of iron chelation therapy are the ongoing clinical trials and uncontrolled clinical use of DFRA in other categories of transfused iron loaded patients such as myelodysplasia and sickle cell disease, and in non-transfused iron loaded patients such as hereditary haemochromatosis, thalassaemia intermedia, and post-transplanted thalassaemia patients. This wider use approach and development undermines patient safety and increases the risk/benefit ratio since any long or short-term benefits on morbidity and mortality from the use of DFRA in other categories of patients and especially in myelodysplasia and sickle cell disease patients are questionable and not yet confirmed[117,118]. Similarly, venesection in hereditary haemochromatosis and post-transplanted thalassaemia patients is a much safer and inexpensive procedure in comparison to DFRA treatment. The same applies to many non-transfused thalassaemia intermedia patients, where chelation therapy with L1 and DF is also safer than DFRA[119]. The wider use of DFRA in the above conditions indicates the pharmaceutical company's marketing potential and the influence it exerts on the risk/benefit assessment of chelation therapy by individual clinicians.

The use of iron chelating drugs in patients with non-iron loaded diseases is another expanding area attracting a lot of interest among clinical investigators and pharmaceutical companies. Similarly, the application of iron chelating drugs could include rare and tropical diseases where no effective treatment is currently available[120-126]. The safety of L1 in non-iron loaded patient categories seems to be higher in comparison to DF and DFRA, as shown from many short and long-term clinical trials in neurodegenerative, renal, infectious and other diseases[62,127]. In contrast, fatal and other serious toxic side effects have been observed in clinical trials using DF, in non-iron loaded patients such as in mucormycosis and rheumatoid arthritis patients[104]. Similarly, in one of the FDA related reports it was estimated that of the 2474 individual fatal cases reported for DFRA at least 500 were not related to transfusional iron overload, but included

cancer, cardiovascular, neurological and other patients with normal iron store levels[100]. Within this context, L1 appears from the various clinical trials with non-iron loaded patients to be relatively safe and promising for wider clinical use as a main, alternative or adjuvant therapy in many diseases, and as a pharmaceutical antioxidant[62,128,129].

The evaluation methods and the controversies which were identified in relation to the development and use of chelating drugs may help in the introduction of new approaches and strategies for the design, development and use of orphan and other drugs. Such academic approaches may benefit the treatment of millions of patients with many other conditions worldwide. Within this context, the paradigm of the design and development of chelating drugs, and especially L1, which was based on academic initiatives, may help to illustrate the need for new strategies in drug development. The use of this approach can increase the accessibility to new drugs due to low drug prices and decrease the morbidity and mortality observed in many diseases in both developed and developing countries.

Drug development based on academic initiatives can minimise costs and increase the prospects of the introduction of new drugs, which can be applied in the treatment of many diseases in both the developing and developed countries. At present the high prices of new patented drugs which are the result of high costs in drug development are not affordable for the vast majority of patients.

In addition to new strategies for the reduction of costs in the production of orphan and other drugs, several other initiatives can be taken to improve patient treatments worldwide. These include further research challenges involving better understanding of the heterogeneity in the underlying mechanisms of disease processes, inter-patient variability in drug responses and better risk/benefit assessment procedures.

CONTROVERSIES IN DRUG DEVELOPMENT AND PATIENT SAFETY

In almost all cases of the development and marketing of new patented drugs, which are mostly undertaken by private multinational pharmaceutical companies, a number of marketing strategies are developed for maximising sales and profit for their product such as advertisements, publications, lobbying and conferences (Figure 1). Within this context, a large marketing plan is constructed involving among others the recruitment of consultants who are usually internationally known influential academics in clinical departments of public hospitals mainly in Western Europe and the United States. These consultants are usually involved in seeding clinical trials and the promotion of their new drug product in publications, conferences, patient organisations and regulatory authorities[20,130].

The market plan also involves arrangements where company representatives personally visit all clinicians who have jurisdiction over potential patient/customers, with the prospect of advertising and promoting their drug to be used by their patients and in most cases offering in exchange different forms of support or gifts ranging from a meal, to the covering of expenses for their participation in conferences where the drug is promoted, consultancies, grants and other benefits (Figure 1).

In many countries with no legal restrictions, pharmaceutical company representatives often offer a percentage of the sale of the drug they promote to clinicians and to other influential persons in health or regulatory authorities. A different marketing strategy is to offer a clinician a "compensation" for "enrolling" patients in clinical trials, with the prospect of the patients involved to remain on the treatment and the government health authorities to pay huge sums of money for the continuation of the expensive new treatments (Figure 1). For example, 5000 euros is paid to the clinicians in charge of the enrolment of each patient for post-marketing monitoring of one of the new patented drugs in Greece.

A number of cases involving bribery of clinicians by pharmaceutical companies have reached the courts, but corruption is so extensive and out of control that in one court case in Germany a limit of 10000 euros was allowed as a donation for such activities for private clinicians, most of whom are working for the National Health System[131-135]. It should be noted that all the benefits paid to the clinicians for drug promotion are included in the cost of drugs as marketing expenses and are paid by the tax payers through the government health authorities (Figure 1). In developed or other countries with legal restrictions on the influence of private organisations, financial support by pharmaceutical companies is provided indirectly through donations to academic, patient, charity and other organisations associated with the supporters or promoters of their drug (Figure 1).

Within the framework of new drug development and marketing, multinational pharmaceutical companies can provide clinicians with financial support for different events and in some cases for research projects and clinical trials involving their drug, under a secrecy agreement[136-139]. Usually, only positive results are allowed to be published by the investigators financed by the pharmaceutical company, which are under the scrutiny of their marketing, legal and medical writer's department[137-140]. Similarly, reports of toxic side effects and studies of low or no efficacy are rarely published. Within this context, pharmaceutical companies influence academic research and academic affairs, including the impact factor of journals, the citations of articles and the citations for authors. Academic consultants of pharmaceutical companies can also influence publications of competing new or generic drugs, by serving in editorial boards or as referees. The unbiased role of journals is also questioned, since almost all journals are businesses and dependent on income from the pharmaceutical industry including advertisements, reprints and conferences[140,141].

Similar influences can also be exerted in other aca-

demic platforms and in medical conferences, especially when the pharmaceutical company is a sponsor. The number of conferences organised and sponsored by a single pharmaceutical company for the promotion of their drug is continuously increasing and support for independent conferences where competing drugs are presented is continuously decreasing.

In addition to targeting clinicians, a similar marketing strategy by pharmaceutical company representatives is also directed to other individuals or organisations, which may influence the sale of their drugs such as patient organisations, academic societies, regulatory authorities and other governmental bodies (Figure 1). For example, most of the conferences related to medicine including the expenses for participation by clinicians, nurses and patients are supported by multinational pharmaceutical companies introducing new drugs. Similarly, pharmaceutical companies are the major sponsors for medical societies, patients' organisations and selected medical departments in academic institutions (Figure 1).

Despite the fact that in some developed countries a number of restrictions have been introduced to reduce the influence of pharmaceutical companies in academic and public institutions, the influence is still evident. Such influences may not appear to be on a direct personal level, but are exerted indirectly, for example by donations or grants to academic institutions and societies[137-141].

In general, the marketing approach is different for each pharmaceutical company, for each country and each "customer" and depends mainly on the local conditions and the potential market scale. This can be illustrated by the variability on the sale figures and the use of the three iron chelating drugs in different countries, hospitals and individual doctors.

The marketing influence of multinational pharmaceutical companies for safeguarding the sale of their new drugs and for generating new income worldwide is evident from many recent events such as the health scare in relation to the spread of A H1N1 influenza and other similar viruses, where vaccines worth billions of dollars were sold worldwide[142]. It should be noted that national governments and the WHO endorsed and advertised the campaign for the pharmaceutical companies to sell and distribute these vaccines worldwide, even when these were not licensed[66,142]. Following the scare warnings some of the national and international advisors in the decision making panels for the approval and supply of the vaccines were later identified also to be consultants for the pharmaceutical companies selling the vaccines[66,142]. This and similar examples illustrate the need for the creation of independent public expert committees, *e.g.*, like NICE in the United Kingdom for safeguarding the rights of patients and for protecting and distributing national health resources according to the health needs of patients and not according to the influences of pharmaceutical companies[143].

There are many other grey areas in the development and sale of pharmaceuticals involving marketing tactics and strategies which are jointly developed between pharmaceutical companies such as price fixing, market sharing, market exclusivity and other arrangements between the companies selling the generic drugs and the new drug or competing drugs. In all of these cases public funds and spending on drugs is misappropriated and pharmaceutical companies make huge profits, which reduce health resources and in many cases may also affect patient safety[20].

The protection and domination plan of the patent monopoly for a new drug product in specific diseases is another major part of the marketing strategy of the patent holder pharmaceutical companies. This may involve the discrediting of competing drugs for example through publication of possible adverse effects by academics supporting their drug or through legal conflicts in relation to exclusivity, or on therapeutic claims made by similar drugs. Other methods used include the acquirement of new patents and delaying tactics in the development of an investigational new drug that may influence the sale of their drug. These marketing and other tactics are pursued not only during the lifetime of a patent, which is usually 25 years, but also when the patent life of a drug is expired. As a result, the price of a drug when it becomes generic (*i.e.*, when there is no patent protection) remains about the same as when it was first introduced.

New patents are usually filed by the proprietor company on the same drug before the initial patent expires. The new patents may involve different drug formulations or related uses or different claims for the same or other diseases. This effort is usually undertaken by the initial patent holder company in order to safeguard the exclusivity of the monopoly, the high sale price and the level of the profits that can be made from the sale of their drug.

The sale price of a drug is another grey area affecting public health funding and drug availability in developed and developing countries. One of the contributory factors for the high price of new drugs is usually the inclusion of the marketing budget in the overall cost for drug development (Figure 1). For example, the costs for the organisation of conferences and the support for physicians to attend such conferences are included in the marketing budget and for fixing the price for the drug. Considering for example that the actual cost of producing and developing a new drug may be negligible, *e.g.*, less than 1 USD/g, the actual retail sale price may be in comparison greater than 1000 USD/g when the drug is under patent protection and produced by a multinational pharmaceutical company in a developed country. Similarly, if the same drug is not protected by a patent then the price could be less than 20 USD/g, if produced by a pharmaceutical company in a developing country. The same also applies when the patent expires and the drug becomes generic.

In general, the high price of drugs is a considerable obstacle in the provision of a better health care system in each country and diminishes the possibilities of supplying new improved drugs for the treatment of patients in developing countries and in some cases in the developed countries.

Many other controversial issues surrounding the development and use of new drugs include toxicity monitoring and differences between regulatory authority procedures among countries. Similar controversies involve the lack of transparency in the reporting of results of clinical trials, ineffective reporting of the adverse effects to the clinicians using the new drug by the pharmaceutical companies and the regulatory authorities, as well as misinformation on the risk/benefit assessment and criteria for the use of the new drug by comparison to generic drugs.

Although many pharmaceutical companies may be aware of the high toxicity of their newly introduced drug, they will continue selling it to acquire as much profit as possible. The new drug will not usually be withdrawn unless the company's profit margins are threatened or may be eliminated due to compensation claims or requests made by the affected patients and their legal representatives[20,100].

The drive for profit by multinational pharmaceutical companies and the lack of strict regulatory procedures or ethical codes endanger the safety of patients and the prospect of introducing optimum treatments. Furthermore, the present system adversely affects the economy of developing countries and health care resources of most developed countries. Within this context, it is highly unlikely that a pharmaceutical company would support any academic research for identifying and decreasing the toxic side effects of their drugs, following regulatory authority approval for clinical use and marketing. The same approach applies to research with generic drugs despite the fact that the manufacturing company involved may still have a sale monopoly or the drug may be tested for a different formulation or patent application.

Drug research, development and availability are relying at present almost exclusively on market forces and pharmaceutical companies' initiatives, marketing policies and decisions. This approach is not in many cases ethical and may not lead to optimal treatments and best patient care solutions. Similarly, the lack of health strategies and policies on drug design, development and use can overall influence patient treatment and safety[144].

There are no transparent procedures or specific ethical rules at present that will safeguard the rights of patients for the safest and most effective treatment for their disease or condition. The awareness and ethical approach for the treatment of each patient is the responsibility of the clinician in charge, who among others should provide the best care and prescribe the safest and most effective treatment and drugs. This responsibility is crucial for life threatening conditions, where the wrong risk/benefit assessment may result in ineffective treatments or serious toxicities and an overall increase in the morbidity and mortality rates. While organisations such as NICE in the United Kingdom may improve patient treatments and decrease costs, new organisations are needed to curb excess profits made by multinational pharmaceutical companies on new patented drugs. Such initiatives can decrease the cost of health care in developed countries and increase accessibility to drugs by patients in developing countries.

Improved measures on transparency regarding the reporting of clinical effectiveness and toxicity may help in the selection of the most appropriate and safe treatments for general, but also for individual patient use. Cost/effectiveness assessment issues are also important and may help orphan patients in developed as well developing countries[143].

Overall, it seems possible that with the appropriate health care policies, patient access to cheaper drugs can increase and waste on resources and health spending decrease. Such policies can benefit millions of orphan and other patients and may help in the treatment and elimination of many orphan diseases.

NEW OUTLOOK IN WORLD HEALTH ISSUES

Hunger, malnutrition, poor sanitation, impure water and lack of medicinal drugs appear to be the main causes of mortality and morbidity worldwide, affecting mostly children and infants in developing countries[28]. These problems can be overcome by increasing the production and supply of food, vaccines and medicinal drugs, as well as by improving water and sanitation technologies[1,2]. Within this context, a substantial reduction in the global mortality and morbidity levels can be achieved provided the appropriate health policies and strategies are implemented for each disease[25-27]. Such strategies should include further research on the mechanisms, drug treatment and prevention of diseases, population control through family planning, reduction of food waste in developed countries and reduction in environmental pollution.

Health resource allocation is a worldwide problem affecting all countries, health services and institutions and most categories of patients. Among these categories are orphan patients with rare, tropical and orphan diseases found both in developed and developing countries, who usually have limited access to treatments and also increased requirements for basic medications. The development of orphan drugs for such diseases can help many millions of patients worldwide. However, present conditions and policies are insufficient for overcoming these problems because of many limitations such as loopholes in regulatory and trade laws, misappropriation of public funds, bias in reporting and many other irregularities all of which undermine the efforts for improving the present status quo and benefiting affected patients (Figure 1)[20].

The present world trade and patent laws mostly benefit developed countries and affect the effective supply of sufficient food and drugs in the developing countries, where the resources are scarce and the appropriate technologies underdeveloped[12,14]. This can be illustrated by the sale of drugs amounting to about 0.5 trillion USD per year by the first twelve richest multinational pharmaceutical companies situated in the United States and Western Europe (Table 2). Such revenues are mainly generated from drugs sold for major common diseases. Interest

in drug development for neglected tropical diseases and rare, orphan diseases is limited, unless pharmaceutical companies are convinced that such projects can result in substantial profit returns[11]. The introduction of orphan drug legislation in developed countries has increased orphan drug development and global orphan drug sales which have steadily increased in the last few years and are now approaching 100 billion USD annually[13].

Market and monetary conditions appear to be the major determinant in government health policy, public health budgets and health resource allocation, which affect, to a great extent, the treatment of patients worldwide. However, it was recently realised by governments in many countries that allowing market forces and the free economy to influence and determine health conditions and resource allocation will be very costly and detrimental to the overall treatment and safety of patients[1,2,7,20].

Within this context, the institution of NICE in the United Kingdom and similar organisations elsewhere in developed countries is a limited step in the right direction, but does not address other major issues such as the pricing of drugs and the influence of pharmaceutical companies on governments, clinicians, pharmacists, regulatory authorities and other organisations. Similarly, it does not address many other problems such as pharmacoeconomic issues, literature bias and misinformation, transparency on drug efficacy and toxicity as well as development of generic drugs and nutraceuticals, and the availability of drugs at a lower cost than developing countries. One of the problems on drug selection and treatments is that NICE and similar organisations rely on published data, which are mostly biased due to the association with pharmaceutical companies and they are not generated by independent clinical investigators[5,19,20,24,50,141].

It appears that as the world population expands and the global economic situation is worsening, financial health resource allocation will become more important and related issues will be discussed on a wider platform in the state authorities, the medical literature and elsewhere. Similarly, initiatives may be taken by government bodies at different levels to decrease or limit the expenditure on drugs and medical devices, without lowering patient safety or treatment standards.

The paradigm of drug design and development of L1, which was mostly the result of academic initiatives and procedures, may prove to be a suitable model for the design of orphan drugs for orphan and rare diseases. This model has been shown to be more successful in comparison to the model of development used by pharmaceutical companies for DFRA, since L1 has been shown to be less toxic and more effective. Furthermore, as a result of transparent procedures a number of prophylactic measures were introduced as soon as the toxic side effects of L1 were identified and reported in the medical literature[17,127].

Transparency on the efficacy, toxicity and costs of the drugs is a major aspect of decision making for health policies and resource allocation. Despite the fact that such issues should be examined by expert public watchdog committees, most decisions rely on the pharmaceutical company's submission data and to a lesser extent on published data by independent investigators. Within this context, a relatively new science was developed, pharmacoeconomics, which is trying to address the cost of drugs and the impact on public health and society in general.

However, research reports suggest that there is evidence of publication bias with about 90% of pharmacoeconomics articles in most journals supporting the drug in question, compared to only 30% in the New England Journal of Medicine[52,141]. Similar articles sponsored by Novartis have been published for DFRA suggesting that DFRA is better value for money than DF, which however took into account much higher daily and weekly doses of DF, excluded the DFRA cost of toxicity monitoring and treatment outcomes as well as other relevant parameters[66,145]. Similar comparisons were also made by a different study sponsored by Apotex, one of the manufacturers of L1 in Western countries and by a company in Thailand, which showed much lower costs using L1 than other chelators[146,147].

Publication bias, misinformation, lack of transparency, selective reporting and other issues surround the publications sponsored by pharmaceutical companies, their academic consultants and medical writers[20,52,141]. Pharmaceutical company sponsored publications usually make excess claims on drug efficacy in contrast to toxicity, which is usually omitted or is scarce. Other marketing methods include misinformation reports on reduced efficacy, high toxicity and high cost of generic and other new competing drugs. Similar strategies, may involve the highlighting of reports of clinical studies using ineffective doses of generic and other new competing drugs or by making false claims on their toxicity.

Publication bias is also the responsibility of the editorial boards of journals, where the risk/benefit assessment for drugs is overtaken by other issues, which may ultimately influence patient treatments. In the case of L1 for example, an editorial dealt with a conflict between an academic and a pharmaceutical company (Apotex) without questioning the motives of the academic or the risk/benefit assessment on the treatment of the thalassaemia patients in developed and developing countries[76]. The same journal ignored its own earlier publications on the efficacy of L1 and its impact on the treatment of thalassaemia patients, probably because L1 was used under its chemical name and before the INN name Deferiprone was registered[69]. This conflict delayed the registration of L1 in the USA and Canada for more than twelve years, which may have resulted in many fatalities related to congestive cardiac failure, since L1 is known to effectively remove iron from the heart and reverse or prevent this form of iron toxicity[50,60,75,92].

There are many other examples of pharmaceutical company marketing policies for driving the market to adopt their product in addition to academic journals, such as the recruitment of patients in seeding trials, who eventually will remain on the treatment with the new drug after the trial, also in swinging opinion in support

of their new drug through conferences, through bribery and other methods (Figure 1)[130-133]. Such methods can influence patient treatments costing the health services billions of dollars, such as in the case of renal dialysis for end-stage renal diseases (ESRD). Emphasis on the prevention of progression of diabetic nephropathy *via* generic drug therapy may minimise such costs[148].

Despite the influence of pharmaceutical companies on public health resource allocation, recently there have been an increasing number of initiatives to change the status quo and overall improve public health care, including the prospect of orphan patient treatments in developing and developed countries. Examples include the successful lobbying by patient organisations for the introduction of L1 in the United States, the court ruling for cheaper imported drugs in India to help local patients and the clinical evaluation of EDTA by NIH in the United States for possible use in patients who suffered myocardial infarction and diabetes[55,149-151]. Further improvements can include the introduction of nutraceuticals, generic drugs and drug combinations in many orphan, rare and other diseases. For example, zinc has been suggested for use as adjunct treatment in infants with serious bacterial infections, which is one of the top causes of global mortality[152]. Similarly, the wider application of L1 and other generic drugs as main, alternative and adjuvant therapies in many conditions may result in more effective and less costly therapeutic options for many categories of orphan and other patients[55].

NEW POLICIES FOR IMPROVING DRUG AND HEALTH DEVELOPMENT

There are increasing prospects of reducing the rate of global mortality and morbidity by adopting specific policies that can have direct effects on many diseases including orphan, rare and tropical diseases. Improvement of food and health resources, better sanitation and drinking water purity, sex education and family planning are some of these policies which can play a major role in such efforts, especially in developing countries[1-3,153]. Similarly, the availability of drugs for the treatment of diseases is another major factor affecting the rate of global mortality and morbidity, especially patients in developing countries.

In contrast, different health policies can be introduced to reduce the rate of mortality and morbidity in the developed countries. Such policies can reduce, for example, environmental pollution, bad dietary habits, excess alcohol consumption and smoking which have been shown to lead to obesity, cancer, cardiovascular and other diseases. A similar policy is to tax such unhealthy habits similar to cigarettes and the relevant tax revenues to be used for subsidizing food supplies for malnourished people, vaccines, medicinal drugs and improve health education.

Health resource allocation can also be improved by reducing public health spending on drugs and diverting resources to other health areas which have an impact on the rate of global mortality and morbidity. This can be achieved through the adoption of policies that can limit excess profits made by multinational pharmaceutical companies and also through other measures that may facilitate the use of the best, safest and less costly treatments for the benefit of patients globally. Such measures and policies may include the sale price of drugs in developing countries to be adjusted as to the per capita ratio of developing to developed countries.

Transparency on the efficacy, toxicity and costs of manufacturing and sale of drugs including orphan drugs is essential for the development, application and safety of drugs in all diseases[66]. Within this context, the risk/benefit assessment of different drugs for each condition should be independently assessed and not rely only on published data, especially on studies sponsored by pharmaceutical companies. Identification of the therapeutic index of new and generic drugs and their impact on the treatment of each condition is also important for decreasing patient morbidity and mortality. Similarly, the implementation of drug safety measures is paramount for patient survival and well-being. In addition to the identification of toxic side effects, research on prophylactic measures and identification of drug antidotes can increase patient safety and survival.

The development of personalised medicine based on ADME, toxicity and other parameters is a further step in the achievement of improved therapeutic interventions[154]. Within this context, randomised clinical trials may not necessarily be the only tool of comparison for competing drugs in each condition. This is particularly important for generic drugs, where it may not be feasible to carry out such studies due to insufficient funding. The most important parameter for comparison in drug application in all diseases is whether the drug in question achieves a full treatment with acceptable level of toxicity. In cases where full treatment cannot be achieved, the effects on morbidity, mortality and cost of the drug under investigation should be compared to other drugs used for the same condition.

The paradigm of the development of L1 and other orphan drugs, which was based on academic and patient initiatives and efforts, appears to be a more successful, less costly and safer method in comparison to the monetary approach of pharmaceutical companies on orphan drugs. In such cases there are transparent procedures and scrutiny by academic peers with ethical instead of monetary motives. Similar academic initiatives involve the institution of drug combination treatments, which are studied and developed by academics independently of pharmaceutical companies and monetary motives[90-93,115]. Generic drug research and applications in other conditions such as the use of EDTA in many non-metal toxicity conditions by thousands of clinicians worldwide in millions of patients is another example of drug development independent of the pharmaceutical companies[55,64,150,151]. The development of other generic drugs and nutraceuticals is also increasing, especially in conditions where treatments are not successful or very expensive. Such approaches in-

clude the development and use of medicinal products in alternative and Chinese medicine.

The role of pharmaceutical companies is essential in manufacturing and developing drugs against diseases. This is a major contribution to society since it helps the treatment of billions of people every day. However, like any other business their main aim is profit and ethical considerations are secondary or absent in their agenda and policy planning. Within this context, their efforts are based on ways to maximise the income from the sale of their drugs. Unless government and regulatory authorities are vigilant and their medicinal drug policies are sufficiently effective, the treatment and safety of patients may be compromised by the activities of pharmaceutical companies including the exploitation of loopholes in regulatory and marketing procedures[20]. Similarly, the development of orphan drugs by pharmaceutical companies is not of major interest unless major financial benefits are clearly secured. Such policies and activities affect health resource allocation and especially orphan patients with orphan or rare diseases.

Drug selection and availability to patients in many cases resembles the sale of a market orientated product, like a chocolate brand or other food commodities. This can be observed for example in chelation therapy, where the drugs used for treatment are related to the marketing success of the manufacturing company and not the patient needs. The responsibility for allowing the manipulation of the drug market by pharmaceutical companies, which affects patient treatment outcomes and safety, lies exclusively with the government, the regulatory authorities and the clinicians. There are many recent examples of commercial company manipulations and government interventions, which may influence health resource allocation and outcomes. Such interventions include fines to settle civil and criminal investigations by the United States government in relation to sales and practices of various drugs by Pfizer totalling 2.3 billion USD, and a 3.0 billion USD fine for similar activities by Glaxo Smith Kline[155,156].

Several other measures and suggestions can also be considered in order to minimise the influence of pharmaceutical companies on patient treatment outcomes and safety and in reducing drug costs. For example, marketing expenses should not be included in the cost estimation and sale price of drugs. Similarly, the entry of pharmaceutical company marketing representatives should not be allowed in public hospitals for drug lobbying purposes, unless transparent procedures are followed and the time used for consultations is outside normal working hours. Manipulation of the drug market and prices and lack of transparency on safety, efficacy and costs should be penalized by the government authorities as shown in the United States, or where there are ineffective or corrupt regulatory authorities these can be replaced as shown, for example, in France[156,157].

The influence of pharmaceutical companies on academia including publications should be more transparent and the publication of studies sponsored by pharmaceutical companies should be cited as advertisements. Similarly, academics associated with pharmaceutical companies or the employees of pharmaceutical companies should not be used as referees or in editorial boards for drug related topics.

Members of public hospitals, local and international health authorities should be prevented from acting as consultants for private pharmaceutical companies unless such activities are through transparent procedures and commercial involvement is outside public service responsibilities. The support received by clinicians from pharmaceutical companies including participation in conferences and clinical trials should also be through transparent procedures. Similarly, results from clinical trials on drugs should be independently reported and not controlled by medical writers and others belonging to the proprietor pharmaceutical company. Public expenditure in relation to doctors' absences for conferences organised or sponsored by pharmaceutical companies should not be considered as further education activities, unless these are organised by independent academic societies or non-profit academic organisations.

These and many other policies and measures may help to decrease the expenditure by health authorities on drugs both in developed and developing countries. Similarly, it may also help in the adoption of more transparent procedures and the improved allocation of health resources which may lead to better patient treatment outcomes and better safety. In the meantime, many research challenges are continuously emerging in many orphan, rare and other diseases which may influence health outcomes, morbidity and mortality as shown by L1 and other drugs[158-162].

The world efforts for better redistribution of global wealth may be the answer to health resource allocation, since the major cause of mortality at present is associated with poverty, malnutrition and starvation. Present estimates suggest that 1% of the world population owns 50% of the global wealth and that more than 3.5 trillion USD are deposited in tax heavens by rich individuals and commercial companies.

CONCLUSION

World health developments including morbidity and mortality outcomes are a reflection of many factors which are affected by health policies in individual countries and globally. Food availability, health provision and education, family planning, disease prevention, nutrition, environmental and monetary influences, genomic and psychological aspects are some of the factors which are in dynamic equilibrium and can influence health levels and outcomes in each country. There is scope for substantial improvements in world health policies and many ethical dilemmas and issues related to health strategies need to be prioritised, readdressed and resolved in each country and also globally. The disease profile and health policies between developed and developing countries are different, with profound financial resource insufficiencies in the latter.

The availability and cost of generic and new medicinal drugs are among the major areas affecting the level of global health care. Monetary, ethical and other issues affect the supply of medicinal drugs for different categories of patients in each country. Health policies, regulatory and marketing procedures can variably influence the risk/benefit assessment, patient safety, drug availability and drug treatment outcomes in each country. Public health and overall national spending are also influenced by such procedures. Reassessment of drug pricing and of regulatory procedures with major emphasis on the development of orphan drugs based on a risk/benefit assessment may help in the treatment of many categories of orphan and rare diseases and millions of orphan patients globally. The criteria for drug development and use and of price levels in each condition should be readdressed and modified to improve patient treatments, drug safety and minimise costs.

The implementation of improved policies on health resource allocation and drug development can lead to the realisation of many major health aims such as the introduction of worldwide and universal health care. Similarly, advances in medical research can lead to the elimination and improved treatment of many diseases, to an overall reduction in the morbidity and mortality rates and an increase in the quality of life for patients worldwide.

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MINIREVIEWS

Dyspepsia and celiac disease: Prevalence, diagnostic tools and therapy

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Abstract

The prevalence of dyspepsia is up to 40% in population-based study. Functional dyspepsia is an exclusion diagnosis and it is classified as a chronic abdominal pain-related functional disorder, characterized by the presence of persistent or recurrent pain or discomfort centered in the upper abdomen, neither relief by defecation, nor association with the onset of a change in stool frequency or form. Celiac disease (CD) is a common autoimmune enteropathy, with a prevalence around 1% in the general population. Its diagnosis includes a serological screening and an upper gastrointestinal endoscopy with multiple biopsies. Gluten-free diet is the only effective treatment. CD diagnosis is often delayed in asymptomatic patients or in individuals with less clinical gastrointestinal symptoms. Several studies performed coeliac disease screening in patients with symptoms suggestive of dyspepsia, showing a biopsy-proved prevalence that ranged from 0.5% to 2%. The typical endoscopic markers of villous atrophy are not sufficiently sensitive, so some endoscopic techniques, such as "water immersion" and confocal endomicroscopy were proposed to improve the diagnostic sensitivity and target biopsies. A recent meta-analysis estimated that the prevalence of CD was higher in patients with dyspepsia, but not in a statistically significant way. However this assumption should be confirmed further larger studies.

Key words: Dyspepsia; Coeliac disease; Upper endoscopy; Villus atrophy; Screening

Core tip: Dyspepsia is classified as a chronic abdominal pain-related functional disorder that affects almost 40% of the population. It can be also a manifestation of celiac disease, an immuno-mediated enteropathy, caused by the ingestion of gluten in genetically predisposed patients. The prevalence of celiac disease among dyspeptic patients has been investigated, with results ranging from 0.5% to 2%. Celiac disease diagnosis requires histological evaluation of villous atrophy on duodenal biopsies specimens. Screening for celiac disease in dyspeptic patients and routinely performing of biopsies during upper gastrointestinal endoscopy, may be useful as part of the diagnostic flow-chart of these patients.

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INTRODUCTION

Dyspepsia is one of the most common gastrointestinal disorders to be faced in clinical practice, with prevalence up to 40% in population-based study[1] so that the economic impact is very high.

When dyspepsia is not a manifestation of an organic

pathology, such as gastroesophageal reflux disease or peptic ulcer disease, then it is classified as functional dyspepsia (FD).

FD markedly reduces patients' quality of life, similarly to mild heart failure and menopause[2]. However FD is an exclusion diagnosis and on the basis of Rome Ⅲ criteria[3], it is defined as the presence of gastroduodenal symptom without evidence of structural disease able to explain the syptoms. Often patients refer to suffer from early satiation or postprandial fullness (postprandial distress syndrome), epigastric pain/discomfort or burning (epigastric pain syndrome).

Pathophysiology of FD is not completely understood yet and several pathophysiological mechanisms have been proposed to underlie symptoms. Central processing of visceral stimuli, low-grade inflammation in the duodenum and genetic factors are the main emerging hypothesis investigated[4]. FD is difficult to manage, because no medication is currently approved in the United States, Canada or the European Union. Many treatments have been proposed (diet, eradication of *H. pylori* and drugs such as prokinetic agents or protonic pump inhibitors)[5] but no one was satisfactory.

Celiac disease (CD) is an auto-immune enteropathy, whose diagnosis is often delayed in asymptomatic patients or in individuals with less clinical gastrointestinal symptoms, such as abdominal bloating, nausea and vomiting. CD diagnosis, according to the American Gastroenterology Association, consists of a serological screening (including anti-transglutaminase, anti-endomisium and anti-deamidated gliadin antibodies) and an upper gastrointestinal endoscopy with multiple duodenal biopsies. Gluten-free diet is the only effective treatment for the disease.

However, although dyspepsia may be a manifestation CD, most of FD patients do not perform serological screening for CD or duodenal biopsies and there are few data about the prevalence of CD in patients with dyspepsia.

Recent studies[6-10] demonstrates that the prevalence of silent CD in patients with dyspepsia is slightly higher than that of the general population, however in one study it resulted rather low[11].

The 40%-60% of subjects with dyspepsia resulted macroscopically normal when performing upper gastrointestinal endoscopy[12]. Unfortunately, the practice of performing biopsies, even in absence of endoscopic alteration of intestinal mucosa, is quite uncommon.

The typical endoscopic markers of villous atrophy include mosaic pattern, scalloping of folds, and a decrease of duodenal folds. However, mostly in less severe cases, CD diagnosis cannot only be performed on these parameters. So, considering that many authors describe these markers as not sufficiently sensitive, some endoscopic techniques, such as "water immersion" and confocal endomicroscopy (CEM) were proposed to improve the diagnostic sensitivity and target biopsies in most damaged mucosal areas[13,14].

DYSPEPSIA AND CELIAC DISEASE

Recent studies demonstrate that the prevalence of CD in patients with dyspepsia is higher than that of the general population[6-10].

Bardella *et al*[6] prospectively enrolled 517 patients suffering from dyspeptic symptoms. All patients were submitted to upper gastrointestinal endoscopy, and six were diagnosed to be celiac (1.2%). Interestingly three patients (50%) had a normal duodenal endoscopic pattern and five of the six celiac patients were young women aged between 20 to 37 years. The authors suggest to perform serological screening for celiac disease especially in young women suffering from dyspepsia.

Lima *et al*[7] reported a CD prevalence of 1.4% in a small series of patients with dyspepsia, both were young women, aged 19 and 25 years respectively. In the paper of Ozaslan *et al*[8] among the 196 investigated patients three were diagnosed to be celiac (1.5%). All were female younger than 52 years, and only two showed abnormal endoscopic findings.

In the manuscript by Giangreco *et al*[9], published in 2008, the role of upper gastrointestinal endoscopy in CD diagnosis was evaluated in patients suffering from FD. The prevalence of CD was 2% (15 patients out of 726 enrolled), higher than the general population one, also considering that patients with an increased risk for CD (such as first degree relatives) were excluded from the study. Among the 15 CD patients (age ranged 20 to 56): 10 were female and only 8 patients presented endoscopic findings suggestive for CD.

Keshavarz *et al*[10] investigated the prevalence of CD among 170 patients with FD. Twelve patients (10 female), suffering form dysmotility-type dyspepsia, tested positive for CD related antibodies, however only two of them showed villous atrophy at the histological evaluation.

Only in the paper by Heikkinen *et al*[11] published in 1995, among the 400 uselected dyspeptic patients enrolled to perform upper gastrointestinal endoscopy, serological evaluation and abdominal ultrasound, CD was diagnosed in 2 patients (both aged less than 64 years). The low prevalence (0.5%) could be due, maybe, to the eterogeneity of the population study, with a higher percentage of aged patients (77% were more than 44 years old) while the most frequent diagnosis in younger patients was lactose intolerance (9%).

In a recent meta-analysis by Ford *et al*[15], the authors provided a pooled prevalence of biopsy-proven CD of 1.0%, similar to that in the general population, when duodenum biopsy was performed as first-line investigation. However when the authors pooled the data from the studies that used the Rome Ⅱ criteria for dyspepsia, the biopsy proved CD was 2%, significantly higher.

CD

CD is a chronic, immuno-mediated enteropathy, caused by ingestion of gluten in susceptible individuals, carrying DQ2 and/or DQ8 HLA. It is characterised by a chronic inflammatory state of the small intestine that recover after gluten withdrawal. The typical changes of the duodenal mucosa include: raised intra-epithelial lymphocyte, crypt hyperplasia and various degree of villous atrophy

as classified by Marsh and modified by Oberhuber *et al*[16] in 1999, that decreased digestion of food and micro- and macronutrients absorption.

CD is common, with a prevalence around 1% in the general population of Western countries[17,18], more frequent in females than males.

Pathogenesis

The pathogenesis is multifactorial, including the interactions between environmental, genetic and immune factors. Gluten, a protein derived from wheat, barley and rye, represents the trigger factor of CD. The alcohol-soluble fraction of gluten, the alpha-gliadin, is rich in prolamine and glutenine that could trigger an immune response, mediated by both innate and adaptative arms of CD patients' mucosal immune system. Genetic susceptibility plays a crucial role in CD pathogenesis, as demonstrated by the increased prevalence in first-degree relatives (9.5%) and siblings (11%)[19]; in the homozygous twins it arises to 75%[20,21]. The genetic basis of celiac disease can be divided between *HLA* and *non-HLA* gene variants[22].

The HLA DQ2 heterodimer is present in 90% of celiac patients, in 5% of the cases the HLA DQ8 heterodimer is present. The HLA DQ2 heterodimer, present in 90% of celiac patients[23], is formed by a beta chain (β) encoded by the allele HLA DQB1 * 02 (HLA DQB1 * 0201 or * 0202) and by an alpha chain (α) encoded by the allele HLA DQA1 * 05. The heterodimer HLA DQ8 is formed by a β chain and an α chain encoded by HLA DQB1 * 0302 and HLA DQA1 * 03 respectively[24].

Genes of the HLA complex can contribute in only 36% of the increased risk of celiac disease in siblings[22], indicating the need for assistance from other *non-HLA* genes[25].

The frequent association of celiac disease with other monogenic diseases may demonstrate the existence of a link with other genes on chromosome 7 (short arm) implicated in Williams syndrome and on chromosome 21 involved in Down syndrome[26].

A fundamental role in the pathogenesis is carried out by an ubiquitous calcium-dependent enzyme, the transglutaminase type 2 (TG2). The TG2 catalyzes the acyl transfer between the γ-carboxamide group of glutamine and the ε-amino group of lysine or primary amine soluble. This mechanism forms gliadin-gliadin macromolecular complexes, which are considered neoepitopes, therefore non-self antigens against which the immune system reacts.

In the presence of a low pH, an abundance of glutamminic residues and scarcity of proteins that bind lysine, TG2 catalyses the deamidation of glutamine[27-29]. Some of these peptides of "deamidated" gluten, because of their negative charge, show a high affinity for the HLA-DQ2 or-DQ8 heterodimer. Once bound to these molecules they activate intestinal mucosa T cells[30-32] and they cause the cytokine production and the begins of the intestinal damage.

Clinical presentation

The first modern description of CD is due to Samuel Gee, an English paediatrician, published in the St. Bartholomew's Hospital Reports of 1888. He recognised CD as a chronic indigestion, occurring in people of all ages, presenting as diarrhoea.

Nowadays clinical presentations of CD may vary from silent to severe malabsorption symptoms (celiac crisis).

Didactically, CD manifestations are divided in: (1) typical: including gastrointestinal symptoms, such as diarrhoea, weight loss, abdominal pain, failure to thrive, abdominal distension and vomiting; (2) atypical: that is for example short stature, iron-deficiency anaemia, dermatitis herpetiformis, delayed puberty; and (3) silent: completely asymptomatic.

A delayed gastric empty and a slow oro-caecal transit has been observed in celiac patients on a gluten containing diet, probably due to abnormal exposure of small bowel unabsorbed starch and fats and to altered neuroimmunomodulation and hormonal deregulation (low levels of cholecystokinin and high levels of peptide YY)[33]. Some authors investigated the transit disorders in patients with untreated CD using the video-capsule endoscopy. Urgesi *et al*[34] found that there was no difference in gastric empting and small bowel transit time between CD patients and control group. However, Ciaccio *et al*[35] observed changes in motility of the small bowel and they speculated that the reduced folds can cause more rapid changes in the position and in the width of the luminal centre.

Diagnosis

CD diagnosis, according to the American Gastroenterology Association, consists of a serological screening and an upper gastrointestinal endoscopy[36].

Nowadays, CD serological screening is recommended for symptomatic patients, or for those people who are at high risk of CD (such as first degree relatives). It encompasses the total serum IgA, the IgA anti-transglutaminase antibodies (AbTG2), IgA anti-endomisium antibodies (EMA) and IgA anti-deamidated gliadin antibodies (DGP).

AbTG2 proved to have a very high sensitivity (98%-100%) and a very good specificity (94%-98%)[37], they are the most widely used for CD screening, even if they can be found in patients affected by other autoimmune diseases[38]. They can be determined both by ELISA or RIA, the latter technique showing a so high sensitivity[39] that it has been used to detect AbTG2 in saliva[40], demonstrating a correlation with CD histological grading and diffusion of duodenal lesions[41].

EMA have a very high specificity (100%), but a lower sensitivity than AbTG2[38]. They are determined by indirect immunofluorescence, using monkey oesophagus sections as substrate.

DGP have been demonstrated to be more sensitive and specific than the old antigliadin antibodies and they are useful especially in children younger than two years

of age[42].

In IgA deficient patients, it is recommended to perform the IgG antibodies, particularly IgG anti-deamidated gliadin antibodies.

The upper gastrointestinal endoscopy with multiple biopsies, both from duodenal bulb and distal duodenum, is the gold standard for diagnosis[36]. The standard endoscopy does not permit the visualization of villous atrophy, even if several macroscopic markers has been related to CD (Figure 1), such as reduction or absence of duodenal folds, scalloping, nodular appearance and mosaic pattern. However the power of these endoscopic markers to predict the villous atrophy is still debated[43,44]. This variability could be due to the absence of macroscopic sign in case of patchy or partial villous atrophy.

In the last few years, new methods have been developed to evaluate with more accuracy the macroscopic appearance of villous pattern during upper gastrointestinal endoscopy.

The water immersion technique (Figure 1C) is an easy procedure that can emphasize the villous pattern. It consist in a first phase of air suction from the lumen, then a second phase of injection of 90-150 mL of water[45]. It has the potential to target biopsies and, eventually, reduce the number of specimen, thanks to its capability of enhancing areas of villous atrophy. An alternative technique is represented by the chromoendoscopy, that uses the dye staining with indigo carmine in enhancing the visualization of the mucosal surface. This endoscopic tool has showed a better accuracy when combined with magnification endoscopy[14].

Narrow band imaging (NBI) is another technology that improves the visualization of the surface of the superficial mucosa and its vascular architecture.

NBI with optical magnification assists in detecting patients with villous atrophy without determining the level of intraepithelial lymphocytosis and crypt hyperplasia[46,47].

Cammarota *et al*[48] reported their experience in the use of I-scan technology during endoscopy for the evaluation of the duodenal villous pattern. It works in real time and permits to switch from standard endoscopy to I-scan view very quickly. The authors reported an accuracy of 100% in detecting total villous atrophy, and suggested a possible role of this technique in targeting biopsies in patchy distribution of lesions. However in the reported study, all the enrolled patients underwent upper gastrointestinal endoscopy for suspicion of malabsorption, so they had a high pre-test probability of duodenal atrophy.

Rokkas *et al*[49] recently published a meta-analysis about the role of video capsule endoscopy in CD diagnosis and reported a pooled sensitivity of the tool of 89%. A normal capsule endoscopy cannot exclude CD, however it could provide information on the extent of the disease, allowing the visualization of not accessible portion of small bowel, even thou the histological evaluation of bioptic samples still remain the gold standard for the diagnosis.

Biopsies taken during endoscopy must be oriented on filter paper, fixed in formalin and embedded in paraffine. After the cut and haematoxylin-eosin staining, an expert pathologist assesses the sections under light microscopy, evaluating the intraepithelial lymphocytes (IEL) count, the villo/crypta ratio and the villous atrophy using the Marsh modified by Oberhuber classification[16]: (1) type 0: normal mucosa with less than 40 IEL/100 enterocites (EC); (2) type 1: infiltrative, that is characterised by normal villous architecture, normal crypt height, but high IEL counts (> 40/100 EC); (3) type 2: hyperplastic, with a normal villous architecture, but but high IEL counts (> 40/100 EC) and crypt hyperplasia; (4) type 3: destructive, in which besides a high IEL counts (> 40/100 EC) and acrypt hyperplasia, it can be also observed a villous atrophy (3a: mild villous atrophy, 3b moderate villous atrophy, 3c: total villous atrophy).

Recently a new classification has ben proposed by Corazza *et al*[50], in order to reduce the inter and itra-observer disagreements and to facilitate the relationship between pathologists and gastroenterologist. It consists of two degrees: (1) A: non-atrophic lesions of the duodenum; and (2) B: atrophic lesions. It is divided into grade B1, that include mild and moderate villous atrophy, and grade B2, with a total villous atrophy.

The intestinal involvement, however, is not always confined to the duodenum. It has been demonstrated that other portions of the gastrointestinal tract are involved, such as the gastric[51], oral[52] and colonic mucosa[53].

The chronic superficial gastritis has been described as the most frequent form of gastritis that occurs in non treated celiac patients[54], followed by lymphocytic gastritis, a form of gastritis of uncertain pathogenesis[55].

Therapy

Gluten-free diet is, at this moment, the only effective treatment for coeliac disease, allowing the healing of intestinal mucosa, the improvement of symptoms and prevents the onset of long-term complications, such as osteoporosis[56] and autoimmune disorders[57,58].

However, many efforts have been made to find an alternative therapy for CD, involving the biotechnology field, which led to a better understanding of the molecular mechanisms of coeliac disease and the identification of pathogenetic pathways that could be targeted by new drugs. Currently the main targets under investigation are[27]: (1) endopeptidases capable to detoxify gluten in order to decrease its immunogenic power; (2) modulation of permeability by the pill AT-1001; (3) block of antigen presentation made by inhibitors of TG2 and HLA-DQ2; (4) inflammation modulation using monoclonal antibodies directed against inflammatory cytokines; (5) block of the recruitment of lymphocytes by molecules that inhibit the migration to the intestinal mucosa; and (6) immunomodulation and induction of gluten tolerance.

In the last few years a new gluten- related syndrome is increasing awareness: the non celiac gluten sensitivity (NCGS). NCGS often overlaps with irritable bowel disease syndrome and for both conditions the diagnosis is

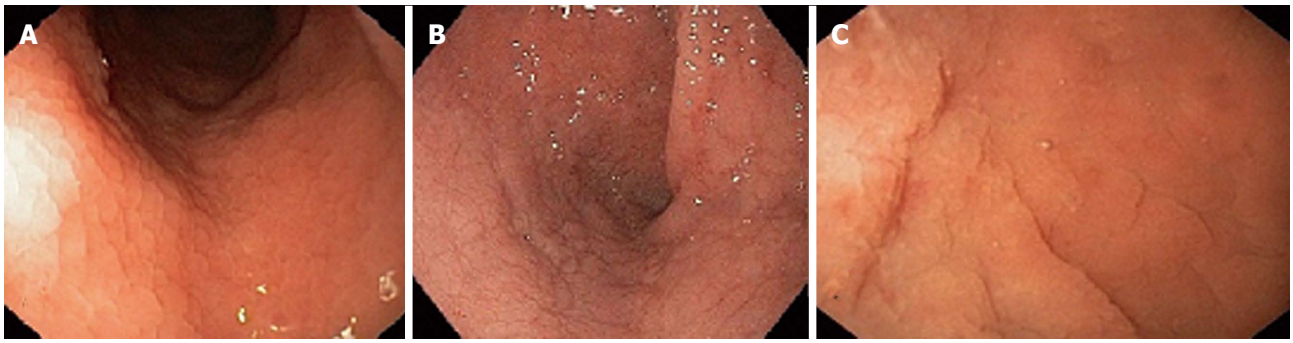

Figure 1 Endoscopic markers of celiac disease. A, B: The typical endoscopic markers of villous atrophy include mosaic pattern, scalloping of folds, and a decrease of duodenal folds; C: Appearance of the duodenum of a celiac patient using the water immersion technique.

based on clinical symptoms.

However it is a still poorly defined syndrome, characterized by the presence of gastrointestinal symptoms, such as bloating, abdominal pain, nausea, gastroesophageal reflux disease, and/or extraintestinal manifestation, tiredness, headache, anxiety, foggy mind and peripheral numbness[59]. The clinical presentation of these symptoms has been associates to the ingestion of gluten, however it has been hypothesize that other wheat proteins, such as amylase trypsin inhibitors, could play a role[60]. Also fermentable oligosaccharides, monosaccharides and disaccharides, contained in wheat, rye, but also in milk, legumes, honey and some vegetables (fennel, beetroot, and chicory) has been proposed to be important in NCGS[61]. NCGS is an exclusion diagnosis, so CD and wheat allergy should be rouled out. Its prevalence is still uncertain, ranging from 3.19%[59] in Italy, to 6% in United States[62] and it is more frequent in female than in male. Further study, including possibly a double blind gluten challenge, should be performed to assess the real prevalence of NCGS.

CONCLUSION

CD diagnosis is often delayed in asymptomatic patients or in individuals with less clinical gastrointestinal symptoms, such as abdominal bloating, nausea and vomiting, despite the many benefits deriving from a prompt identification.

Based on these assumptions, several studies performed coeliac disease screening in patients with symptoms suggestive of dyspepsia, showing a biopsy-proved prevalence that ranged from 0.5% to 2%[6-11]. Interestingly, the subgroup of dyspeptic patients at highest risk comprised young women, aged from 20 to 37 years (RR of CD 3.22)[6].

The 40%-60% of subjects with dyspepsia resulted macroscopically normal when performing upper gastrointestinal endoscopy[63,64]. Unfortunately, the practice of performing biopsies, even in absence of endoscopic alteration of intestinal mucosa, is quite uncommon.

The typical endoscopic markers of villous atrophy include mosaic pattern, scalloping of folds, and a decrease of duodenal folds (Figure 1). However, mostly in less severe cases, CD diagnosis cannot only be performed on these parameters. So, considering that many authors describe these markers as not sufficiently sensitive, some endoscopic techniques, such as "water immersion" and CEM were proposed to improve the diagnostic sensitivity and target biopsies in most damaged mucosal areas[12-14].

A recent meta-analysis by Ford *et al*[15] evaluated the yield of diagnostic testing for CD in patients affected by dyspepsia. The pooled prevalence of positive celiac serology ranged from 6% to 8%. The author pooled the data from literature and estimated that the prevalence of positive celiac serology ranged from 6% to 8%, the biopsy-proved CD prevalence was also higher in patients with dyspepsia, approximately 2%, than controls, but not in a statistically significant way. However, due to several limits that affected the paper, such as presence of study based only in tertiary care, this assumption should be confirmed further larger and, possibly, case-control studies.

In conclusion screening for CD in patients suffering from dyspeptic symptoms, as defined by Rome Ⅲ criteria, and routinely performing of biopsies during upper GI endoscopy, may be useful as part of the diagnostic flow-chart of these patients, considering the benefits of a promptly beginning of a gluten-free diet, even thou further, well-defined and case-control studies on a larger population could definitively assess if CD prevalence is higher in dyspeptic patients.

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Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express t test as t (in italics), F test as F (in italics), chi square test as χ^2

(in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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