

# Journal of the Malta College of Pharmacy Practice



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# I am a Pharmacist

I am a specialist in medications  
I supply medicines and pharmaceuticals to those who need them.  
I prepare and compound special dosage forms.  
I control the storage and preservation of all medications in my care.

I am a custodian of medical information  
My library is a ready store of drug knowledge.  
My files contain thousands of specific drug names  
and tens of thousands of facts about them.  
My records include the medication and health history of entire families.  
My journals and meetings report advances in pharmacy from around the world.

I am a companion of the physician  
I am a partner in the case of every patient who takes any kind of medication.  
I am a consultant on the merits of different therapeutic agents.  
I am the connecting link between physician and patient  
and the final check on the safety of medicines.

I am a counsellor to the patient  
I help the patient understand the proper use of prescription medication.  
I assist in the patient's choice of non-prescription drug or in  
the decision to consult a physician.  
I advise the patient on matters of prescription storage and potency

I am a guardian of public health  
My pharmacy is a centre for health care information.  
I encourage and promote sound personal health practices.  
My services are available to all at all times.

This is my calling. This is my pride

Anon

# Community pharmacists: An expression of gratitude

**Maria Cordina** B. Pharm (Hons) *Melit.* PhD (QUB)

Editor

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One of the main advantages of choosing to study pharmacy, is that it leads to a highly flexible profession, providing the graduate with multiple options to choose from. Switching from one career path to another is also relatively common in local practice. While working conditions are a prime contributor to the choice of career path, additional factors such as personality-job fit also play a very important part. In terms of work related personality, pharmacists working in different areas exhibit distinct traits.<sup>1</sup>

A study conducted locally illustrated that when comparing major areas of practice, those whose primary career choice was community practice rated high in the trait of Personal Relations, demonstrating that they have faith and trust in people, are tolerant and understanding. These traits show that they are predisposed to caring.<sup>1</sup> Other significant traits in community pharmacists, but which also features prominently in all pharmacists, are those of Cautiousness and Responsibility. These individuals are persevering, determined and reliable; they consider matters very carefully before taking a decision and are not inclined to take chances or run risks. These traits are also very similar to those found in US pharmacists.<sup>2</sup>

While multiple career options are available, community pharmacy either as a primary or secondary occupation is still the most popular.<sup>3</sup> Community pharmacists offer a sterling service to the community. Studies conducted locally have shown their commitment and dedication to their clients and comparative studies have also demonstrated that their practice is akin to their international colleagues.<sup>4</sup>

Community pharmacists practice in every town and village, they are integral members of their community and the vast majority not only know their patients but also entire families and extended families. This, coupled with the ease of access and relatively informal environment in which they practice make them instrumental in the delivery of patient care. Due to these factors and informal

conversation community pharmacists conduct a number of 'invisible' services, which may not have a clear definition and are most certainly not remunerated, yet these services rank as one of their most vital functions. Community pharmacists are very often the first port of call for patients, they are therefore able to guide individuals accordingly through advice, selection of appropriate medication or referral to the appropriate health care professional. At times, just listening and making the individual feel that his/her needs or situation is understood is a significant intervention.

Dispensing of medicines brings with it significant responsibilities, be they legal, ethical or in terms of patient care. Once again, most of these responsibilities may not be fully appreciated by those external to the profession. Striving to ensure that the medication is taken appropriately, identification of any cases of drug misadventure and monitoring of patients are but a few ways in which pharmacists contribute to their patients' safety and positive health outcomes on a daily basis.

Many pharmacists are also very actively engaged in public health activities and conduct campaigns to educate their clients, promote health and prevent disease. This is done in a rather personalised manner which promises to have a better impact.

Pharmacists find tremendous satisfaction from their interventions and interactions with patients. As with every other profession, at times, they find themselves in situations where they do not feel professionally respected and appreciated, yet they still provide the best possible service to their patients. In these circumstances pharmacists should be fully and publically supported. A show of support not only restores the pharmacists' confidence but also benefits the profession.

Community pharmacists should receive all the training and necessary support to continue to provide their sterling, yet often invisible, service to their community. They clearly respond to the needs of their

community as outlined by WHO's European health policy framework *Health 2020* and the strategic document *Priorities for health system strengthening in the WHO European Region 2015-2020: walking the talk on people centeredness*.<sup>5,6</sup> The Malta College of Pharmacy Practice endeavours to support pharmacists to offer even more innovative services through the provision of Continuing Professional Development programmes.

The Malta College of Pharmacy Practice would therefore like to take this opportunity to thank all pharmacists who practice, or have practiced, in the community for their invaluable contribution to society.

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# “Food allergy/intolerance testing” in dermatology - science or hype?

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## Educational aims

- To understand food allergies, their presentation and to differentiate it from food intolerance
- To clarify the proper diagnosis and management of food allergies and give a brief overview of the tests available and their appropriateness
- To outline the association of food with common skin conditions namely eczema, acne and urticaria

## Key words

Food, allergy, intolerance, eczema, acne, urticaria

## Abstract

**Food allergies are becoming an increasingly recognized clinical problem and patients frequently ask if their health complaints are related to their diet. There has been a recent phenomenon of food allergy or intolerance testing leading to dietary alterations and restrictions. This article gives a brief overview of the latest evidence and current guidelines for proper diagnosis and management. A distinction between food allergies and food intolerance or sensitivity should be made. It focuses on the association of the common skin conditions: eczema, acne and urticaria, to food and whether dietary changes are indicated. The currently available tests and their appropriateness are also discussed.**

## Introduction

Health care providers should be aware of food allergies and food intolerance, their presenting symptoms, diagnosis and management. In the past years a variety of tests have been made easily available and it is important to know how to use these tools appropriately. We should be able to educate patients and follow appropriate diagnostic and management pathways to give the best care based on published research.

Food allergies have become a common clinical problem in the Western world. Studies report that they are likely to affect nearly 5% of adults and 8% of children with increasing

prevalence.<sup>1</sup> Research in this area is growing and guidelines have been published by various organizations on how to investigate and manage these patients.

This article gives a brief overview on the differences between food allergies and food intolerance, the tests available, their use and misuse. It also gives an outline of the food associations with commonly implicated skin conditions namely eczema, acne and urticaria. It is based on the latest evidence and guidelines and aims to give the latest updates on a topic which has received increased public interest.

## Food Allergies and testing

### *What is a food allergy? How does it present?*

Food allergy is defined as an immune system reaction that occurs soon after eating a certain food. Even a tiny amount of the allergy-causing food can trigger signs and symptoms. The food allergen causes an immunoglobulin E (IgE) – mediated response and symptoms typically manifest within a few minutes to a couple of hours after ingestion.

The clinical features can be mild to severe and include symptoms such as skin rashes, a stuffy or itchy nose, sneezing, itchy and tearful eyes, wheezing and chest tightness. It might also present with gastrointestinal symptoms such as vomiting, stomach cramps and diarrhoea. More severe symptoms include angioedema or swelling, hoarseness, troublesome breathing and anaphylaxis.

While any food can trigger an allergic response, common culprits include cow's milk, eggs, wheat, soy, peanut, tree nuts, fish and shellfish.<sup>2</sup>

### *Food allergy vs food intolerance/sensitivity*

Food intolerance/sensitivity should not be confused or labelled as a food allergy. Food intolerance occurs when a person has difficulty digesting a particular food and can lead to symptoms such as intestinal gas, abdominal pain or diarrhoea. An example includes lactose intolerance due to the deficient enzyme lactase. Food intolerance involves the digestive system and is not immune mediated whilst food allergies involve the immune system.<sup>3</sup>

### *How to diagnose and tests available*

A detailed clinical history is critical to correct diagnosis. Laboratory tests should support clinical diagnosis and not vice versa. Available laboratory tests have limitations not least poor positive predictive value and limited repertoire.<sup>4</sup>

Diagnosis of IgE-mediated food allergy is typically made using the clinical history in combination with skin prick testing (SPT) and/or immunoassays of serum food-specific IgE (sIgE) levels. These tests tend to be sensitive tools in the detection of IgE-mediated food allergy, but have a number of disadvantages, including that positive test results to tolerated foods are not uncommon; and test results do not accurately predict the severity of an allergic reaction.<sup>5</sup>

The EAACI (European Academy of Allergy and Clinical Immunology) food allergy guidelines recommendations on the diagnosis of food allergy include a careful and detailed

dietary history and standardised tests.<sup>6</sup> Specific IgE blood testing should be guided by the history and large screening panels are not recommended.

Skin prick testing demonstrates an allergic response to a specific allergen. In conjunction with an allergy focused history, SPT can help to confirm the presence of an allergy to either a food or inhaled substance (allergen). The skin prick test introduces a tiny amount of allergen into the skin, eliciting a small, localised allergic response, in the form of a wheal (bump) and flare (redness) at the site of testing.

Interestingly in a recent meta-analysis to determine the accuracy of tests to diagnose food allergy, the authors concluded that SPT and sIgE testing have good sensitivity but poor specificity for diagnosing clinically confirmed food allergy (Table 1). They highlight the fact that evidence base is limited and weak and result interpretation can be difficult. This underlines the importance of using these tests in a clinical context and keeping in mind their poor specificity.<sup>7</sup>

Component-resolved diagnostic testing is a new methodology which utilises purified or recombinant allergens for identification of specific molecules causing sensitisation or allergy. This is mentioned in the guidelines but is not widely available and further research on its clinical relevance is needed.<sup>5,6</sup>

Elimination diets for diagnostic purposes and an oral food challenge can also be considered after careful patient selection. These should be undertaken with caution and with emergency support cover.<sup>6</sup>

Recently there has been a surge of various other tests offered in the community to diagnose food intolerance. These tests include total immunoglobulin G (IgG) antibody binding to each food and also the IgG subclass 4 (IgG4) binding may be measured. IgG antibodies signify exposure to products, not allergy. Some research suggests that IgG may actually be a marker for food tolerance, not intolerance.<sup>8</sup>

Patients are given lists of foods which they are allergic to, instructions on which foods to avoid and possible medical associations. At times the diets suggested are extremely difficult to follow and very restricting since multiple allergens are listed. Recently, testing kits have become available and are directly sold to consumers. In addition these tests can be quite expensive.

**Table 1: Showing good sensitivity but poor specificity of skin prick testing (SPT) and specific Immunglobulin (sIgE)**

		Sensitivity	Specificity
Cow's milk	SPT	88%	68%
	sIgE	87%	48%
Egg	SPT	92%	58%
	sIgE	93%	49%
Wheat	SPT	73%	73%
	sIgE	83%	43%
Peanut	SPT	95%	61%
	sIgE	96%	59%

#### Evidence and guidelines

Various official associations have elected to issue a formal statement supporting the opinions expressed by the American Academy of Allergy Asthma and Immunology (AAAAI) and by the European Academy of Allergy and Clinical Immunology (EAACI) regarding the use of IgG testing.<sup>9,10</sup> Both of these organizations warn about the inappropriate measurement of food-specific IgG or IgG4 to suggest the presence or potential of adverse reactions to food. Recent guidelines emphasize that such testing plays no role in the diagnosis of food allergy or intolerance.<sup>11</sup>

For example, IgG measurements cannot be correlated with any clinical symptoms or disease. Food-specific IgG4 levels indicate that the atopic individual has been repeatedly exposed to high doses of food components, which are recognized as foreign proteins by the immune system. Therefore, EAACI gave a clear recommendation not to use these tests.

Interestingly the CSACI (Canadian Society of Allergy and Clinical Immunology) has issued a statement with a number of concerns. These included the fact that tests were widely available through complementary health providers, paramedical clinics, and some physicians. Furthermore testing kits were being sold directly to customers and are very expensive with lack of supporting evidence. Such tests might lead to exclusion diets which might cause malnutrition and poor growth in children. It also went further to discourage their use and recommended health care providers not to offer such testing.<sup>9</sup>

#### Misuse of food allergy tests

Unconventional tests are becoming increasingly common and, according to the latest evidence and research, cannot be recommended. These tests might lead to patients being misinformed and labelled as allergic to specific foods. This can cause considerable impairment in quality of life due to exaggerated restricted diets. These diets can be harmful to patients leading to malnutrition and deficiencies especially in children. Inappropriate dietary advice based on food allergy testing can also lead to micronutrient deficiency. These include vitamins, minerals, phytochemicals and antioxidants. They are all essential components of skin structure responsible for multiple biological functions. Such deficiencies can lead to skin barrier and function abnormalities.<sup>12</sup>

IgG testing has already been discussed. Other examples include bioresonance (electromagnetic waves used to diagnose and treat human illness), Vega electrodermal tests, iridology (determines information on patient's health based on iris characteristics) and hair analysis amongst many others. These tests are not currently validated and cannot be recommended in diagnosing food allergy.

#### Probiotics

Probiotics have been investigated as another option for the management of patients with food allergy, particularly cow's milk allergy, either added to formulas or given as a supplement.

Evidence that probiotic supplements have preventative or therapeutic activity for

food allergy is lacking and further research is needed to make recommendations in this area.<sup>13</sup>

Probiotics are not an effective treatment for eczema and may, in fact, carry a small risk of adverse events such as infections and bowel ischaemia.<sup>14</sup>

## Diet and the skin

### Eczema

Health practitioners are constantly asked by patients if certain foods could be the cause or exacerbating their eczema. This is a common concern of parents with atopic children. It should be noted that many children outgrow atopic dermatitis (eczema) by the time they reach adolescence.

In a 5-year multicenter study in infants age 3-18 months it found that even in reported mild cases of atopic dermatitis (AD), roughly 15% of infants had definite food allergies.<sup>15</sup> Patients with AD typically have higher levels of immunoglobulin E (IgE) antibodies. Elevated IgE antibodies are evidence only for sensitization to a food but are not proof of a food allergy. It is postulated that the presence of antibodies is a consequence of the pruritic nature of AD, causing children to scratch their skin, allowing food allergens to be absorbed via this disrupted skin barrier, and inducing the development of antibodies. AD usually comes on in infancy, before any possible food reactions.

The evidence suggests that in most cases food exclusion does not prevent or improve atopic dermatitis.<sup>16</sup> Some diets may actually be harmful to the skin and be associated with higher eczema prevalence in the United States. E.g. Herbal therapy, Homeopathic therapy, Vegan diet.<sup>17</sup>

On the other hand several studies have found an association between clinical food allergy and AD. Diet elimination trials in patients who are clinically allergic to eggs have shown promise in reducing symptoms. Elimination of certain foods (chocolate, cheese, coffee, yoghurt) in a subgroup of patients was found to be beneficial.<sup>18</sup>

Research clearly shows that food allergies are commoner in AD patients. Wheat, milk, soy, fish, eggs and peanuts are the commonest culprits. There was no benefit of an egg and milk-free diet in unselected participants with atopic eczema.<sup>19</sup>

It is still common to see food allergy testing done or referrals after a single bout of atopic dermatitis. This is neither practical nor useful.

The NIAID (National institute of allergies and infectious diseases) guidelines recommend two indications for consideration of food allergy testing (milk, egg, peanut, wheat, and soy) in children younger than 5 years with moderate-to-severe AD:

- A child who has persistent AD despite optimized management; or
- A child who has a *reliable* history of an immediate reaction after eating a specific food.

Before embarking on these tests, parents should be educated about food allergies and that positive tests are not necessarily diagnostic. In some cases dietary restrictions can lead to malnutrition and nutrient deficiencies adversely affecting growth and causing harm.<sup>20</sup>

### Patch Testing

Another pitfall is the referral of patients with eczema for patch testing to exclude food allergies. Dermatologists apply patch tests in patients with dermatitis, to find out whether their skin condition may be caused or aggravated by a contact allergy. Patch tests are not the same as skin prick tests. Patch testing helps identify which substances may be causing a delayed-type allergic reaction (Type IV hypersensitivity reaction) in a patient.

A range of substances can be used for patch testing. A routine screen such as the European Baseline Series of allergens is applied to nearly every patient, together with specific tests appropriate to the individual. These allergens are applied on the back and may identify allergens causing contact dermatitis. It is intended to produce a local allergic reaction on a small area of the patient's back.

The top allergens from 2005-06 were: nickel sulfate, Myroxylon pereirae (Balsam of Peru), fragrance mix I, quaternium-15, neomycin, bacitracin, formaldehyde, cobalt chloride, methyl dibromoglutaronitrile/ phenoxyethanol, p-phenylenediamine (PPD), potassium dichromate, carba mix, thiuram mix, diazolidinyl urea, and 2-bromo-2-nitropropane-1,3-diol.<sup>21</sup>

It is interesting to note that some foods and preservatives contain these substances e.g. nickel and paraben. This might lead to a systemic contact dermatitis; when a person who is already sensitized to a substance through skin contact is exposed to that substance (allergen) via a systemic route. An example is the Latex fruit syndrome; patients who are sensitized and allergic to latex

develop symptoms when eating certain fruits such as avocado, banana, chestnut, kiwi, peach, tomato, potato, bell pepper, turnip, zucchini and cassava. This is due to cross reactivity where similar structural epitopes exist in different allergens.

In summary, there is scanty evidence to justify the use of food allergy tests in most patients with atopic dermatitis. Elimination diets based on these tests are not recommended and might actually be harmful. Patient reassurance and education is paramount to quell this misconception. Patch tests are indicated when there is a suspicion of allergic contact dermatitis.

### Acne

Acne is a common skin condition mostly seen in adolescents and can cause considerable distress. Invariably most patients with acne and their relatives ask if certain foods could cause or exacerbate acne. Some patients will be convinced that certain specific foods cause worsening of their acne. What does the current evidence say?

Studies have shown a positive association between the intake of skimmed milk and acne.<sup>22</sup> The role of chocolate and other dietary factors in acne development has also been reported but not enough evidence is present to date. A particular study reported that acne is absent in populations consuming low glycaemic load and no consumption of milk or dairy products e.g. Eskimo, Okinawa islanders. Genetic differences could explain these findings.<sup>23</sup> Two randomized controlled studies have provided evidence for the beneficial therapeutic effects of low glycaemic load diets.<sup>24</sup>

There is little evidence to suggest a definite pathogenic role of specific foods in relation to acne. Advising patients to restrict particular foods is not based on robust scientific evidence. A balanced healthy diet should be recommended.

### Urticaria

Urticaria is a skin condition characterized by wheals which last less than 24 hours and/or angioedema. It is important to differentiate between acute (< 6 weeks) and chronic urticaria (> 6 weeks).

Urticaria can be triggered by a variety of factors causing histamine release. These include infections, drugs, stress, food allergies and insect bites amongst others. In a study up to 63% of patients with acute urticaria suspect food as the eliciting factor but this is much lower in clinical practice.<sup>25</sup>

In more than half of the cases a cause is never found. A detailed history should help elucidate the cause especially in individuals with repeated episodes. Testing for food allergies should be guided by the clinical context and 'mass' testing should be avoided.

In patients with chronic urticaria (lasting more than 6 weeks) food is rarely the cause. This should only be suspected in patients who suffer from intermittent attacks of whealing lasting for a few hours shortly after the ingestion of food.

## Conclusion

Attributing skin diseases to food allergies or intolerance should be undertaken with caution. In recent years there has been increased public concern and misconceptions regarding this subject. Patients constantly asked if certain skin diseases could be attributed to food and a recent phenomenon of unregulated food allergy testing has emerged. These tests might lead to unnecessary rigorous diet regimes without any proven benefit, in addition to being expensive. Health practitioners should be aware of food allergies, intolerance/sensitivity and follow a structured diagnostic and management pathway.

Current evidence suggests that a detailed history is invaluable in diagnosis of food allergies. Proper validated tests can help elucidate these allergies but should be guided within the clinical context. Serum IgE testing and skin prick testing are first line investigations but still have significant false positive and false negative results and should be interpreted with caution. Elimination diets and oral food challenge tests should be undertaken under specialist care and in selected patients.

Food allergies should be distinguished from food intolerance/sensitivity. At present, there are no reliable or validated tests for the diagnosis of food intolerance. Even though IgG testing has become increasingly common there is no scientific rationale behind them and there is no proven correlation between results and symptoms. Various international official immunology organisations have issued statements against IgG testing. Moreover these tests can lead to harmful diets, especially in children, causing malnutrition and retarded growth.

Currently there is limited evidence linking common skin conditions such as eczema and acne to particular food allergens. Acute urticaria is associated with certain foods but this is grossly overestimated by affected patients.

## Key points

- Food allergies and food intolerance are distinct
- Diagnosis should be based on a detailed clinical history aided with appropriate testing and not vice versa
- Based on the latest evidence and published research IgG measurements for food allergies or intolerance cannot be recommended
- Patch testing is used to identify allergens in allergic contact dermatitis and not food allergies
- In general, restrictive diets are not recommended in eczema and acne since there is not any robust evidence. Acute urticaria can be triggered by food and should be investigated appropriately

Future considerations could include increased public awareness and educational campaigns. Involving all the related specialties to draft and implement local guidelines on the proper use of food allergy testing would also be beneficial.

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# Urticaria – diagnosis and management

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## Educational aims

- To make a more confident clinical diagnosis of urticaria
- To increase familiarity with the commonest causes and triggers of urticaria
- To update the knowledge on management of urticaria

## Key words

Urticaria, angioedema, oral antihistamines, omalizumab

## Abstract

**Urticaria is a common and characteristic skin condition presenting with wheals and/or angioedema. It may be acute or chronic and has various causes and triggers. Patients with urticaria are often referred to allergy clinics to find out 'what they are allergic to'. Urticaria may impact significantly on a patient's quality of life. Its management involves identifying and removing causes or triggers, together with oral antihistamines as first line treatment for symptomatic control. Omalizumab is a recently licensed expensive treatment option for patients with urticaria resistant to oral antihistamines, and has shown a high success rate and good safety profile.**

## Introduction

**Urticaria**, also known as 'nettle rash' or 'hives', is a reaction pattern that gets its name from the Latin word for nettle (*urtica*). The nettle is a weed with stinging hairs that causes contact urticaria. Urticaria describes a reaction pattern with characteristic features and may have many causes or triggers. It is described as acute if it lasts less than 6 weeks, or chronic if it lasts 6 weeks or longer. Some patients have episodic acute intermittent urticaria lasting for hours or days and recurring over months or years.

Urticaria is a very common condition, occurring across all age ranges. It has a lifetime prevalence of approximately 20% in the general population. The chronic form affects 1-3% of the population.<sup>1</sup> When severe and extensive, it often prompts patients to seek treatment in the emergency department; in fact, it is the most common skin disease treated in the emergency department. Patients with urticaria may also seek advice from their pharmacist. Most new-onset urticaria resolves spontaneously within days or a few weeks. However, at least 20% of chronic urticaria patients with symptoms severe enough to warrant hospital referral remain symptomatic 10 years after first presentation.<sup>2</sup> Although rarely life-threatening, chronic urticaria leads to both misery and embarrassment and has a significant impact on an individual's quality of life.<sup>3</sup>

Box 1 summarizes the main clinical features of urticaria.<sup>4-6</sup> It is typical of patients with urticaria to have no evident lesions at the time of their doctor's visit. In this situation, a good history is sufficient to make the diagnosis. Patients may also bring photos of their rash to the clinic.

The characteristic urticarial wheals affect the superficial skin layers (papillary dermis). When the submucosa, the deeper reticular dermis and subcutaneous tissues are involved, the resulting deep swelling is called **angioedema**. This is often most notable in the eyelids and lips. These swellings can be painful rather than itchy. Urticarial wheals and angioedema often coexist, but either can occur separately. Unlike wheals that individually resolve within 24 hours, angioedematous swellings can persist for a few days. Disfiguring when they occur in the skin, they can be extremely alarming and occasionally life-threatening when they occur in the oropharynx.

Urticaria needs to be differentiated from other medical conditions where

### Box 1: Clinical features of urticaria

- Pink, non-scaly, itchy or sometimes burning swellings (wheals) that can occur anywhere on the body and blanch with pressure.
- Lesions of urticaria can be polymorphic and vary from several millimetres to large, continuous plaques.
- Individual wheals do not last longer than 24 hours, fading without a trace and without scars, but new wheals may continue to appear for days, months or even years.
- Patients with urticaria tend to rub their skin rather than scratch, so heavily scratched skin is rarely, if ever, a consequence of urticaria.
- Patients show no or minimal systemic symptoms. Patients often feel fatigued, especially during relapses, but headache, dizziness, syncope, or respiratory, gastrointestinal or arthralgic symptoms are rare.

wheals, angioedema, or both can occur as a symptom, for example skin prick test, anaphylaxis, or hereditary angioedema.

### Pathogenesis

The *mast cell* is the primary agent in the pathogenesis of urticaria. Mast cell stimulation results in the release of both preformed (*histamine*) and newly formed mediators (prostaglandins) from cytoplasmic granules, which cause wheal formation, vasodilatation, oedema (due to increased microvascular permeability) and erythema. Mast cells release chemoattractants for other cells (for example eosinophils and neutrophils) that are also involved in wheal formation. A number of agents may be involved in the pathogenesis of urticaria, which may explain why antihistamines are not always effective therapy.<sup>7</sup>

Release of mediators by mast cells may be caused by both immune and nonimmune mechanisms. All mast cells express high-affinity IgE receptors (FceR1s) that enable their involvement in IgE-dependent allergic reactions. When IgE forms a complex with FceR1 on the mast cell to which an allergen binds, degranulation occurs. Examples of IgE-mediated urticaria include acute urticaria secondary to foods (for example peanuts, eggs, shellfish), animal dander, stinging insects, some medications (for example beta-lactam antibiotics) and latex.

Mast cell degranulation also occurs through a variety of other mechanisms. Some agents, such as opioids and radiocontrast media, cause mast cell degranulation directly through nonimmunologic means. In chronic spontaneous urticaria (CSU, previously called chronic idiopathic urticaria), there appears to be persistent activation of mast cells in the skin, but the precise mechanism is unknown. Functional auto-antibodies against the FceR1 on the mast cell surface have been

demonstrated in 30–40% of patients with chronic urticaria suggesting an autoimmune basis. This variant of chronic urticaria is described as chronic autoimmune urticaria (CAU). Patients with CAU tend to follow a more aggressive course and often require more aggressive therapy.<sup>8</sup>

The commonest type of angioedema without wheals is histaminergic.

Angioedema without wheals is a cardinal feature of hereditary angioedema (HAE), which is bradykinin-mediated and typically involves subcutaneous sites, gut and larynx. In Types I and II HAE, levels of C4 and C1 inhibitor (functional and/or antigenic) are low.<sup>9</sup>

### Commonest causes and triggers of urticaria and angioedema

#### Acute urticaria

A definitive inciting agent can be identified in about 50% of cases of acute urticaria and angioedema. Infections, medications and food were identified as the commonest causes.<sup>4</sup> Acute urticaria is considered to be a classical manifestation of viral infection in general, especially in children, but also in adults.<sup>10</sup> When the trigger is a viral upper respiratory tract infection (URTI), the patient should be informed of the self-limiting duration of the disorder. Other viruses (for example hepatitis or herpes viruses) or infections (including streptococcal infections and chronic parasitic infections) may also be possible triggers.

#### Drug triggers

Acute urticarial reactions from drugs are common. The commonest drugs to trigger urticaria are nonsteroidal anti-inflammatory drugs (NSAIDs), salicylates, codeine and antibiotics, especially penicillins, cephalosporins, tetracyclines and sulphonamides. Urticaria usually comes on within a few hours of exposure to the drug. A notable exception is angioedema secondary to angiotensin converting enzyme (ACE) inhibitors (refer to Box 2).<sup>5</sup>

### Box 2: Angiotensin converting enzyme (ACE) inhibitor-induced angioedema

- ACE inhibitors can cause angioedema without wheals resulting in airway compromise. The patient usually presents with swelling of the tongue, but the lips, pharynx, larynx and viscera may also be involved.
- Fatalities are reported and, hence, it is mandatory to recommend that the ACE inhibitor is withdrawn.
- ACE inhibitors are contraindicated in individuals with a history of angioedema with or without wheals.
- The mechanism underlying the angioedema is likely to be due to the reduced metabolism of bradykinin.
- Angioedema associated with angiotensin receptor blockers has been occasionally reported and hence their use in individuals with ACE inhibitor-related angioedema has been questioned but is not contraindicated.
- The incidence of ACE inhibitor-induced angioedema may be as high as 0.68%.
- Most cases were initially thought to occur in the first weeks of treatment, but it is now appreciated that later onset angioedema, occurring after many years of uneventful drug use, is quite common.
- The episodes of angioedema may persist for several months after withdrawal of the ACE inhibitor without undermining the validity of the drug-related diagnosis.
- Individuals who do not improve even after several months of stopping the ACE inhibitor are likely to have an alternative explanation for their angioedema and were coincidentally taking an ACE inhibitor.
- There are no routine investigations to distinguish responders from non-responders to ACE inhibitor withdrawal. If the ACE inhibitor is responsible but is not withdrawn, the attacks may become more severe and frequent.

### Food triggers

Acute urticarial reactions to food are believed to be common and many go unreported. Food allergy should be considered in acute urticaria and urticaria in children. Such foods as tree nuts, peanuts, eggs, shellfish, and tomatoes should be considered (the involvement of food additives or preservatives is controversial). Urticarial reactions may not be to the basic nutrient but to other constituents such as spices. Usually, reactions occur within minutes or a few hours but sometimes allergic urticaria develops many hours after food ingestion. This may occur due to slow absorption or metabolism of food, or because the mechanism is IgG-mediated. Urticaria occurs consistently after every exposure to the problem food. A careful history is normally adequate to determine if a particular food is causing urticaria in a patient. Testing for serum IgE to the food is occasionally performed to help confirm a clinical suspicion. Rarely, allergic reactions to food may occur only if intake is followed by exercise, with neither the food nor exercise alone inducing wheals. Substances reported to cause this include wheat, hazelnuts and shellfish. Box 3 summarizes some important features of IgE-mediated food-induced urticaria/angioedema.<sup>5</sup>

### Chronic urticaria

Most cases of chronic urticaria are thought to have an autoimmune origin. Autoimmune conditions (including thyroid disease, vitiligo, insulin-dependent diabetes, rheumatoid arthritis, and pernicious anaemia) are associated more frequently with chronic urticaria patients having functional autoantibodies than in those without autoantibodies. URTI and psychological factors, including stressful events, are thought to aggravate chronic urticaria. Rarely is food allergy the cause of chronic urticaria and food can typically be excluded on the basis of the clinical history. Alcohol can aggravate chronic urticaria by its effect of vasodilation.<sup>5</sup>

#### Box 3: IgE-mediated food-induced urticaria/angioedema

- Symptoms typically occur reproducibly within 60 min of exposure to the offending food rather than coming on overnight or being present first thing in the morning.
- Symptoms do not last several days.
- Additional symptoms are usually present, such as oropharyngeal itching and discomfort, wheezing, vomiting or abdominal pain.

### Inducible urticarias

Inducible urticarias are responsible for approximately 20-30% of cases of chronic urticaria. In some patients, the triggering stimuli are the predominant cause of the condition, whereas in other patients it is an incidental factor in a case of chronic urticaria. Inducible urticarias are reproducible with the appropriate stimuli and can be identified with a thorough history and sometimes challenge testing. In many patients, the condition gradually improves and clears after several years, for example after 2-3 years, but the duration is usually quite unpredictable.<sup>11</sup>

- Patients with *symptomatic dermographism* (writing on the skin) can be diagnosed in an office setting by stroking the skin with a firm object, such as a tongue depressor. This action provokes a typical, itchy, wheal-and-flare response within a few minutes, usually resolving within an hour.
- Patients with *cholinergic urticaria* get numerous small pruritic wheals after sweating, for example following exercise or emotion.
- Patients with *delayed pressure urticaria (DPU)* suffer from erythema and swelling, associated with itch and/or pain, typically 4-6 hours after a pressure stimulus.
  - Examples of pressure stimuli include standing, walking, sitting on a hard surface, using tools (such as a screwdriver or a hammer), hand-clapping, carrying a handbag or wearing tight-fitting clothes, especially at the waistline. Patients with DPU may be significantly limited in activities of daily life.
  - Lesions can occur anywhere, but are especially common on the hands and feet. They may persist for three days and may be associated with flu-like symptoms.
- Patients with *cold urticaria* develop an urticarial rash and/or angioedema

within 2-5 minutes of being exposed to cold, cold water, cold wind and cold objects. Symptoms usually last for a few hours. In very severe cases, hypotension, shock, collapse and even death may occur, often after swimming in cold water.

- Cold urticaria may be primary (idiopathic) or secondary to an underlying haematologic (for example cryoglobulinaemia or chronic lymphocytic leukaemia) or infectious disease (for example varicella or glandular fever); most cases are idiopathic.
- *Contact urticaria* refers to the onset of urticaria within 30-60 minutes of contact with an inciting agent, for example latex, plants (including the stinging nettle), animals (caterpillars, dander), medications and food (for example fish, garlic, onions, tomato).
- Other forms of inducible urticaria include *aquagenic urticaria*, *vibratory urticaria* and *solar urticaria*.

#### Some conditions that may be confused with urticaria

- *Arthropod bites*. Pruritic papules and papulovesicles with a central punctum appear on exposed skin and usually take more than 24 hours to resolve. Sometimes arthropod bites trigger a hypersensitivity reaction called *papular urticaria*. In this case, the itchy red papules can come up even under tight clothes, in areas away from the bites.
- *Polymorphic light eruption (PLE)*. This is a reaction to the sun, coming up after minutes or hours of sun exposure, typically on skin that is having its first exposure to the spring or summer sun. Lesions last for several days. PLE usually becomes less of a problem as the summer progresses, as the skin gets 'hardened' to the sun. PLE is much commoner than solar urticaria, in which wheals develop within 5-10 minutes of sun exposure and usually resolve within an hour.
- *Urticarial vasculitis*. In this variant of vasculitis, lesions look like urticarial wheals, but they burn rather than itch, last longer than 24 hours and tend to leave a bruise-like stain on fading. Urticarial vasculitis is usually confirmed by taking a skin biopsy and is investigated as for other forms of vasculitis.

- *Scombroid food poisoning.* This is a foodborne illness that results from eating spoiled (decayed) fish, especially mackerel, tuna, bluefish, mahi-mahi, bonito, sardines, anchovies, and related species of fish that were inadequately refrigerated or preserved after being caught. It resembles an allergic reaction, but is actually due to histidine that exists naturally in many types of fish, which is broken down by bacteria into histamine at temperatures above 16°C. Histamine is not destroyed by normal cooking temperatures, so even properly cooked fish can be affected. Symptoms consist of skin flushing (especially of the face, spreading to the torso), facial sweating, throbbing headache, burning-peppery taste sensations in the mouth and throat, abdominal cramps, nausea, diarrhoea, palpitations, a sense of unease, and, rarely, collapse or loss of vision. Symptoms usually occur within 10–30 minutes of ingesting the fish and are generally self-limited, but respond quickly to oral antihistamines.<sup>12</sup>

### Clinical history and examination

A detailed history in a patient with urticaria and/or angioedema is essential. Note should be made of the nature, site and duration of individual wheals, and whether they are itchy or painful. The circumstances of onset and any triggers should be noted, together with the frequency and pattern of recurrence. A family history and detailed drug history are important, as well as response to previously attempted treatments. The clinical history often identifies relevant triggers and directs any further investigations.

### Laboratory investigations

The diagnosis is based primarily on the clinical presentation. The need for investigations to elucidate a possible underlying cause should be guided by the history, but may not be necessary in patients showing a good response to antihistamines. Complete blood count (CBC) and erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) are often performed routinely in patients with chronic urticaria.<sup>6</sup>

In patients with angioedema without urticaria, and who are not on ACE inhibitors, it is important to rule out C1 esterase inhibitor deficiency. A normal plasma C4 during an attack or normal C4, C1 inhibitor, and C1 inhibitor function between attacks, will typically exclude this.<sup>5</sup>

### Management

In patients with acute urticaria and associated shortness of breath, suggesting respiratory involvement and a diagnosis of angioedema, the patient should be monitored in the emergency department until normal airway function is restored. Adrenaline should be used when laryngeal angioedema is suspected.

An adrenaline autoinjector is rarely required and should only be considered if there is a history of significant angioedema affecting the upper airway (rare in angioedema with urticaria). The patient should then be shown how to use the device and provided with a written self-management protocol. For patients with a known history of hereditary angioedema, C1 esterase inhibitor concentrate, ecallantide, or icatibant should be administered as soon as an angioedema attack is recognized.

Acute urticaria restricted to the skin and chronic urticaria do not require hospitalization and can be managed with outpatient care. Inpatient care for angioedema is usually not necessary when timely treatment is administered.<sup>5,6</sup>

### General measures

Any triggering foods or drugs identified from the history should be withdrawn. Aggravating factors such as heat, tight clothing, stress, overtiredness and alcohol, as well as trigger stimuli for physical urticaria should be avoided if possible. It is important to reassure anxious patients that the eruption is not a hallmark of cancer, HIV infection or other underlying disease.

Certain medications, such as aspirin and NSAIDs, are reported to exacerbate urticaria in patients with chronic urticaria resulting from other causes. Their avoidance in favour of paracetamol as an analgesic should usually be recommended because these drugs aggravate chronic urticaria in about 30% of patients. Patients taking low-dose aspirin for its antithrombotic properties can usually continue regular treatment, although non-aspirin alternatives, such as clopidogrel, are available. It is good practice to recommend avoidance of codeine and other opiates in view of the enhanced skin test reactions to codeine found in chronic urticaria, but the value of this is unclear. It is common to see exacerbations of chronic urticaria at the time of minor viral infections, and it may be difficult

to differentiate between a flare caused by the illness and a flare caused by medication taken for it.<sup>5,6</sup>

### Oral antihistamines

Oral antihistamines active against the H1 receptor remain the mainstay of treatment in patients with urticaria, providing control of symptoms.<sup>3,4</sup> There are several on the market. First-generation (sedating) antihistamines and their recommended adult doses include chlorphenamine (4mg every 4–6 hours), diphenhydramine (25–50mg every 4–6 hours), hydroxyzine (25–100mg daily) and promethazine (25mg nocte). Second-generation (non-sedating or minimally sedating) antihistamines with their licensed adult doses include acrivastine (8mg, 1–3 times a day), bilastine (20mg daily), cetirizine (10mg daily), desloratadine (5mg daily), fexofenadine (180mg daily), levocetirizine (5mg daily), loratadine (10mg daily), mizolastine (10mg daily) and rupatadine (10mg daily).

Individual patient responses and side-effects to antihistamines vary. Due to the absence of head-to-head comparisons in clinical trials, none can be recommended over others, but non-sedating or minimally sedating antihistamines are generally preferred. The older first-generation sedating antihistamines have pronounced anticholinergic effects and sedative actions on the central nervous system, which last longer than 12 hours, whereas the antipruritic effects last only for 4–6 hours. Many interactions have been described for these sedating antihistamines with alcohol and drugs affecting the central nervous system. They can also interfere with rapid eye movement sleep and impact on learning and performance.<sup>13</sup>

**It is recommended that modern second-generation H1-antihistamines are to be used as first-line treatment of urticaria because of their good safety profile.**

The usual once-daily dose may be increased incrementally in resistant cases up to 3–4 times the recommended dose (off-label). Updosing with a single antihistamine is preferable to mixing different antihistamines. Patients should be advised that antihistamines work best when they are taken regularly.<sup>14</sup> In patients with chronic urticaria, treatment for 6 or even 12 months is advised, with gradual withdrawal over a period of weeks. Safety data is available for

#### Box 4: Oral antihistamines in children

- Cetirizine and desloratadine are licensed for the treatment of chronic urticaria in children from 1 year of age.
- Loratadine and levocetirizine are licensed for the treatment of children 2 years and older.
- Acrivastine, bilastine, fexofenadine, mizolastine and rupatadine are licensed for use in children over 12 years.
- Desloratadine, levocetirizine, loratadine and cetirizine are available in syrup formulations.
- The metabolism of cetirizine in children is different to that in adults; hence, this drug should be taken twice daily.
- First-generation sedating antihistamines should be avoided due to the risk of psychomotor impairment, impacting on the child's safety and education. Those licensed for use in childhood include diphenhydramine, hydroxyzine, promethazine and chlorphenamine.

oral antihistamines taken continuously for several years. For patients with infrequent symptoms, treatment may be taken as required or even prophylactically (for example prior to an important event such as a wedding or a work presentation).

#### A trial of up to fourfold dose of modern second-generation H1-antihistamines is recommended as second-line in the algorithm of treatment.

Box 4 summarizes the use of oral antihistamines in children, while Box 5 indicates the recommended antihistamines in pregnancy and during breast-feeding.<sup>5</sup>

#### Treatment in resistant cases

Oral corticosteroids may occasionally be required in short rescue courses for angioedema affecting the mouth or for severe exacerbations of chronic urticaria that have not responded to full-dose antihistamines. Examples are 30-40 mg of prednisolone daily for 1-3 days reducing to zero over 10 days, or 30-40 mg daily for 3-7 days. Prolonged daily treatment with oral corticosteroids nearly always leads to severe systemic toxicity accompanied by poor control of urticaria and severe rebound on attempts to withdraw.<sup>5,6</sup>

Third-line treatment for resistant cases includes cyclosporine, montelukast and omalizumab. Efficacy of cyclosporin A in combination with a modern second-generation H1-antihistamine has been shown in placebo-controlled trials as well as open controlled trials. This drug does have a high incidence of adverse effects, but it has a far better risk/benefit ratio than long-term use of oral corticosteroids.

is recommended for 6 months, but typically relapses occur when treatment is discontinued.<sup>15,16</sup> Figure 1 illustrates the EAACI/GA2LEN/EDF/WAO (2013 revision and update) recommended treatment algorithm for urticaria.<sup>6</sup>

Some other (off-label) treatment options for resistant cases include dapsone, mycophenolate mofetil and methotrexate. Tranexamic acid may benefit patients with antihistamine-resistant angioedema without wheals. Due to the migrating nature of urticarial wheals, topical steroids are not indicated. Cooling antipruritic lotions such as 2% menthol in aqueous cream can, however, be soothing.

#### Conclusion

In patients with urticaria, the history and physical examination are crucial while undirected laboratory examination is typically fruitless. Although acute urticaria often has an identifiable trigger (foods, drugs, virus), chronic urticaria frustratingly tends to remain idiopathic. About 30–40% of patients with chronic idiopathic disease appear to have an autoimmune aetiology. Second-generation H1 receptor antihistamines represent the first-line therapy for urticaria.

Anti-leukotrienes such as montelukast may be useful in urticaria patients who are sensitive to aspirin. *Omalizumab* is a recently licensed humanized monoclonal anti-IgE antibody indicated in patients with spontaneous and autoimmune chronic urticaria who have persistent symptoms despite high-dose antihistamines. It is expensive and requires monthly injections but appears well-tolerated. It is effective in approximately 80% of individuals with persistent and resistant symptoms, leading to a rapid improvement. Currently, treatment

#### Box 5: Oral antihistamines in pregnancy and breastfeeding

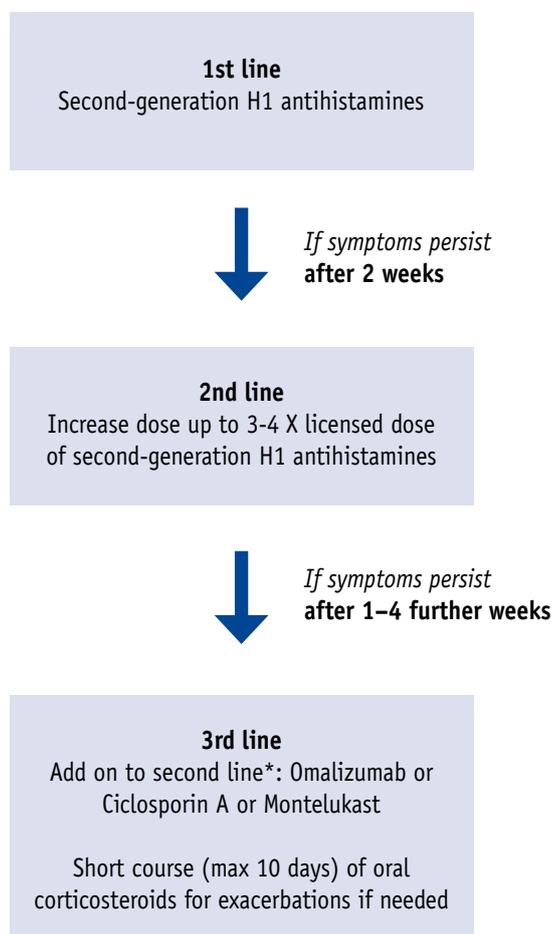
- If an antihistamine is required in pregnancy, the lowest dose of loratadine, cetirizine or chlorphenamine should be used. Loratadine and cetirizine have been assigned a category B by the US FDA. Hydroxyzine is specifically contraindicated in early pregnancy.
- If an antihistamine is required during breastfeeding, it is recommended that either cetirizine or loratadine are taken at the lowest dose. Whenever possible, chlorphenamine should be avoided during breastfeeding as it may cause drowsiness and poor feeding.

#### Key points

- A diagnosis of urticaria is usually easily made from the clinical history
- Triggers and aggravating factors can often be elicited by taking a good history without the need for testing
- Any potential triggers or aggravating factors should be removed where possible
- Modern second-generation oral antihistamines are indicated as first-line treatment in patients with urticaria, with up dosing if necessary
- Omalizumab (anti-IgE) is a new treatment option in patients who do not respond to oral antihistamines or second-line treatment options

**Figure 1: The EAACI/GA2LEN/EDF/WAO (2013 revision and update) recommended treatment algorithm for urticaria<sup>6</sup>**

*\*The order of third-line treatments does not reflect preference*



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# Overview of the pathogenesis and management of postmenopausal osteoporosis

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## Educational aims

- To review the pathogenesis of osteoporosis
- To identify risk factors for postmenopausal osteoporosis
- To update healthcare professionals on the management of postmenopausal osteoporosis

## Key words

postmenopausal women, osteoporosis, bone density, pharmacological therapy, fracture

## Abstract

**Postmenopausal osteoporosis is a silent systemic progressive disease characterised by a decrease in bone mass per unit volume, compromising the physical strength of the skeleton and enhancing susceptibility to fractures on minor trauma. The progressive loss of bone tissue occurs as a result of an imbalance between bone formation and bone resorption subsequent to oestrogen deficiency. The aim of this overview is to shed light on the pathophysiology, aetiology and diagnostic techniques for this metabolic bone disorder. It demonstrates current treatment options and evaluates the emerging pharmacological therapies in the management of postmenopausal osteoporosis.**

## Introduction

Osteoporosis is a silent systemic progressive disease characterised by a decrease in bone mass per unit volume, compromising the physical strength of the skeleton and enhancing susceptibility to fractures on minor trauma.<sup>1, 2</sup>

## Osteoporosis can be classified into 2 categories

- **Primary osteoporosis (Type I)** refers to the idiopathic form of osteoporosis occurring as a consequence of the normal process of aging.<sup>3,4</sup> Postmenopausal osteoporosis affects one third of all women and is caused by a decline in sex hormones as a consequence of menopause.<sup>5,6,7</sup>
- **Secondary osteoporosis (Type II)** occurs as a result of chronic conditions such as rheumatoid arthritis and diabetes mellitus, adverse effects of medications or nutritional deficiencies which enhance bone loss and interfere with peak bone mass attained at maturity.<sup>8</sup>

## Aetiology

Bone remodelling is a lifelong continuous process whereby bone resorption and bone formation are kept at a balance so as to maintain a normal bone mass. At a cellular level, bone remodelling is dependent on osteoblasts and osteoclasts whilst osteocytes and bone lining cells maintain homeostasis by controlling the bone's microenvironment.<sup>9</sup> Osteoclasts, originating from the monocyte macrophage lineage, are responsible for bone resorption and reside on calcified bone surface.<sup>10</sup> Osteoblasts, originating from precursors of mesenchymal cells, are responsible for bone formation and mineralisation of bone matrix by deposition of the organic matrix called osteoid.<sup>11</sup>

In the presence of stress or microdamage, osteocytes will stimulate the remodelling cycle and start the process of bone repair. Upon stimulation, osteoclasts assemble on the bones' surface held tightly by fimbriated organelles and secrete proteolytic enzymes initiating the process of bone resorption and bone degradation.<sup>10,12</sup> Following resorption, a process known as formation follows whereby osteoblasts secrete both the matrix vesicles which are highly rich in the enzyme alkaline phosphatase, and collagen to produce an unmineralised matrix known as osteoid. Upon osteoid maturation, crystals of hydroxyapatite are deposited on the matrix vesicles leading to bone mineralisation.<sup>12,13</sup> This whole process of bone remodelling occurs every 3 to 6 months.<sup>3,5</sup>

At maturity when peak bone mass is reached, an imbalance exists between bone

resorption by osteoclasts and bone formation by osteoblasts, forming the basis of menopause-related bone loss leading to accelerated bone loss and skeletal fragility.<sup>5</sup> It is proposed that oestrogen deficiency results in a rise in the number of osteoclasts and an increase in the depth of resorption, stimulating more bone turnover sites and thus increasing the number of bone remodelling units.<sup>2,5,14</sup> Oestrogen deficiency results in weakening of the response of osteoblasts to mechanical stimuli hence affecting bone repair.<sup>15</sup> This is known as uncoupling of the bone remodelling process<sup>16</sup> leading to low bone mass and microarchitectural disintegration of bone tissue, with a resultant increase in bone weakness.<sup>17</sup>

### Signs and symptoms

Postmenopausal osteoporosis is referred to as a silent epidemic as bone loss itself produces no symptoms until a fracture occurs. Women with osteoporosis have a higher incidence of fractures than non-osteoporotic women when exposed to equal trauma.<sup>18</sup> A fracture is classified as osteoporotic if it occurs in individuals over 50 years of age and is associated with low bone mineral density (BMD) at the fracture site.<sup>15</sup> The most frequent fractures are those occurring at the wrist (most frequently the distal radius), vertebrae and hip (proximal femur).<sup>14,19</sup>

Colles' fracture is a fracture of the distal radius with dorsal displacement producing the "dinner fork deformity". This type of fracture occurs due to a fall on the outstretched hand.<sup>14</sup> Fracture at the wrist is regarded as an early warning sign for the presence of postmenopausal osteoporosis.<sup>2</sup>

Vertebral osteoporotic fractures affect approximately one in four postmenopausal women.<sup>20</sup> These occur when one or more vertebra of the spinal column (most commonly in the T8 to L4 regions) collapse spontaneously with normal activities such as coughing or sneezing.<sup>21</sup> This results in shortening of the length of the spinal column and in the development of the characteristic dorsal kyphosis known as dowager's hump.<sup>3</sup>

Fracture of the neck of the femur is the most severe repercussion of postmenopausal osteoporosis. Pain, disability and hospitalisation are among the consequences of such a fracture.<sup>22</sup> Surgical complications appear in one third of the patients.<sup>2</sup> Hip fractures cause 20% of the affected individuals to die within a year<sup>19</sup>, 40% become incapable to carry out their daily needs, 20% bedridden, whilst 20% may require to stay in nursing homes for some period of time.<sup>22</sup>

### Risk factors

Risk factors for osteoporosis are those which affect peak bone mass, influence bone loss or else interfere in some way with calcium homeostasis.<sup>5</sup> A single risk factor will not cause osteoporosis but in combination these are known to increase the rate of bone loss.<sup>16</sup>

#### Unmodifiable risk factors include:

- **Age**  
Bone mineral density is on the increase until the mid-twenties when the peak bone density is reached. However, after a period of stability, it starts to decline as from the mid-forties. The rate of bone loss in premenopausal women is less than 1% each year. However, in the early postmenopausal years, a phase of accelerated bone loss ensues in which the rate of bone loss may reach a maximum of 5% each year. Bone mineral density declines with advancing age resulting in a higher risk of osteoporotic fracture.<sup>5,6</sup>
- **Gender**  
Females are at a higher risk for osteoporosis due to smaller bones and consequently lower peak bone mass when compared to males. Additionally, in menopause as a result of a decline in the ovarian hormones, women tend to lose bone at a faster rate than males.<sup>23</sup>
- **Ethnicity**  
Postmenopausal osteoporosis is more common in white or Asian women than among the dark-skinned population.<sup>23</sup>
- **Family history**  
Being a polygenic disorder, several genes are thought to have a role in determining women's rate of bone loss and the inflammatory bone turnover.<sup>5,24</sup>
- **Reproductive History**  
An exponential rate of bone loss occurs after early or surgical menopause which then decreases after 4 years to a rate equal to that of premenopausal women.<sup>5</sup>

Multiple reproductive factors such as parity, age at menarche and menopause, duration of menopause, age at first pregnancy and length of lactation period are known to affect bone mineral density with their effect being debatable. Gur *et al.*, reported that the number of pregnancies is inversely related to BMD and this is correlated with the calcium demands during pregnancy. In contrary to this, other studies reported that high parity is protective against osteoporosis as a result of a rise in circulating level of oestrogen in the third trimester of pregnancy, weight gain and elevated calcium intake. Interpregnancy interval period of less than 2 years, especially if

less than 1 year, is associated with increased risk for osteoporosis. Pregnancy before 27 years of age is negatively correlated to BMD as a result of competition between mother and baby for calcium adversely affecting the mother's skeleton. The effect of breastfeeding on BMD is controversial.<sup>25,26</sup>

#### Potentially modifiable risk factors include:

- **Body mass index**  
Overweight individuals exert more physical stress on the skeleton. However as overweight individuals possess a greater number of fat cells, more oestrogen is produced through peripheral conversion of androstenedione in adipose tissue. This elevated oestrogen level may protect such women from postmenopausal osteoporosis by slowing the rate of bone loss.<sup>5</sup> Low body mass index increases the risk for fractures, especially hip fractures. The correlation between BMI and fracture risk is non-linear with BMI <20kg/m<sup>2</sup> linked with higher risk of fractures.<sup>27</sup>
- **Dietary Calcium levels and Vitamin D**  
Diet deficient in calcium results in a decline in bone formation causing low peak bone mass and exerts no effect on the rate of bone loss.<sup>28</sup> Vitamin D aids in calcium absorption and in preserving bone integrity.<sup>5</sup>
- **Caffeine and Alcohol**  
Caffeine and alcohol both promote calcium transfer from bone to plasma.
- **Exercise**  
Sedentary lifestyle increases the risk of osteoporosis. The bone density in the spine and the hip are improved following fast walking exercise by promoting the action of osteoblasts.<sup>28</sup>
- **Smoking**  
Decreased bone mass and higher rate of bone loss are observed in cigarette smokers.<sup>23</sup> Smoking interferes with the hormone calcitonin responsible for calcium metabolism<sup>5</sup> and may also decrease circulating endogenous oestrogen levels.<sup>28,29</sup>

### Pathophysiology

#### Effect of oestrogen on the skeletal system

Low plasma calcium, due to low intake or impaired calcium intestinal absorption, stimulates chief cells of the parathyroid gland to secrete parathyroid hormone (PTH).<sup>9</sup> In order to maintain calcium homeostasis, PTH indirectly stimulates osteoclasts which engage in bone resorption causing calcium mobilisation from bone and thus bone demineralisation. Additionally, PTH upregulates the enzyme 1-alpha-hydroxylase essential for the activation

of the inactive form of Vitamin D to its active form, 1,25-dihydroxycholecalciferol. The latter elevates calcium absorption in the intestine. PTH also acts on the kidneys to increase tubular calcium reabsorption. It has been proposed that sex steroids such as oestrogen, androgens and progesterone inhibit the activity of PTH on bone, decreasing bone resorption.<sup>14,30</sup> Oestrogen has the ability to alter the sensitivity of bone to PTH without changing its sensitivity to other target organs, such as the gut and the kidneys.<sup>16</sup>

During menopause, as oestrogen production decreases, bone becomes more sensitive to PTH. As a consequence, the inhibition of PTH on bone resorption declines and for a certain level of PTH, higher calcium mobilisation from bone occurs. This leads to an increase in plasma calcium which in turn causes a decline in the level of PTH. Renal tubular resorption of calcium decreases causing higher urinary excretion of calcium while the production of 1-alpha hydroxylase decreases, causing a reduction of active vitamin D with less absorption of calcium from gut.<sup>18</sup>

In menopause, a decline in the body's efficiency to make use of dietary calcium together with a decrease in calcium reabsorption from the renal tubule, stimulate bone remodelling in attempt to provide a constant amount of calcium to non-osseous tissues. As the majority of plasma calcium is derived from bone, there is a constant bone loss each year.<sup>18</sup>

Calcitonin is a hormone synthesised by the parafollicular cells of the thyroid gland in response to an increase in plasma calcium. Calcitonin has opposite effects to PTH. It suppresses both the production of new osteoclasts and it also inhibits their activity. Calcitonin causes less calcium tubular reabsorption in the kidneys and elevates urinary excretion of calcium.<sup>14</sup> Oestrogen elevates calcitonin levels needed to prevent bone loss.<sup>18</sup>

### Effect of oestrogen on fibrocartilage

Twenty percent of the spinal column height is attributable to the intervertebral disc. The roles of the intervertebral disc in permitting mobility of the spine and acting as a "shock absorber" are most compromised in osteoporotic compression fracture.<sup>31,32</sup>

In pre-menopausal women, the nucleus pulposus and annulus fibrosus of the intervertebral disc contain collagen type IV, II and IX, elastin, glycosaminoglycans and water. These constituents provide visco-elastic properties and a discoid anatomy to the intervertebral discs.<sup>30,32</sup> As the woman advances in her postmenopausal years, a more pronounced difference in collagen types is observed.<sup>31</sup>

Menopause has an effect on connective tissue.<sup>31</sup> Aging causes diminished circulation to the nucleus pulposus leading to a series of modifications in discs' components. These variations include a decrease in glycosaminoglycans and elastin content, dehydration and formation of collagen types I, III and VI replacing previous collagen types. Disc fibrosis and degeneration cause loss of intervertebral disc height interfering with its function as a vital tissue pump.<sup>33</sup>

### Diagnostic techniques for bone mass measurement

#### Radiographic techniques

In postmenopausal women, bone mineral density can vary according to the skeletal site. Postmenopausal women may present with normal bone mineral density in one part of the body and low bone mineral density in another body part. The primary site where bone loss occurs is the spine (L1 or L2-L4) as it is the region where the greatest trabecular bone remodelling occurs. In women up to 65 years of age, the ideal body region to measure bone mineral density should be the spine as it is the first site of bone loss.<sup>34,35</sup>

BMD testing is advised in patients with dual-energy X-ray absorptiometry (DXA) in women aged 65 years of age and older and in postmenopausal women younger than 65 years of age but with clinical risk factors for fracture. Patient should then have BMD testing at least every 2 years as follow-up.<sup>36,37</sup>

Several direct methods for measuring bone mineral densities are available.<sup>16</sup> Dual-energy X-ray Absorptiometry (DXA) is mainly used to determine bone mass in the spine (mainly in the lumbar regions), the hip or the total skeleton. Dual-Photon Absorptiometry (DPA) uses a beam of photons with discrete energy peaks in which one of them is absorbed by soft tissue and the other by bone. DXA uses an X-ray beam with

discrete energy peaks in which one of them is absorbed by soft tissue and the other by bone DXA is regarded as the gold standard for diagnosing postmenopausal osteoporosis as it provides both accurate and precise measurements.<sup>2,35</sup>

### Interpreting a DXA bone density result

The different bone mineral density tests are generally reported in the form of a T-score or Z-score. The T-score, is the number of standard deviations (SD) by which the bone mineral density (BMD) of the patient (Equation 1) varies when compared to that of the average young adult (30 years old) of the same gender and ethnicity. The Z-score is the number of standard deviations the bone mineral density measurement varies when compared to a mean bone mineral density of the same age, gender and ethnicity (Equation 2). This score determines whether a secondary underlying cause is actually contributing to bone loss.<sup>6,35</sup> In accordance to the International Society for Clinical Densitometry (ISCD), the WHO classification shall only be recommended to be used in clinical practice for postmenopausal women and in men 50 years and older.<sup>38</sup>

Quantitative ultrasonography (QUS) is used to assess skeletal status of the calcaneus and the phalanges. As QUS makes use of ultrasound waves, no radiation load is involved, making it a safe and a non-invasive technique. QUS can also be used in conjunction with DXA to provide a better prediction of fracture risk.<sup>23,34,39</sup>

### Biochemical markers of bone turnover

Biomarkers are sensitive tools to assess bone resorption and formation. These are actually used to monitor treatment of osteoporotic patients rather than to diagnose osteoporosis itself. The level of biochemical markers fluctuate within few months following initiation

**Table 1: World Health Organisation (WHO) classification<sup>11</sup>**

T-score is equal or greater than -1 SD	Normal
T score between -1 SD to -2.4 SD	Osteopenia
T-score is equal or lower than -2.5 SD	Osteoporosis

$$T \text{ score} = \frac{\text{BMD} - \text{population peak}}{\text{SD of population peak BMD}}$$

#### Equation 1: T-score<sup>6</sup>

$$Z \text{ score} = \frac{\text{Patient's BMD} - \text{population age-related}}{\text{SD of population age-related BMD}}$$

#### Equation 2: Z-score<sup>6</sup>

of treatment. Postmenopausal women are characterised by an increase in bone resorption markers and a decline in bone formation makers indicating the extent of uncoupling of bone formation and resorption present in postmenopausal osteoporosis.<sup>6,40,41</sup>

Biomarkers of bone formation represent osteoblastic activity and these are by-products of collagen synthesis, matrix proteins or osteoblastic enzymes.<sup>40</sup> These biomarkers include serum total alkaline phosphatase, osteocalcin and procollagen type I. These markers can be measured directly from circulation by a serum or plasma sample.<sup>42</sup>

Biomarkers of bone resorption represent osteoclastic activity and are mainly degradation products of type I collagen.<sup>40</sup> Fragments resulting from osteoclastic degeneration of bone as well as from disintegration of the organic matrix are used as biochemical markers for bone resorption. These fragments generally possess post-translational modifications of collagen which make them useless for utilisation by the body.<sup>42</sup> Disintegrated products resulting from enzymatic hydrolysis of type I collagen are among the most effective markers for bone resorption.<sup>41</sup> These collagen degradation products are released in circulation and then excreted in urine.<sup>6</sup> N-telopeptide, C-telopeptides and related pyridinoline cross-links of bone type I collagen are used as markers of bone resorption in osteoporosis.<sup>41</sup>

### Fracture risk calculators

New diagnostic techniques have been devised to assess the absolute risk of developing an osteoporotic fracture. FRAX<sup>®</sup> algorithm and the Garvan nomogram are web-based tools which integrate the clinical risk factors with BMD measured at the neck of the femur. FRAX<sup>®</sup> algorithm can predict a 10-year risk of hip fracture and other major osteoporotic fractures. The latter refers to a clinical spine, forearm, hip or humerus fracture. The fracture risk is calculated based on clinical risk factors with or without femoral neck BMD.<sup>35</sup>

### Treatment

The primary goal of treating postmenopausal osteoporosis is to mediate bone repair, alleviate bone strength and decrease the frequency of both vertebral and non-vertebral fractures which account for significant morbidity and mortality.<sup>43,44,45</sup>

The management of postmenopausal osteoporotic women involves both pharmacological and non-pharmacologic treatments.<sup>43</sup>

### Pharmacological interventions

**Table 2: Pharmacological agents<sup>48</sup>**

Anti-resorptive	Anabolic	Anti-resorptive & anabolic
HRT	Teriparatide	Strontium ranelate
Calcitonin	Parathyroid hormone	
Bisphosphonates		
SERMs		
Cathepsin K inhibitor (odanacatib)		
Denosumab		

Pharmacological therapy shall be considered in postmenopausal women and men aged 50 years and older with history of hip or vertebral fracture, T-score of -2.5 or lower at the femoral neck or spine without any secondary causes and in patients with T-score between -1 and -2.5 at femoral neck or spine who have a 10 year probability of hip fracture of  $\geq 3$  or any osteoporotic fracture of  $\geq 20\%$  based upon the United states using the WHO FRAX.<sup>46</sup> As displayed in table 2, treatment can be categorised into antiresorptive drugs which delay bone resorption and anabolic drugs which promote bone formation.<sup>47</sup>

- **Hormone Replacement Therapy (HRT)**

Hormone replacement therapy involves the administration of physiologic levels of oestrogen and progestin to replace and artificially boost the hormones which decline during menopause.<sup>14</sup>

Epidemiology shows that a short time frame with HRT decreases the occurrence of osteoporotic fractures.<sup>14</sup> It has been determined that undergoing oestrogen therapy for 5 years reduces vertebral fracture by 60% whilst hip fracture can decline by 50%.<sup>2,49</sup>

Generally by oestrogen treatment, bone turnover is reduced by half, decreasing postmenopausal bone loss and lowering the incidence of an osteoporotic fracture.<sup>50</sup> However, once treatment with HRT stops, oestrogen level declines and protection against osteoporosis is lost again. Most studies suggest that bone loss will progress at the same rate as before HRT treatment. Thus the accelerated bone loss during menopause is postponed by the duration of HRT treatment.<sup>2,14</sup>

As long term duration of therapy is needed for the prevention of postmenopausal osteoporosis, HRT has been associated with various risks which limit its attraction as treatment option.<sup>19</sup>

The long term adverse effects implicated for HRT include carcinoma mainly of the breast, endometrium and colorectal as well cardiovascular diseases such as coronary heart disease, cerebrovascular accident and pulmonary embolism.<sup>51,52</sup>

Regulatory authorities state that HRT should not be used as first-line treatment in prophylaxis as long term risks may outweigh the potential benefits.<sup>6,40,45</sup> Thus HRT should only be considered when other treatment options have proved to be unsuccessful.<sup>45,53,54</sup>

- **Teriparatide**

Teriparatide is a recombinant synthetic form of the natural human hormone, PTH which is administered subcutaneously. It is known to enhance bone formation by activating osteoblasts and increase bone mineral density.<sup>14,55</sup> Teriparatide enhances calcium intestinal absorption as well as calcium reabsorption from the kidney. With its anabolic action, it decreases significantly vertebral and non-vertebral fractures but not hip fracture.<sup>56</sup> Teriparatide is used mainly in severe osteoporotic patients with a high fracture risk.<sup>2</sup>

- **Bisphosphonates**

Bisphosphonates are anti-resorptive drugs<sup>2</sup> which act by adsorbing onto hydroxyapatite crystals in bone and exert a potent inhibitory effect on bone resorption rate maintaining BMD. Bisphosphonates can be used both as a prophylaxis and as treatment for osteoporosis as they decrease the rate of bone loss and fracture risk in osteoporotic patients.<sup>53</sup> In fact, bisphosphonates decrease both vertebral and non-vertebral fractures up to 50% while hip fractures are decreased by 20%. Bisphosphonates also enhance BMD at both the hip and the spine in a dose-dependent way.<sup>57</sup>

Bisphosphonates can be divided into two main groups: non-nitrogen containing bisphosphonates and nitrogen containing

bisphosphonates. Etidronate is a non-nitrogen containing bisphosphonate. The nitrogen containing bisphosphonates include alendronate, risedronate and ibandronate. Alendronate, risedronate and etidronate are most clinically used in corticosteroid-induced osteoporosis to decrease both vertebral and non-vertebral fractures.<sup>6, 55</sup> Osteonecrosis of the jaw<sup>53</sup> and gastro-intestinal damage are among the adverse effects which limit the use of oral bisphosphonates.<sup>19,56</sup> Administering bisphosphonates such as ibandronate and zoledronic acid intravenously enhance their potency in inhibiting bone remodelling however they are linked to transient flu-like symptoms and self-limited myalgia.<sup>57</sup>

- **Strontium ranelate**

Strontium ranelate is a dual action bone agent as it has an anti-resorptive and anabolic action.<sup>56</sup> It inhibits the osteoclasts recruitment and augments osteoblasts proliferation and differentiation.<sup>58</sup> Thus, with strontium ranelate therapy bone resorption is suppressed while bone formation is stimulated. Both hip and vertebral fractures are decreased with such a treatment.<sup>56</sup>

- **Selective oestrogen receptor modulators (SERMs)**

SERMs are non-steroidal compounds with tissue-specific activity having oestrogenic effects in certain tissues and anti-oestrogenic effects in others. They bind to oestrogen receptors within the bone and change the receptor conformation to aid in the binding of co-regulatory proteins and activate target genes.<sup>59</sup> Raloxifene is an anti-resorptive agent which prevents osteoporosis-related vertebral fracture. It decreases vertebral fractures by 30-50% and increases bone mineral density at the hip and the spine by 0.5% to 1.0% respectively.<sup>6,34,45</sup>

- **Calcitriol**

Calcitriol (1,25-dihydroxycholecalciferol) is an active vitamin D metabolite used to treat low calcium levels in postmenopausal women. Conflicting data has emerged regarding the effect of calcitriol on bone.<sup>6</sup> Studies have shown that calcitriol aids in calcium intestinal absorption and reduces the rate of vertebral fracture whereas it has no effect on hip fracture.<sup>45</sup>

- **Vitamin D and calcium supplementation**

During infancy, childhood and adolescence calcium intake is of utmost importance as calcium is required for peak bone mass to be achieved. Postmenopausal women have the highest calcium requirements

with a recommended daily intake of at least 1000-1200mg. Providing adequate amount of dietary or supplemental calcium and vitamin D to postmenopausal women particularly the elderly serves as a baseline treatment of postmenopausal osteoporosis<sup>56</sup> as a decrease in intestinal calcium absorption is noted with advancing age.<sup>60</sup>

Calcium supplementation may lower the rate of bone loss following two years of treatment however during the initial 5 years of menopause, it exerts little effect on bone loss as it can be attributed to the decline in oestrogen synthesis.<sup>18,47</sup> Calcium supplementation is regarded as an adjunct to other treatment regimens unless sufficient dietary intake is ensured.<sup>56</sup>

Institutionalised or housebound patients who are rarely exposed to the natural sunlight and persons who chronically use sunscreen products particularly with high sun protection factors may possess vitamin D deficiency. Thus Vitamin D supplementation is essential in these patients. Vitamin D may decrease the risk of hip and non-vertebral fractures in ambulatory elderly patients only if an oral supplementation of 700-800IU is administered each day.<sup>6,61</sup>

Vitamin D and calcium supplementation increase BMD and decrease the risk of fracture. The latter is negated by some randomised controlled clinical trials.<sup>57</sup> In addition to calcium, other elements including copper, boron, magnesium, manganese, phosphorus, potassium and zinc are associated with improving bone health. Aluminium, cadmium and lead appear to affect BMD negatively.<sup>62</sup>

- **Fluoride**

The use of fluoride in the treatment of osteoporosis is argumentative.<sup>2,63</sup> Fluoride treatment activates osteoblasts resulting in bone formation in trabecular part of bone while long term treatment enhances the mass of the central skeleton.<sup>16</sup>

- **Denosumab**

Denosumab is a Receptor Activator of Nuclear Factor kappa- $\beta$  ligand (RANKL) - targeted monoclonal antibody.<sup>64</sup> By binding with high affinity and high-specificity to RANK, it suppresses the binding of RANKL to receptor Activator of Nuclear Factor kappa- $\beta$  (RANK)<sup>64,65</sup> and hence, blocks the development, differentiation, activation and survival of osteoclasts. This leads to a decline in bone resorption and promotes BMD. Its fast onset of action and its constant prolonged reversible activity make denosumab a better therapeutic agent than the current treatment options.<sup>65-68</sup>

Administration of denosumab subcutaneously once every 6 months for 36 months decreases the risk of vertebral, non-vertebral and hip fractures in osteoporotic women.<sup>68</sup> However, two major drawbacks of using denosumab as a treatment of postmenopausal osteoporosis are the potential formation of infections and higher risk of malignancy.<sup>69</sup>

- **Calcitonin**

Calcitonin is a peptide hormone synthesised by the thyroid gland. Calcitonin has been withdrawn for the treatment of osteoporosis as it has been found that long term use increases the risk of cancer.<sup>70</sup>

### Potential novel pharmacological agents

- **Cathepsin K inhibitors**

Cathepsin K is an acid activated cysteine protease,<sup>71</sup> required for the degradation of the organic matrix.<sup>72</sup> It exerts its function specifically on bone type I collagen under acidic conditions.<sup>73,74</sup>

Odanacatib is the most advanced cathepsin K inhibitor undergoing clinical trials.<sup>47</sup> It decreases bone resorption not by decreasing the presence of osteoclasts or their activity but by selectively suppressing the degradation of matrix protein. Substantial dose-related increase in BMD was observed in lumbar spine, total-hip, femoral neck and one-third of the radius during the second year of treatment. Headaches, flu-like symptoms and abdominal discomfort are amongst the adverse effects of odanacatib.<sup>74,75</sup>

The novel cathepsin-K inhibitor, ONO-5334 is still undergoing clinical investigation. It seems to enhance BMD in the lumbar spine with an improved effect on the femur and induces no severe side effects.<sup>47,76,77</sup>

- **Calcilytic drugs (MK-5442)**

MK-5442 is a calcilytic drug which targets calcium sensing receptors (CaSR antagonist) of the parathyroid glands. It provokes a hypocalcaemic environment hence stimulating pulsed PTH secretion resulting in a net anabolic situation. MK-5442 is still currently undergoing clinical trials.<sup>47,78,79</sup> MK-5442 has not been shown to improve BMD in patients already on bisphosphonates therapy and hence should not be considered to be used in combination with bisphosphonates.<sup>80</sup>

- **Wnt / $\beta$ -catenin pathway antagonists**

Osteoblastic differentiation and subsequently bone formation can be induced by Wnt-dependent nuclear accumulation of  $\beta$ -catenin.

Possible future therapeutic agents include BHO-880 which suppress Wnt pathways by Dickkopf-1 antibodies and Romosozumab

and blosozumab by targeting sclerostin antibodies. These medications are still under study as potential therapeutic agents for postmenopausal osteoporosis.<sup>47,80</sup>

### Alternative and complementary therapy

#### • Phytoestrogens

As some women have fear of the adverse effects produced by some drugs, alternative methods to prevent and treat postmenopausal osteoporosis are sought. Phytoestrogens are plant-derived compounds which function like oestrogen. Classes of phytoestrogens include isoflavones present in soy beans and soy products, lignans present in oilseeds and coumestans found in alfalfa and red clover. These phytoestrogens exert a protective effect on bone by retarding bone resorption and maintaining skeletal integrity. Phytoestrogens promote osteogenesis when taken at low concentrations and suppress osteogenesis at higher doses.<sup>6</sup>

### Conclusion

A high index of clinical suspicion of postmenopausal osteoporosis is required as it may go unnoticed until a fracture occurs. Lifestyle measures that include the adequate intake of calcium and vitamin D, 30-minutes exercise at least three times a week and smoking cessation and limit alcohol intake aim to reduce bone loss in postmenopausal women. Counseling on fall prevention to women at risk shall be employed. The choice of pharmacological therapy depends on multiple factors including the severity of the osteoporosis and the risk for fractures. Preventing and treating post-menopausal osteoporosis decreases the social, emotional and financial burden of an osteoporotic fracture.<sup>46</sup>

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### Key points

- Post-menopausal osteoporosis is a silent progressive disease until a fracture occurs
- Bone mineral density is helpful in assessing bone health in women. Timely intervention can prevent bone loss and its associated complications
- BMD testing is advised in women aged 65 years of age and older and in postmenopausal women younger than 65 years of age with clinical risk factors for fracture
- Prevention of osteoporosis involves proper nutrition, exercise, lifestyle and early screening
- The management of postmenopausal osteoporotic women involves both pharmacological and non-pharmacologic treatments

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# Cystitis and its management

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## Educational aims

- To provide an overview of urinary tract infections and cystitis, underlying the difference between the latter and interstitial cystitis
- To outline the major causes of cystitis and the signs and symptoms that are associated with it
- To describe the different tests that are used to diagnose an episode of cystitis
- To highlight the various classes of medications that are used to prevent and treat cystitis
- To offer a series of recommendations for lifestyle modifications that will help prevent further episodes

## Key words

urinary tract infection, cystitis, dipstick test, alkalinising agents, antibiotics, probiotics, cranberry juice

## Abstract

**Urinary tract infections are very common. They are responsible for over 6 million patient visits to doctors per year in the United States.<sup>1</sup> These include over 2 million cases that are attributed to cystitis which is the most common urinary tract infection. Cystitis can be defined as an inflammation of the bladder and it may result in pain and discomfort. Women tend to suffer from this condition more than men, although the chance of men acquiring cystitis increases with age. Untreated cystitis can lead to kidney infection, even kidney damage. Treatment for cystitis ranges from over-the-counter medications to antibiotics if the cause is an infection.<sup>2</sup>**

## Introduction

Urinary tract infections (UTIs) are divided into lower and upper tract infections. Lower UTIs (LUTIs) include the bladder and urethra, whereas upper UTIs involve the kidneys. Many a time, the infection migrates from one part of the tract to another. Most UTIs are caused by bacteria, although sexually transmitted pathogens, mycobacteria, fungi and parasites also give rise to such infections.<sup>3</sup>

Bacterial UTIs involve any part of the urinary tract and may be asymptomatic or characterized by the symptoms that are normally associated with these infections. Diagnosis focuses on the doctor taking a detailed history of the presenting complaints by the patient, together with a urine analysis and urine culture. Treatment usually involves the use of antibiotics. When considering adults up to 50 years of age, bacterial UTIs are much more common in females. The incidence of these infections increases in both male and female patients over 50 years of age. On the other hand, the female:male ratio decreases, a reason being an increased frequency of prostate disease.<sup>3</sup>

The urinary tract is normally a sterile environment and this is very often maintained due to various reasons which may include the acidity of the urine, emptying of the bladder at micturition and various immunological and mucosal barriers. Most UTIs occur when the pathogenic bacteria ascend the urethra to the bladder.<sup>3</sup> A bacterial infection of the urethra, urethritis, is mainly caused by the sexually transmitted pathogens, *Chlamydia trachomatis*, and *Neisseria gonorrhoea*.<sup>1</sup>

Cystitis is the term used to describe a bladder inflammation that is very often due to an infection which is usually of bacterial origin. Examples of such micro-organisms include *Escherichia coli* (70-95% of all cases) and *Klebsiella pneumonia*. A bladder infection may become a serious health problem if not treated, as the infection will otherwise spread to the kidneys. This may result in renal failure or pyelonephritis.<sup>3</sup> Due to complications of cystitis, mortality rates can be as high as 1% in men and 3% in women.<sup>1</sup>

Non-infective episodes of cystitis are rare and they may be due to:<sup>1</sup>

- radiation therapy
- certain medicines such as cyclophosphamide; this is thought to be due to the metabolites that are excreted in the urine - effects appear to be

**Table 1: Typical signs and symptoms of cystitis**

- A strong, persistent urge to urinate
- Haematuria
- Dysuria
- Lower back and/or abdominal pain and discomfort
- Fever
- Malaise
- Pressure in lower pelvis

related to the dose of medication taken and to the duration of therapy

- the long-term use of a catheter
- hypersensitivity to certain chemicals that can be found in spermicidal gels, feminine hygiene sprays and shower gels or bath foams.

Interstitial cystitis (IC), also known as painful bladder syndrome, should not be confused with cystitis. IC is a chronic bladder inflammation that is not bacterial in origin. It affects both sexes and known causes are sexual intercourse, mental and/or physical stress and menses in women. IC may occur in association with other conditions, such as sinusitis, hay fever, fibromyalgia, migraines and food allergies.<sup>4</sup> Treatment does not involve antibiotics but requires personalized detailed patient education. Patients must be well informed on potential trigger factors. This will help patients enjoy long periods of remission and a better quality of life.

### Risk factors

Cystitis commonly occurs in females; about 20% of women, sooner or later, develop a UTI.<sup>5</sup> This is mainly due to women having a shorter urethra than men. Women aged 18-30 years are very prone to getting cystitis; sexually active females are at a greater risk of developing cystitis as sexual intercourse can result in bacteria being pushed into the urethra. Hormonal changes that occur in pregnancy and the use of diaphragms also attribute to an increased risk of cystitis. Altered hormonal levels in postmenopausal women and a bladder or uterine prolapse may cause incomplete bladder emptying are also associated with cystitis.<sup>1</sup> Cystitis may also arise as a complication of another illness, an example being diabetes.

On the other hand, cystitis is quite rare in younger men and children. Whenever a man presents with symptoms pertaining to cystitis he should be immediately referred to a doctor as the symptoms may be indicative of an underlying pathology, such as stones in

the bladder or an enlarged prostate.<sup>2</sup>

Children are very susceptible to kidney and bladder damage as a result of a urinary tract infection. A UTI in children may be an indication of structural abnormalities within the urinary tract and it hence merits further investigations by a urologist.

### Signs and symptoms

The symptoms of cystitis are very similar to the ones an individual experiences when suffering from acute urethritis that arises due to sexually transmitted diseases.<sup>3</sup> The symptoms of vaginitis are also very similar to those of cystitis, although the former is often characterized by the presence of vaginal odour, dyspareunia and vaginal discharge.<sup>5</sup> It is thus of major importance that other conditions are ruled out before treating an individual for cystitis.

Typical signs and symptoms pertaining to cystitis are presented in Table 1.

Catheterised and elderly patients may present with atypical symptoms which will unfortunately delay a correct diagnosis and hence the appropriate treatment to be given.<sup>6</sup> Such atypical symptoms are presented in Table 2.

### Diagnosis

Various diagnostic studies confirm cystitis. These include a urine dipstick test, urinalysis and a bacterial culture. Imaging studies are not indicated in the routine evaluation of cystitis.<sup>5</sup> A dipstick test is usually sufficient to diagnose an episode of cystitis. This may be performed by a pharmacist or doctor and is very cost-effective and convenient to carry

out. Ideally, a mid-stream urine is used to perform the test to remove the commensal flora in order to avoid contamination.<sup>7</sup>

The dipstick test may result in microscopic haematuria, proteinuria, a positive nitrate test and a positive leukocyte esterase test. These are all indicative of a UTI. The most accurate indicators - 98% - of an acute uncomplicated episode of cystitis in symptomatic individuals are the presence of nitrites and leukocyte esterase in the urine sample.<sup>7</sup> According to the guidelines issued by the local National Antibiotic Committee for antibiotic use in LUTIs in the community setting, a negative dipstick test excludes a UTI, including cystitis. On the other hand, a positive test does not necessarily confirm a UTI but the presence of leukocyte esterase and nitrites may indicate the presence of a UTI.<sup>8</sup>

The dipstick test makes use of the Kastle-Meyer test which detects the peroxidase activity of red blood cells. In this test, the chemical indicator, phenolphthalein is used to detect the possible presence of haemoglobin. Haemoglobin catalyses the oxidation of the colourless reduced form of phenolphthalein into phenolphthalein. The latter is visible as a bright violet colour. False-positive results may result due to a contaminated specimen container or due to the presence of semen in the urine. Blood in the urine may also be due to vaginal bleeding or bleeding haemorrhoids.<sup>7</sup> The detection of haematuria can therefore give rise to a high rate of false positives and also false negatives. For this reason, this test is unreliable and cannot be considered in isolation.<sup>7</sup>

Normal urine contains very little protein which consists mainly of low-molecular-weight serum proteins that have been filtered by the glomerulus and proteins that are produced in the genitourinary tract. The albumin content of urine is normally low because most of this protein is not filtered at the glomerulus. A large amount

**Table 2: Atypical signs and symptoms of cystitis**

- Abdominal pain
- Nausea and vomiting
- Urinary retention
- Altered mental state
- Worsening in the control of diabetes
- Rigors
- New-onset incontinence

of any filtered albumin is reabsorbed by the tubules. Other proteins found in urine may include small amounts of tubular microglobulins. The urinary dipstick test only detects the presence of albumin. A negative protein result therefore does not rule out the presence of globulins. This test is therefore also unreliable and of little diagnostic value.<sup>7</sup>

The urine dipstick test to check for proteinuria is based on the fact that certain indicators vary in colour in the presence of protein even though the pH of the medium remains the same. This occurs because proteins tend to accept hydrogen ions from the indicator on the test strip. The dipstick test is very sensitive to albumin because albumin contains more amino groups than other proteins. For this reason, albumin can accept more hydrogen ions. Indicators appear yellow in the absence of protein but as the protein concentration increases, the colour change progresses through various shades of green.<sup>7</sup>

Nitrites are not normally present in the urine. They are produced when Gram negative bacteria, examples being *Escherichia coli*, *Enterobacter* and *Klebsiella* reduce dietary nitrates to nitrites. In order for the nitrate test to be reliable, the urine specimen should be one that has been present in the bladder for at least four hours so that sufficient time elapses for the nitrate to nitrite conversion to take place. Negative results in the presence of clinical symptoms can be obtained and this may be due to the presence of non nitrate-reducing micro-organisms. Also, some bacteria can convert nitrite to nitrogen and this too will give a false negative result.<sup>7</sup>

White blood cells contain an enzyme called leukocyte esterase. This is released when the white blood cells undergo lysis. Very few white blood cells are usually present in the urine for the dipstick test to be positive. However, when there is a large number of white blood cells present in the urine, as in the case of a UTI, a positive result is obtained. The reaction that takes place is based on the fact that the leukocyte esterase catalyses the hydrolysis of an ester of indolecarboxylic acid on the reagent strip. As a result, the indoxyl that is liberated combines with a diazonium salt. A violet coloured azole dye is subsequently obtained.<sup>7</sup>

Urine microscopy may be necessary if the dipstick test is negative and the patients are experiencing the clinical symptoms that are normally associated with cystitis. Some patients - immunosuppressed

patients, pregnant women, children under three years of age, individuals who have recurrent UTIs, those who have had a recent urinary tract intervention and those who are of an advanced age - who experience the symptoms of cystitis must be subject to a urine culture test at all costs as the dipstick test is considered to be unreliable.<sup>1,7</sup> Bacterial culture is not usually necessary to confirm the diagnosis of an episode of cystitis in a non-pregnant woman. If however she does not respond to first-line treatment, her urine should be cultured so that the appropriate medication will be prescribed.<sup>7</sup>

Microscopy is used to determine haematuria, bacteriuria and pyuria. Haematuria is not always present in cystitis and it can also be associated with other conditions, an example being neoplasia. Bacteriuria, especially with pyuria, highly suggests an infection whereas pyuria occurring in isolation is an indicator of a urinary tract inflammation; it however does not confirm the presence of an infection.<sup>7</sup>

#### Treatment

Pharmacotherapy in cystitis aims to:

- provide symptomatic relief to the patients
- eradicate the infection
- prevent complications - early treatment is recommended to reduce the risk of complications.

The diagnosis and management of uncomplicated acute episodes of cystitis are relatively straightforward to handle. On the other hand, recurrent and complicated infections require more specialized assessment. Without any treatment, 25-42% of uncomplicated episodes of cystitis in women will resolve spontaneously. The chance of these women developing pyelonephritis is around 2%.<sup>5</sup>

The treatment for cystitis varies as it depends on the underlying cause. Cystitis that occurs because of radiation therapy involves adequate hydration to flush out any irritants and also pain management. For chemical cystitis it is recommended to discontinue the use of the irritating products.

At times, it is impossible for some patients to visit their doctors. Studies have demonstrated that there may be incidences when women who self-diagnose a UTI are treated safely via telephone management.<sup>5</sup> Women who have previously suffered from

acute uncomplicated cystitis are usually correct in determining when they are suffering from another episode.

#### Paracetamol and Non-steroidal anti-inflammatory drugs (NSAIDs)

These medications act as antipyretics and also reduce the pain or discomfort experienced by the patient. NSAIDs reduce the production of prostaglandins by inhibiting cyclo-oxygenase. They vary in their selectivity for inhibiting the different types of cyclo-oxygenase; the NSAIDs that are selective cyclo-oxygenase-2 inhibitors, such examples being etoricoxib and celecoxib, are associated with less gastrointestinal intolerance. They hence reduce the risk of peptic ulceration. In the elderly and other high risk patients, the use of a proton pump inhibitor (PPI) is highly recommended together with the cyclo-oxygenase-2 inhibitor. It is of utmost importance that on dispensing, the pharmacist informs the patient to take the NSAIDs after food in order to further reduce gastrointestinal upset. On the other hand, the PPI is ideally taken one hour before breakfast.

#### Alkalinising agents

Alkalinising agents include sodium bicarbonate, sodium citrate, potassium citrate and sodium carbonate. Although there is no clinical evidence to support their use, sources claim that they actually relieve discomfort.<sup>2</sup>

The recommended dosage for the preparations that are available locally is one sachet three times a day for two days. If the symptoms do not subside, patients ought to be referred to a doctor as further investigations and/or antibiotic therapy may be required.

Since the sodium content in the sachets is relatively high, these preparations should be avoided in individuals who are on a diet that requires a restricted salt intake. They should also be avoided in hypertensive patients as they will cause fluid retention and further increase the blood pressure. Diabetics, pregnant women, patients who have heart disease and/or renal failure should also avoid these products as these patients, as previously noted, should be referred immediately.<sup>2</sup> It is of major importance that patients who are on lithium therapy should not be given these sachets because sodium is preferentially absorbed by the kidney. Lithium excretion is hence

**Table 3: Maltese guidelines to treat UTIs in the community**

UTI in pregnancy	First line: nitrofurantoin Amoxicillin if organism is sensitive	50-100mg qds x 7 days 500mg tds x 7 days
UTI in children	Second line: cephalexin LUTI: coamoxiclav	500mg tds x 7 days 15mg/kg/dose tds x 3 days
LUTI in men and women (no fever)	Second line: cefuroxime If pH< 7: nitrofurantoin If pH>7: coamoxiclav	10-15mg/kg/dose bd x 3 days 50-100mg qds; female x 3 days and males x 7 days 625mg tds; female x 3 days and males x 7 days

increased thus resulting in reduced plasma concentrations.<sup>9</sup>

These sachets must also be used with caution in patients who are on medications that require an acidic urine to be excreted.<sup>10</sup> Patients taking certain medications that increase the potassium level, such as potassium sparing diuretics, aldosterone antagonists and angiotensin converting enzyme inhibitors, should consult the doctor before taking the potassium-salt containing sachets because of the risk of hyperkalaemia.<sup>2</sup>

#### **D-Mannose**

According to The National Institute for Health and Care Excellence Guidelines, D-Mannose is the most effective over-the-counter supplement for preventing and treating urinary tract infections. Similar to glucose in structure, D-mannose is a naturally occurring sugar and can be found in several fruits such as apples, blueberries, and cranberries. D-mannose is effective because it attaches itself to *Escherichia coli* and as a result, the bacteria are eliminated from the body during micturition. Even if taken in large quantities, D-mannose does not cause any adverse effects. It is safe in diabetics and can be easily taken by patients who have to avoid sugar.<sup>11</sup> The preparation that is available locally should be taken twice daily for a week and ideally should be continued even after the infection subsides so as to ensure complete elimination of the bacteria in the urinary tract. Individuals who are prone to recurrent urinary tract infections can take D-mannose on a regular basis as a means of prevention.

#### **Antibiotics**

According to the 2010 Infectious Diseases Society of America (IDSA) guidelines, no antibiotic is considered as being ideal for treating acute uncomplicated episodes of cystitis. The choice of antibiotic depends on several factors and these include the medication's efficacy, any associated adverse

side effects and the resistance the microorganisms exhibit.<sup>5</sup> The prevalence of antibiotic-resistant infections tends to be higher in patients who suffer from recurrent infections and who have taken various antibiotics due to other illnesses. Physicians should also consider ease of availability, cost and individual patient factors, such as a history of allergy. Although published guidelines offer choices for the various antibiotics that can be prescribed by doctors, studies have shown that prescribing practices vary tremendously.

Since most cases of cystitis are caused by *Escherichia coli*, it is of major importance that this microbe is not resistant to the antibiotic that is chosen for empirical treatment. The resistance patterns of *Escherichia coli* vary considerably between countries. A specific recommended treatment protocol may therefore not be suitable for all regions.<sup>12</sup>

According to the IDSA guidelines, the antibiotics that are to be considered as the treatment of choice for uncomplicated and acute episodes of cystitis in women include nitrofurantoin or trimethoprim-sulfamethoxazole or fosfomycin. Fluoroquinolones are usually used to treat complicated episodes of cystitis and should not be used as a first line for the empirical treatment of LUTIs. Their use should be guided by culture and sensitivity results. Beta-lactam antibiotics may be prescribed when other recommended medications cannot be used. For instance, fosfomycin and nitrofurantoin should be avoided in patients who suffer from a possible early episode of pyelonephritis.<sup>5</sup>

The Maltese National Antibiotic Committee issued treatment guidelines for the treatment of UTIs in the community.<sup>8</sup> These are presented in Table 3.

The local guidelines stipulate that in women and children, a three day course of antibiotics is usually sufficient to cure an

uncomplicated acute case of cystitis whereas males and pregnant women should be treated for at least 7 days. Children suffering from upper UTIs should also be treated for 7 to 10 days.<sup>8</sup>

It is clearly shown that the local guidelines differ from the 2010 IDSA guidelines. Co-trimoxazole, for example is presently no longer indicated for the empirical treatment of LUTIs. This contrasts with the local widespread use of this drug over twenty years ago.

Pharmacists are in a key position in advising the patient on the proper administration of the medication. It is to be noted that on average, patients will begin experiencing symptom relief within 36 hours of commencing the treatment. The whole course of antibiotics must be taken even if the patient feels better before completion.

#### **Nitrofurantoin monohydrate**

Nitrofurantoin is highly effective against *Escherichia coli*, many Gram negative bacteria and Gram positive cocci. The duration of therapy for nitrofurantoin has been reduced to five days in the 2010 IDSA guidelines. This varies from the stipulated seven day treatment recommended in the previous 1999 guidelines.<sup>5</sup> Nitrofurantoin is generally well tolerated, with no significant effects on vaginal flora. It is contraindicated in patient with impaired renal function and possible adverse effects include nausea, vomiting, hypersensitivity, peripheral neuropathy, hepatitis and haemolytic anaemia.<sup>9</sup> Nitrofurantoin lacks significant drug interactions although administration with alkalisating agents renders the antimicrobial ineffective due to the alkaline pH. The pharmacist should advise the patient to take the medication with food and not to get alarmed if the urine is coloured yellow or brown. Nitrofurantoin is unlikely to cause problems to the foetus if given for a short period in pregnancy. It however cannot be given at term or during breastfeeding.

### **Trimethoprim–sulfamethoxazole**

These two antibiotics are used in combination due to their synergistic activity. They interfere with two consecutive steps in the biosynthesis of nucleic acids and proteins that are essential to many bacteria.<sup>5</sup> Trimethoprim inhibits dihydrofolate reductase; the synthesis of tetrahydrofolic acid from dihydrofolic acid is therefore inhibited. Sulfamethoxazole, on the other hand, inhibits the synthesis of dihydrofolic acid by competing with para-aminobenzoic acid. Co-trimoxazole should not be prescribed to individuals who have already taken it in the previous three months for cystitis. This antibiotic has been associated with rare, but serious adverse effects which include Stevens-Johnson syndrome, photosensitivity reactions and blood dyscrasias. More common adverse effects include diarrhoea, nausea and headache.<sup>9</sup> The pharmacist ought to advise the patient regarding adequate fluid intake whilst taking the antibiotic and to also avoid direct sunlight. Patients taking warfarin should not be prescribed co-trimoxazole because of the increased anticoagulant effect due to trimethoprim.<sup>9</sup> There is also an increased risk of hyperkalaemia when trimethoprim is given with angiotensin converting enzyme inhibitors or angiotensin II receptor antagonists.<sup>9</sup>

### **Fosfomycin tromethamine**

In the United States fosfomycin is not widely available because it is considered to be less effective than standard short-course regimens. This antibiotic inhibits bacterial cell wall synthesis and also reduces bacterial adherence to the urinary tract. Adverse effects may include diarrhoea, nausea, vomiting, drowsiness and pruritus.<sup>5</sup> The pharmacist should advise the patient to add the sachet contents to a glass of cold water, to stir it and drink it straight away, ideally in the evening, with or without food. Symptoms should improve within two to three days after taking the medication; if symptoms persist or worsen, the doctor must be contacted. It is very important to inform the patient not to use more than one sachet as more packets will not make the medication work better. On the other hand, adverse effects will be increased. It would be very useful to limit the local use of this new medication because fosfomycin is active against most local multidrug resistant strains of Gram negative organisms. It will therefore save on the use of second line antibiotics, such as carbapenems within the hospital setting.

### **Quinolones**

Quinolones are very effective against Gram negative bacteria and to a lesser extent also inhibit Gram positive bacteria. Unfortunately, resistance to quinolones is on the increase.<sup>5</sup> The use of low dose ciprofloxacin is replacing older quinolones (norfloxacin) due to the former's better pharmacokinetic properties. Quinolones should be used with caution in patients who have a history of epilepsy since a main adverse effect is that they may induce convulsions. Other adverse effects may include rash, headache, restlessness, an Achilles tendon rupture especially in patients who are older than 60 years and a QT interval prolongation. The community pharmacist should advise the patient to take the medications after food and to avoid direct sunlight due to photosensitivity reactions. Dairy products should not be taken with quinolones as the absorption of the medication will be reduced. Very important interactions involving quinolones include those with warfarin – where the anticoagulant effect is enhanced – and NSAIDs – where the risk of convulsions is increased if they are given concomitantly.<sup>9</sup> Also, ciprofloxacin should not be given with calcium supplements and alkalisating agents due to the risk of chelation and crystalluria respectively.

### **Beta-lactam antibiotics**

Cephalosporins are rarely indicated for LUTIs because of increased resistance by Gram negative bacteria. Amoxicillin should be avoided as much as possible because resistance to this agent is also very high. Beta-lactam antibiotics tend to have inferior efficacy and more associated adverse effects than nitrofurantoin.<sup>5</sup> They therefore should be used with extreme caution in uncomplicated cases of cystitis. The pharmacist should advise the patient regarding possible adverse effects which can be avoided; the individual should take probiotics with the antibiotic so as to prevent diarrhoea and use an intimate wash to help avoid vaginal candidiasis.

Women who suffer from more than three recurrent UTIs a year should take prophylactic antibiotics in addition to behavioural modification. Women whose recurrent UTIs are associated with sexual intercourse should take a single dose of an effective antibiotic as post-coital prophylaxis.<sup>5</sup> Local guidelines recommend a 50mg nitrofurantoin STAT dose. Prophylactic antibiotic use should not be taken for

more than six to twelve months due to the risk of bacterial resistance and also due to the occurrence of adverse effects, such as gastrointestinal effects and rashes.<sup>10,13</sup> Patients should be well informed about the fact that the antibiotic prophylaxis is not usually a life-long treatment. The medication, however, is to be taken for the required period of time so as to allow adequate healing of the bladder to take place and it should ideally be taken at night when urine flow is rather low.<sup>10</sup> Men and patients who have an indwelling catheter should not take a daily dose of prophylactic antibiotic therapy unless the medications are prescribed by a urologist, microbiologist or nephrologist.<sup>14</sup>

### **Probiotics**

Probiotics can be defined as, “live microorganisms, which when administered in adequate amounts confer a health benefit on the host”.<sup>15</sup> The micro-organisms that inhabit the vaginal tract play a very important role in the prevention of infections and also in the maintenance of good health. About 50 different types of microbial species inhabit the vagina. The species that are present in the vaginal mucosa vary between premenopausal and postmenopausal women. The microbial flora of a healthy premenopausal woman is normally dominated by the *Lactobacillus* species. Various factors such as hormonal changes (especially in oestrogen levels) and vaginal pH can affect the colonization of the *Lactobacilli* in the vagina. Spermicides for example, lead to a loss of *Lactobacilli* and alter the pH.<sup>15</sup> The growth of Gram-negative organisms is therefore stimulated, thus resulting in cystitis.

*Lactobacilli* are required in the vaginal mucosa for various reasons - they produce antibacterial materials, an example being hydrogen peroxide, so as to limit pathogen growth. They also produce biosurfactants that inhibit pathogen adherence to the mucosa and attract macrophages, leucocytes and other host defences to the particular area.<sup>15</sup>

Clinical trials have demonstrated that a number of strains of *Lactobacilli* are very effective at helping to treat and prevent cystitis. Taking *Lactobacillus* probiotics daily as prevention for cystitis offers advantages over long term preventive antibiotic therapy; probiotics do not cause antibiotic resistance and unlike antibiotics allow re-colonization of bacteria to take place.<sup>15</sup>

### Cranberry oral preparations

The mechanism of action of cranberries as a prevention of cystitis has not been fully understood. Cranberries contain water (as their main constituent) and carbohydrates. Benzoic acid in cranberry juice is excreted in urine as hippuric acid which is a bacteriostatic agent; it has the potential to acidify urine. Studies have also shown that in petri dishes, cranberry metabolites prevent *Escherichia coli* from adhering to other bacteria, hence limiting its ability to grow and multiply.<sup>10,16</sup> Despite this, results of studies are rather inconsistent and the required dose to be taken is unclear.<sup>10</sup> Women should be advised that high strength cranberry capsules are more effective than cranberry juice. Flavonoids, which are constituents of cranberries, have an effect on the cytochrome P450 drug-metabolizing enzyme. Flavonoids together with the salicylate content of the juice enhance the anticoagulant effect of coumarins, so concomitant use with warfarin should be avoided.<sup>16</sup> Cranberry juice has also been reported to delay the absorption of Beta-lactam antibiotics.

It is important to point out that both IDSA and Maltese National guidelines do not mention the use of probiotics and cranberry tablets.

### Lifestyle recommendations

Lifestyle changes that will surely help reduce the severity and incidence of cystitis include:

- avoiding perfumed soaps, tight clothing and other potential irritants, such as deodorant sprays
- maintaining good toilet hygiene - wiping from front to back after a bowel movement helps prevent bacteria in the anal region from spreading to the vagina and urethra
- drinking large quantities of water (about 2L a day) so as to flush out the bladder and reduce the acidity of the urine by dilution
- avoiding foods and drinks that contribute to cystitis, examples being alcohol, spices, chocolate, caffeine,

### Key points

- Episodes of cystitis are quite common in females but they are relatively rare in men and children
- A woman is said to have recurrent UTIs if she suffers from three episodes in the past twelve months or two in the previous six months
- Prophylactic antibiotic therapy should be taken at night when the urine flow is low; ideally, it should not be taken for more than six to twelve months
- When possible, urine specimens should be collected before initiating antibiotic therapy so as to avoid interference with laboratory findings

citrus beverages, tomatoes, vitamin C and citrus fruits

- urinating frequently without delay
- not using a spermicide but considering alternative methods of contraception
- taking showers rather than baths
- using tampons for periods
- wearing cotton underwear
- using an oestrogen cream in some postmenopausal women

### Conclusion

Men, children under 16 years of age, pregnant women and individuals suffering from certain conditions that include diabetes, heart and/or renal disease should be immediately referred to a physician if they suffer from symptoms pertaining to cystitis. A specific recommended treatment protocol is not suitable for all countries because the resistance patterns of *Escherichia coli* vary considerably.

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# Insulin therapy in adult type 1 diabetes patients: Multiple Dose Insulin Injection (MDI) or Continuous Subcutaneous Insulin Infusion (CSII)

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## Educational aims

- To give an overview of MDI therapy and introduce associated technologies
- To highlight the various types of insulin pumps available
- To summarise clinical evidence regarding treatment with MDI and CSII in type 1 diabetes patients

## Key words

type 1 diabetes, multiple dose insulin injection, continuous subcutaneous insulin infusion, continuous glucose monitors, sensor-augmented insulin pumps, closed loop insulin delivery systems

## Abstract

**Insulin therapy in type 1 diabetes is recommended as multiple dose injections (MDI) or continuous subcutaneous insulin infusion (CSII) to deliver a basal background insulin dose together with bolus insulin doses prior to meals. Use of automated bolus calculators and / or continuous glucose monitors with MDI have been shown to improve glycaemic control in type 1 diabetes patients. Several models of insulin pumps with various features are available. CSII offers greater flexibility with a reduced number of injections but in view of its complexity, patients who will benefit from this therapy need to be carefully selected.**

## Introduction

In type 1 diabetes, autoimmune destruction of the beta cells of the pancreas results in insulin deficiency and insulin dependence. The ultimate aim of treatment is to provide type 1 diabetes patients with an insulin therapy which mimics the physiological basal and meal-related insulin secretion from the pancreas and maintain normoglycaemia thus preventing the development of complications. The latest clinical guidance on the diagnosis and management of type 1 diabetes in adults issued by the National Institute for Health and Care Excellence (NICE) in August 2015<sup>1</sup> and the Standards of Medical Care in Diabetes 2016 issued by the American Diabetes Association<sup>2</sup> both recommend insulin therapy in the form of multiple dose insulin (MDI) injection of a basal-bolus regimen or continuous subcutaneous insulin infusion (CSII) for adult type 1 diabetes patients.

## Multiple Dose Insulin (MDI) Injection

MDI regime includes a long acting insulin injected once or twice daily (the basal insulin), together with a rapid acting insulin injected at meal times (the bolus insulin) translating to at least 3 to 4 insulin injections per day. To achieve optimal glycaemic control with this regimen, the rapid acting insulin dose should be calculated according to the carbohydrate content of meals, the glucose level before the meal and the expected level of physical activity.<sup>2</sup> This highlights the importance of educating the patient who is being considered for MDI on the various aspects of diabetes self-management including carbohydrate counting, frequent blood glucose monitoring, pre-meal and post-meal glucose targets, and insulin dose adjustments in relation to exercise and general health status. Education minimises the episodes of hypoglycaemia that are more likely to occur with intensive insulin regimes.<sup>3</sup> Use of insulin analogues, rather than human sequence insulin, for the MDI regimen has been associated with decreased rates of hypoglycaemia. Type 1 diabetes patients, who reported at least 2 episodes of severe hypoglycaemia in the previous 12 months, had a reduced rate of severe hypoglycaemic episodes by 0.51 episodes (95% CI 0.19-0.84) per patient-year or 29% (95% CI 11-48; p=0.010) when treated with insulin analogues for one year as compared to treatment with human sequence insulin for one year.<sup>4</sup>



With the availability of automated bolus calculators, the dose of insulin that is required prior to meals is recommended to the patient and generated using patient-specific data combined with meal-specific data. The patient specific data is pre-set and includes carbohydrate to insulin ratios, insulin sensitivity factors and blood glucose targets. On the other hand, meal-specific data includes carbohydrate intake and pre-meal glucose levels. Automated bolus calculators facilitate the complex and laborious task of calculating bolus insulin doses prior to each meal.<sup>5</sup> In the multicentre randomised controlled trial ABACUS, the use of a bolus advisor in patients with diabetes on MDI therapy was associated with a significantly greater percentage of patients who achieved >0.5% reduction in HbA1c (56.0 vs 34.4%;  $P < 0.01$ ) without an increase in severe hypoglycaemia and significantly greater treatment satisfaction when compared to patients with diabetes on MDI therapy who manually calculated the bolus insulin dose.<sup>6</sup> Type 1 diabetes patients on MDI therapy who used an automated bolus calculator had decreased fear of hypoglycaemia and increased confidence in bolus dose accuracy in a separate study.<sup>7</sup>

Another tool that has been shown to be beneficial in self-management of type 1 diabetes is real time continuous glucose monitors (CGM). These display the current interstitial glucose level from a subcutaneous glucose sensor, provide glucose trends based on changes in previous glucose readings and alarm at pre-set glucose values and following rapid changes in glucose readings. These devices still require sensor calibration with self-monitoring of blood glucose (SMBG) at least 12 hourly. In a randomised controlled trial comparing CGM to SMBG in patients with type 1 diabetes with poor metabolic control treated with MDI or CSII, the CGM group showed improved glycaemic control with 50% of patients achieving a 1% minimum reduction in HbA1c and 25% of patients achieving a 2% minimum reduction

in HbA1c.<sup>8</sup> The Juvenile Diabetes Research Foundation (JDRF) CGM randomised trial, also registered improvement in glycaemic control with CGM use, without an increase in severe hypoglycaemia, when compared to SMBG (mean difference in change in HbA1c  $-0.53\%$ ; 95% CI,  $-0.71$  to  $-0.35$ ,  $P < 0.001$ ) in type 1 diabetes patients >25 years of age on MDI or CSII with a baseline HbA1c of 7-10%.<sup>9</sup> The greatest reductions in HbA1c associated with CGM use were documented in patients who had the highest HbA1c and in frequent users of CGM.<sup>10</sup>

### Continuous subcutaneous insulin infusion (CSII)

Delivery of CSII requires an external pump device which includes an insulin pump, an insulin storage reservoir and an insulin infusion set consisting of a tubing set and a cannula for subcutaneous insertion. The pump delivers a basal rate of insulin, which can be programmed to vary according to different basal insulin requirement throughout the day and night, with bolus doses at meal times triggered manually by the patient. The delivery of the bolus can be of various types and duration including normal (standard), extended (square wave) and dual wave, depending on the macronutrient composition of the meal. There are several models of insulin pumps available with various features, alerts and alarms. Bolus calculators can be integrated in insulin pumps with the insulin on board (IOB) calculation available in most insulin pump models. The IOB is the calculation of how much insulin is still active from previous bolus doses.

Sensor-augmented insulin pumps (SAP), which incorporate continuous subcutaneous glucose monitors, have been shown to improve glycaemic control with frequent CGM use, but whether the associated risk of hypoglycaemia is increased or not is unclear. In a randomised treat-to-target study of patients with type 1 diabetes, use of a SAP for 6 months was associated with a

significant reduction in HbA1c ( $P = 0.0456$ ) in patients who utilised the CGM sensors for >60% of the time, and an increased number of severe hypoglycaemic events (11 events in the SAP group vs 4 events in the CSII and SMBG group) ( $P = 0.04$ ), when compared to insulin pump therapy with SMBG. The probability of a 0.5% reduction in HbA1c was increased by 41% for each 10% increase in CGM sensor compliance.<sup>11</sup> Significant improvement in HbA1c in patients using SAP who utilised CGM for  $\geq 70\%$  of the time ( $P = 0.004$ ) (SAP group  $-0.96 \pm 0.93\%$ ,  $P < 0.001$ ; CSII and SBGM group  $-0.55 \pm 0.93\%$ ,  $P < 0.001$ ) was confirmed in the RealTrend Study which studied patients during their initial 6 months of insulin pump therapy with SAP compared to CSII and SBGM. No associated increase in hypoglycaemic events was reported in this study.<sup>12</sup>

SAPs with the added function of low-glucose suspension have been associated with improved rates of hypoglycaemia. These devices stop insulin delivery automatically, for up to 2 hours, once the glucose level falls below a certain threshold. The rate of moderate and severe hypoglycaemia was found to be reduced in type 1 diabetes patients on SAP with low glucose suspension compared to SAP only, with an adjusted incidence rate per 100 patient-month of 34.2 (95% CI, 22.0-53.3) for patients using SAP only and 9.5 (95% CI, 5.2-17.4) for patients using SAP with low-glucose suspension.<sup>13</sup> In the ASPIRE In-Home study, the rate and severity of nocturnal hypoglycaemia was also documented to be decreased in type 1 diabetes patients using SAP with low-glucose suspension who had improving or low HbA1c at baseline, compared with SAP alone.<sup>14</sup>

Closed Loop (CL) insulin delivery systems or 'Artificial Pancreas' comprise a CSII, a CGM and complex algorithms to control glucose and ensure safety, incorporated in one device or separately, fully automated or combining user input with automated periods of insulin administration.<sup>15</sup> During periods of automated insulin administration, the rate of insulin delivery is determined by real-time interstitial glucose levels which feed into a control algorithm.<sup>16</sup> Use of CL insulin delivery system day-and-night for 12 weeks, in 33 adult type 1 diabetes patients resulted in a lower mean glucose level (difference,  $-0.6\text{mmol/L}$ ; 95% CI,  $-17$  to  $-6$ ;  $P < 0.001$ ), lower mean HbA1c (difference,  $-0.3\%$ ; 95% CI,  $-0.5$  to  $-0.1$ ;  $P = 0.002$ ), less time spent with glucose  $< 3.5\text{mmol/L}$  (39% lower; 95% CI, 24 to 51;  $P < 0.001$ ) and more time spent

in target glucose range (95% confidence interval [CI], 8.1 to 13.8) ( $P < 0.001$ ) when compared to SAP therapy.<sup>17</sup>

While CSII offers the advantages of greater flexibility with variable rates of basal insulin delivery and frequent boluses of various type and duration with fewer injections, it is also associated with a number of possible complications including cannula site reactions and infections, insulin infusion blockage and pump malfunction.

With the complexity of intensive insulin therapy by CSII and with the possibility of associated complications which might result in severe adverse events like diabetes ketoacidosis, CSII therapy requires the selection of highly motivated patients who will benefit from this mode of insulin delivery safely and effectively. CSII therapy should be initiated in the setting of a highly specialised multidisciplinary diabetes management team whose members are specifically trained in insulin pump therapy to provide selected patients with the required support, education and training on the safe use of insulin pumps.

### Multiple Dose Injection (MDI) vs Continuous subcutaneous insulin infusion (CSII)

Several systematic reviews and meta-analyses have been carried out to compare insulin treatment with MDI or CSII in patients with type 1 diabetes. A Cochrane systematic review which included 23 studies found a significant difference in HbA1c (weighted mean difference -0.3% (95% confidence interval -0.1 to -0.4), reduced severe hypoglycaemia and better quality of life in

### Key points

- Insulin therapy in type 1 diabetes is recommended as multiple dose insulin (MDI) injections or as continuous subcutaneous insulin infusion (CSII)
- MDI consists of a once or twice daily basal long acting insulin dose and a bolus rapid acting insulin dose prior to meals, calculated according to the carbohydrate content of meals, the glucose level before the meal and the expected level of physical activity
- Automated bolus calculators and / or continuous glucose monitors have been shown to improve glycaemic control in type 1 diabetes patients on MDI
- Treatment with CSII has been associated with improved glycaemic control and reduced rates of severe hypoglycaemia when compared to MDI in observational studies
- CSII should only be initiated in the setting of a highly specialised multidisciplinary diabetes management team whose members are specifically trained in insulin pump therapy
- CSII therapy requires the selection of highly motivated patients who will benefit from this mode of insulin delivery safely and effectively

patients using CSII compared to MDI.<sup>18</sup> In a previous systematic review and meta-analysis, Fatourechci showed that adult patients with type 1 diabetes on CSII had slightly reduced HbA1c (random-effects weighted mean difference, -0.2%; 95% confidence interval (CI), -0.3, -0.1) with no difference in the risk of hypoglycaemia when compared to patients on MDI.<sup>19</sup> In a meta-analysis by Pickup et al, the rate of severe hypoglycaemia was less in patients on CSII with the greatest reduction noted in patients with the highest initial rates of severe hypoglycaemia on MDI and in patients with long diabetes duration. The greatest reduction in HbA1c was observed in patients who had highest HbA1c levels on MDI.<sup>20</sup> Adult type 1 diabetes patients on CSII were found to have a greater reduction in HbA1c without increased hypoglycaemia rates and

with a reduced total daily insulin dose when compared to patients on MDI, in a systematic review and meta-analysis by Jeitler *et al.*<sup>21</sup> These meta-analyses are limited since older studies using old insulin pump technology, studies of short duration, small studies and studies comparing MDI therapy with human sequence insulin to insulin analogues in CSII were often included in the analysis.

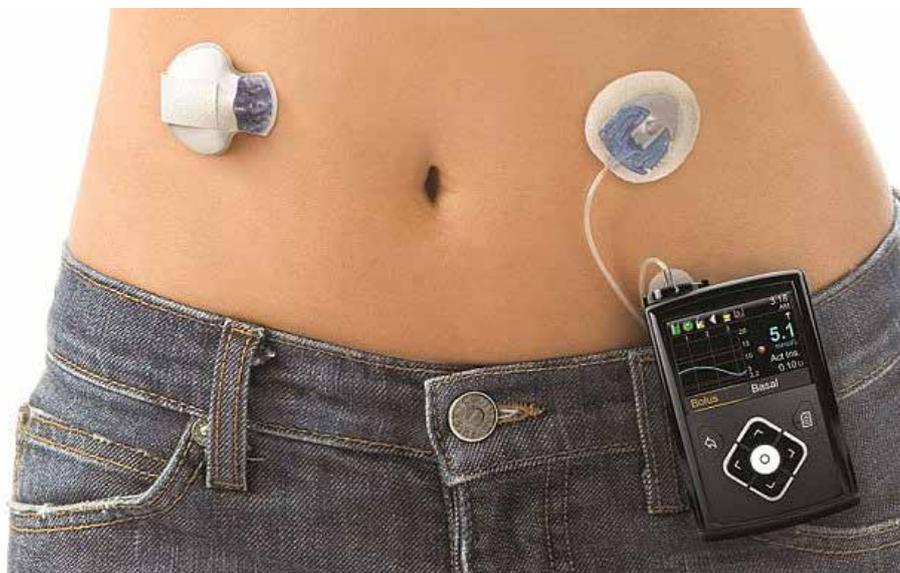
Evidence from observational studies points towards reduced HbA1c levels and statistically significant reduced rates of severe hypoglycaemia with CSII when compared to MDI.<sup>22</sup>

The ongoing Relative Effectiveness of Pumps Over MDI and Structured Education (REPOSE) trial, a randomised controlled trial of 280 adult type 1 diabetes patients, recruited from 7 UK centres, assigned to either analogue MDI or analogue CSII following standard structured training in insulin adjustment and followed up for 2 years,<sup>23</sup> is expected to address several limitations of previous randomised controlled trials of MDI vs CSII, highlighting risks and benefits of both therapies.

Current guidelines for CSII therapy in adult type 1 diabetes patients recommend treatment with CSII in patients who have persistently high HbA1c despite intensive therapy with MDI and in patients with disabling hypoglycaemia.<sup>24,25</sup> The American AACE/ACE recommendations for CSII therapy are broader but also specify characteristics of patients who would not benefit from pump therapy.<sup>25</sup>

### Conclusion

Type 1 diabetes is a chronic disease that requires a lot of input from the patient



together with regular review and support by the specialised diabetes teams who provide intensive education and training and recommend the most suitable insulin type, insulin delivery and accurate, reliable and safe technologies that best serve each individual patient based on clinical evidence and tailored to the patient's lifestyle, commitment, motivation, skills and expectations while striving to reduce disease burden and preserve quality of life.

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# Pregnancy and asthma

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## Educational aims

- To provide information on the management of asthma during pregnancy
- To emphasize that benefits of asthma medication outweigh the risks
- To highlight the importance of a multidisciplinary approach in the care of a pregnant asthmatic

## Key words

asthma, pregnancy, inhaled corticosteroids,  $\beta_2$ -agonists, exacerbation, complications

## Abstract

**Asthma is the most common chronic condition encountered in pregnancy. Due to lack of evidence on the adverse effects of asthma medication during pregnancy, there is persistent concern regarding use of asthma-related medication throughout the pregnancy, management of exacerbations and asthma management during labour. Several respiratory-related physiological changes are said to occur during pregnancy which could possibly worsen or improve asthma, and asthma as well as its treatment can affect pregnancy outcomes. The aim of this paper is to summarize current literature findings on clinical management issues encountered in this cohort of patients.**

## Introduction

Asthma is a chronic inflammatory disease of the airways that is characterized by increased airway responsiveness to multiple stimuli. It is considered to be the most common chronic condition encountered in pregnancy.<sup>1</sup> The prevalence of asthma during pregnancy in Europe has been estimated to be between 4% and 8%.<sup>2</sup> Asthma-related morbidity and mortality rates during pregnancy are comparable to those in the general population. Mortality from asthma in the United States is calculated to be 2.1 persons per 100,000 population.<sup>1</sup> No local or European data has been identified in the literature regarding mortality. Many physiological and anatomical changes of pregnancy affect the respiratory system. These changes often affect the presentation and management of the various respiratory illnesses in pregnancy, including asthma,<sup>3</sup> and asthma and its treatment can affect pregnancy outcomes.<sup>4</sup> Pregnancy can have a variable impact on asthma, and there is no general rule to predict in whom it is going to be better, stable or worse.<sup>5</sup> The National Asthma Education and Prevention Program (NAEPP) "Working Group Report on Managing Asthma During Pregnancy: Recommendations for Pharmacologic Treatment—Update 2004"<sup>4</sup> emphasizes that maintaining adequate control of asthma during pregnancy is important for the health and well-being of both the mother and her baby.<sup>6</sup>

## Differential Diagnosis

A number of conditions that can mimic symptoms of asthma in pregnant patients include the following:

- **Amniotic fluid embolism<sup>1</sup>**  
Amniotic fluid embolism is a rare event which is precipitated when amniotic fluid is able to breach the physical barrier between the maternal and foetal environments as a result of uterine trauma. It can take a variety of clinical presentations but a classical presenting sign is dyspnoea, which in the acute situation can mimic an acute exacerbation of asthma, particularly in patients who are known to suffer from asthma.<sup>7</sup>
- **Acute congestive heart failure, secondary to peripartum cardiomyopathy<sup>1</sup>**  
Peripartum cardiomyopathy is an uncommon disorder associated with pregnancy in which the heart dilates and weakens, leading to symptoms of

heart failure. This condition may be difficult to diagnose because symptoms of heart failure can mimic those of pregnancy as well as other respiratory conditions such as asthma.<sup>8</sup>

- **Physiologic dyspnoea of pregnancy<sup>1</sup>**  
Dyspnoea is commonly reported by 70% of healthy pregnant women during their daily living activities, starting from the very first trimester of gestation. Possible explanations could be an increased awareness of the new sensation of the physiological hyperventilation associated with pregnancy or an increased central perception of respiratory discomfort or a combination of these two. This should be a diagnosis of exclusion after pulmonary and cardiac causes for the dyspnoea have been excluded.<sup>9</sup>

### Pathophysiology

Asthma control often changes during pregnancy; in approximately one-third of women asthma symptoms worsen, in one-third they improve, and in the remaining one-third they remain unchanged.<sup>10</sup>

Pregnancy has a significant effect on the respiratory physiology of a woman. While the respiratory rate and vital capacity does not change in pregnancy, tidal volume, minute ventilation (40%), and minute oxygen uptake (20%) increase, with a resultant decrease in functional residual capacity and residual volume of air as a consequence of the elevated diaphragm. In addition, airway conductance is increased and total pulmonary resistance is reduced, possibly as a result of the influence of progesterone. The consequence of these physiologic changes is a hyperventilatory picture as a normal state of affairs in the later half of pregnancy. This results in the picture of a chronic respiratory alkalosis during pregnancy, with a decreased partial pressure of carbon dioxide ( $pCO_2$ ), decreased bicarbonate, and increased pH. A normal  $pCO_2$  in a pregnant patient may signal impending respiratory failure. The increased minute ventilation and improved pulmonary function in pregnancy promote more efficient gas exchange from the maternal lungs to the blood. Therefore, changes in respiratory status occur more rapidly in pregnant patients than in non-pregnant patients.<sup>1</sup>

### Non-pharmacological management Non-pregnant asthmatics

Asthmatic patients should be strongly advised to quit smoking and smoking

cessation advice given as necessary. Weight loss in overweight patients has many health benefits, and should be supported in people with asthma since, if successful, it may lead to improvements in asthma symptoms. Allergen avoidance and elimination of exposure to trigger factors is of optimal importance. The practitioner must also ensure that the patient is adherent with existing therapies. Inhaler technique should also be checked and explained as necessary. The use of a spacer should be strongly encouraged.

### Pregnant asthmatics

Non-pharmacological management is very similar to non-pregnant patients. Pharmacologic as well as cognitive interventions should be offered to pregnant women to assist in smoking cessation, in view of the known risks associated with cigarette smoking.<sup>3</sup> Avoidance of exposure to allergens as well as any situation which could potentially trigger asthma symptoms should be avoided. The importance of a good inhaler technique and spacer use should be emphasized.

### Pharmacological management

#### Non-pregnant asthmatics

Before initiating asthma medication, practitioners should address non-pharmacological measures. A short acting  $\beta_2$ -agonists (SABA) is recommended for symptom relief, on an as needed basis, in the mildest cases of asthma. Inhaled corticosteroids (ICS) are the recommended preventer drug for adults for achieving overall treatment goals. If asthma symptoms remain uncontrolled, add-on therapy to ICS in adults is an inhaled long-acting  $\beta_2$ -agonist (LABA), which should be considered before going above a dose of 400 micrograms beclomethasone or equivalent per day and certainly before going above 800 micrograms. If asthma control remains suboptimal after the addition of LABA, then the dose of ICS should be increased to 800 micrograms/day, if not already on these doses. A leukotriene receptor antagonist should also be considered at any stage after initiation of an ICS. Theophyllines might also be considered at times.<sup>4</sup>

### Pregnant asthmatics

There is much concern amongst pregnant asthmatic patients regarding the use of their asthma medications. In fact lack of adherence to therapy is a frequent

occurrence, because such patients are worried about medication effects on the fetus.<sup>1</sup> Current available literature shows that the management of asthma remains largely unchanged compared to the non-pregnant state.<sup>3</sup> Differences were found in the prevalence of prescribing of asthma medications during and surrounding pregnancy in Europe from data recorded in seven European population-based databases. Inhaled  $\beta_2$ -agonists and ICS were, however, the most popular therapeutic regimens in all databases.<sup>2</sup>

According to the BTS guidelines, the following drugs should be used as normal during pregnancy: SABA, LABA, ICS, oral and intravenous theophyllines. They also recommend that systemic corticosteroids should also be used as normal when indicated during pregnancy for severe asthma. If leukotriene receptor antagonists are required to achieve adequate control of asthma then they should not be withheld during pregnancy.<sup>4</sup> ICS prevent exacerbations of asthma during pregnancy, and their cessation during pregnancy is a significant risk factor for exacerbations.<sup>10</sup> ICS are the preferred treatment for long-term control medication. Budesonide is the preferred ICS because more data is available on using budesonide in pregnant women than is available on other ICS, and the data is reassuring. However, no data indicates that the other ICS preparations are unsafe during pregnancy. Garne et al also concluded that the use of ICS during the first trimester of pregnancy seems to be safe in relation to the risk for a range of specific major congenital anomalies.<sup>11</sup> It has also been recommended that a low priority should be placed on stepping down treatment (however guided) until after delivery.<sup>10</sup> There is minimal published data on the use of leukotriene receptor antagonists during pregnancy; however, animal safety data submitted to the Food and Drug Administration (FDA) is reassuring.<sup>6</sup> Treatment to control symptoms and hence minimize adverse outcomes from exacerbations using recommended asthma medications is considered of utmost importance.

### Acute exacerbations

During pregnancy, exacerbations of asthma which require medical intervention occur in about 20% of women, with approximately 6% of women requiring hospitalization. Severe asthma appears to be the biggest risk factor for exacerbations during pregnancy

**Table 1: Adverse perinatal outcomes that have been associated with uncontrolled asthma during pregnancy**

**Adverse perinatal outcomes**

Pre-eclampsia
Pregnancy-induced hypertension
Uterine haemorrhage
Preterm labour
Premature birth
Congenital anomalies
Intra-uterine growth restriction
Low birth weight
Breech presentation
Neonatal hypoglycemia
Neonatal seizures

although some have noted that respiratory viral infections are also a common cause. Pregnant women appear also to be particularly susceptible to the effects of viral respiratory infections, including influenza.<sup>10</sup> This could be explained by the changes in cell-mediated immunity during pregnancy which may lead to exacerbations of asthma.<sup>12</sup> Non-adherence to asthma treatment could also be an important cause. It is safer for pregnant women who have asthma to be treated with asthma medications than to have asthma symptoms and exacerbations. Monitoring and making appropriate adjustments in therapy may be required to maintain lung function and, hence, blood oxygenation that ensures oxygen supply to the fetus.<sup>6</sup> Exacerbations are common in pregnancy, particularly in the second trimester. Exacerbations and poor asthma control during pregnancy may be due to mechanical or hormonal changes, or to cessation or reduction of asthma medications due to concerns by the mother. Exacerbations and poor symptom control are associated with worse outcomes, as described below, for both the baby and the mother.<sup>10</sup>

The British Thoracic Society (BTS) guidelines recommend that during an exacerbation, pregnant mothers should be administered drug therapy as for non-pregnant patients.<sup>4</sup> Acute severe asthma in pregnancy is an emergency situation and should be treated aggressively with nebulized SABA, oxygen and early administration of systemic corticosteroids.<sup>10</sup>

Asthma exacerbation during pregnancy is not associated with adverse pregnancy outcomes while managed appropriately with systemic corticosteroids.<sup>13</sup> Magnesium sulphate can also be administered if necessary. Delivery of high flow oxygen immediately to maintain oxygen saturations of 94 to 98% is recommended together with continuous foetal monitoring,<sup>4</sup> so as to avoid foetal hypoxia. Respiratory infections should be monitored and managed appropriately during pregnancy.<sup>10</sup> Infectious respiratory illness, including pneumonia and tuberculosis, are similarly managed in pregnancy with antibiotics, although special attention may be needed for antibiotic choices with more pregnancy safety data.<sup>3</sup>

When mechanical ventilation is necessary, consideration should be given to the maternal haemodynamics of pregnancy and foetal oxygenation. Maintaining maternal oxygen saturation above 95% is recommended to sustain optimal foetal oxygenation.<sup>3</sup>

It is also recommended that there should be close liaison between the respiratory physician and obstetrician, with early referral to critical care physicians for women with acute severe asthma.<sup>4</sup> Prevention of exacerbations is essential to reduce the risk of complications and poor outcome.<sup>14</sup>

**Management during labour**

During labour and delivery, usual controller medications should be taken, with a SABA, such as salbutamol, if needed.<sup>10</sup> Acute exacerbations during labour and delivery are uncommon,<sup>4</sup> but bronchoconstriction may be induced by hyperventilation during labour, and should be managed with SABA. Neonatal hypoglycemia may be seen, especially in preterm babies, when high doses of beta<sub>2</sub>-agonists have been given within the last 48 hours prior to delivery. If high doses of SABA have been given during labour and delivery, blood glucose levels should be monitored in the baby (especially if preterm) for the first 24 hours.<sup>10</sup>

If anaesthesia is required, regional blockade is preferable to general anaesthesia. In the absence of an acute severe asthma attack, caesarean section should be reserved for the usual obstetric indications. Prostaglandin F<sub>2α</sub> should be used with caution because of the risk of inducing bronchoconstriction.<sup>4</sup>

Women receiving steroid tablets at a dose exceeding prednisolone 7.5 mg per day for more than 2 weeks prior to delivery should receive parenteral hydrocortisone 100 mg 6–8 hourly during labour.<sup>4</sup>

**Monitoring**

Pregnancy profoundly affects asthma-related health care use.<sup>13</sup> It is recommended that pregnant asthmatics should be monitored closely, particularly those patients with moderate or severe asthma to keep their asthma well controlled.<sup>4</sup> Monthly evaluations of asthma history and pulmonary function (spirometry is preferred, but measurement with a peak flow meter is generally sufficient) are recommended.<sup>6</sup> Regular evaluation will allow the opportunity to step-up treatment as necessary.

**Outcomes and complications**

Although women with mild asthma are unlikely to have problems, patients with severe asthma are at greater risk of deterioration. The deterioration risk is highest in the last portion of a pregnancy. In fact, severe and/or poorly controlled asthma has been associated with numerous adverse perinatal outcomes as per Table 1.<sup>1,5,6,14,15</sup>

This risk of giving birth to a small or preterm infant appears to be small and may be minimized by good control of asthma. Studies have indicated that low-birth-weight infants are more common in women with daily symptoms or low expiratory flow than in women without asthma.<sup>1</sup>

**Table 2: Adverse maternal outcomes have been associated with uncontrolled asthma during pregnancy**

**Adverse outcomes in pregnant asthmatics**

Respiratory failure +/- mechanical ventilation
Barotrauma
Complications of systemic corticosteroid use
Mortality

**Table 3: Recommended interventions by health care professionals to pregnant asthmatics**

Dispel any fears/misconceptions about use of asthma medication
Emphasize the importance of adherence to therapy
Highlight the risk of non-adherence to therapy for mother and foetus
Arrange regular follow-ups to identify symptoms of uncontrolled asthma
Monitor inhaler technique
Emphasize the importance of using a spacer
Advise smoking cessation as necessary
Offer smoking cessation techniques as necessary
Provide the patient with an asthma self-management plan

Asthma can also lead to complications in pregnant women as per table 2.<sup>1</sup>

According to Blais *et al*, only severe asthma exacerbations were found to significantly increase the risk of congenital malformations in this representative study.<sup>16</sup> However, it is uncertain whether this risk is associated with the exacerbation itself or the treatment given.<sup>2</sup> Being able to identify complications associated with asthma during pregnancy is of great importance in providing appropriate asthma management and medical care to these pregnant women, which may have lifelong consequences for their offspring.<sup>15</sup> Hence the importance of advising adherence with asthma medications, with the aim of achieving the best asthma control possible.

### Breastfeeding

Asthmatic women should be encouraged to breastfeed. Asthma medications should be taken as normal during lactation.<sup>4</sup>

### Key points

- The management of asthma during pregnancy remains largely unchanged compared to a non-pregnant patient
- For both the mother and baby, the advantages of achieving asthma control and avoiding exacerbations appear to outweigh the potential risks related to asthma medication
- Stepping down of treatment should be reserved for the post-partum period.
- In the case of an asthma exacerbation during pregnancy, this should be treated aggressively
- Pregnant women should be advised that an acute asthma attack is rare in labour but if this occurs, they should continue their usual asthma medications throughout labour

### General advice

- Women should be advised about the importance of maintaining good control of their asthma during pregnancy to avoid problems for both mother and baby.
- Pregnant asthmatics should be advised that poorly controlled asthma, and exacerbations, provide a much greater risk to their baby than do asthma medication.
- Smokers should be advised about the dangers for themselves and their babies and should be given appropriate smoking cessation advice.
- Table 3 highlights a list of recommended interventions by health care professionals to pregnant asthmatics.

### Conclusion

The management of asthma during pregnancy remains mainly unchanged compared to a non-pregnant patient. The advantages of achieving asthma control and avoiding exacerbations appear to

outweigh the potential risks related to asthma medication. Stepping down of treatment during pregnancy should be reserved for the post-partum period. In case of an exacerbation, this should be treated aggressively.

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# Molecular classification of colorectal cancer

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## Educational aims

- To describe the current molecular markers used in colorectal cancer
- To give an overview of the various colorectal cancer molecular classifications published in the past five years
- To describe the first consortium colorectal cancer taxonomy

## Key words

colorectal cancer, molecular classification, gene expression, biomarkers, colorectal cancer taxonomy

## Abstract

**Colorectal cancer (CRC) is a heterogeneous disease with several clinical, pathological, and molecular presentations. A comprehensive and unifying molecular classification would be useful for genotype-phenotype correlations, to better understand disease progression, and to predict responses to treatment. Such a classification would be helpful for quickly and efficiently translating results from the laboratory to the clinic and closing the gap between research breakthroughs and actually implementing them clinically. In November 2015, an international consortium consisting of six expert groups published the first consensus on molecular subtypes of colorectal cancer, by bringing together six previously published CRC classifications.**

## Introduction

Cancers have traditionally been classified clinically and pathologically based on stage and grade. Stage is closely associated with patient prognosis, generally defined as progression-free survival (PFS) and overall survival (OS). Although prognosis has been shown to be dependent on local tumour involvement, regional lymph node metastasis, lympho-vascular invasion, positive surgical margins, preoperative elevation of CEA (a circulating tumour marker), high tumour grade, and tumour budding, responses to treatment are still difficult to predict in specific scenarios, particularly the metastatic setting.<sup>1</sup>

The Union of International Cancer Control (UICC) and American Joint Committee on Cancer (AJCC) Tumour Node Metastasis (TNM) systems are the most commonly used classifications for colorectal cancer staging. The TNM classification was developed in the 1940s and codes the depth of primary tumour (T) invasion into or beyond the colorectum (invasion of or adherence to adjacent organs or structures), the number of regional lymph nodes involved (N), and the presence or absence of distant metastasis (M).<sup>2</sup> The classification allows grouping of these three indicators and provides a "stage-grouping".<sup>3</sup> The TNM classification is a dual system and comprises a clinical (pre-treatment) classification (usually referred to as cTNM) and a pathological (post-surgical histopathological) classification (pTNM).<sup>4</sup> As a rule, surgical decisions are based on cTNM classification, while post-surgical management and prognosis are established using the pTNM classification.

In 1932, the Dukes' staging system was proposed for rectal tumours that classified tumours pathologically into three different stages: A to C.<sup>5</sup> Dukes established that the extent of local tumour invasion (A – least, C – most) was prognostic and reliably predicted operative mortality. This classification was then adapted to colon cancer and further variations were introduced, namely the introduction of Stage D (distant metastasis) and the Modified Astler-Coller (MAC) classification.<sup>5</sup> Today, the Dukes' staging system and the MAC classification have been superseded by the UICC and AJCC TNM systems and subsequently their use is highly discouraged.

## Molecular markers currently used in colorectal cancer

With further advances in biomedicine, it has become possible to classify tumours based on their molecular characteristics. Three main cancer pathways are implicated in CRC molecular classification: microsatellite instability (MSI), chromosomal instability (CIN), and the CpG island methylation phenotype (CIMP). MSI is characterised by small insertions or deletions in repetitive DNA sequences (microsatellites).<sup>6</sup> When MSI is not present, a tumour is defined as microsatellite stable (MSS). CIN is defined as an elevated frequency of whole-chromosome missegregation, and CIMP occurs when there is methylation of the CpG islands within the promoter region and is associated with transcriptional silencing.<sup>7,8</sup> In 2008, Ogino and Goel proposed a revised molecular classification based on the five subgroups proposed by Jass in 2007 that consisted of six subgroups: Group 1: MSI-H CIMP-High (10%); Group 2: MSI-H CIMP – Low/O (5%); Group 3: MSI-L/MSS CIMP-High (5-10%); Group 4: MSI-L CIMP-Low (5%); Group 5: MSS CIMP-Low (30 to 35%); Group 6: MSI-L/MSS CIMP-O (~40%).<sup>6,9</sup> This molecular classification depends on common DNA markers together with *BRAF* and *KRAS* mutational status.<sup>10</sup>

With the advent of high-throughput molecular technology, we are now generating a vast amount of genomic, transcriptomic, proteomic, and metabolomic data that are facilitating the comprehensive molecular characterisation of cancer. The aim is to identify a robust molecular signature that can be applied in the clinic and help to identify the best treatment and care for the individual. Furthermore, it would be helpful to establish molecular signatures capable of predicting individuals at higher risk of developing the disease who hence must take preventative measures (diagnostic biomarkers). Molecular signatures would also be beneficial in guiding clinicians with respect to predicting recurrence of the disease following surgery (surveillance biomarkers). Other beneficial molecular signatures are prognostic biomarkers, which give an indication of the likely progression of disease, and predictive biomarkers, which predict responses to treatment. This has been best demonstrated in breast cancer, in which the molecular classification has been useful for both diagnosis and treatment.<sup>11-14</sup>

However, the molecular characterisation of colorectal cancer has lagged behind breast cancer in which, in some instances, diagnosis

and treatment are based on the expression of particular molecules, e.g. expression of the estrogen receptor (ER) to indicate anti-estrogen therapy or amplification of the human epidermal growth factor receptor 2 (HER2) for HER2-directed therapy.<sup>15</sup> This progress in breast cancer has in no small part been driven by its molecular classification. Nonetheless, there are still clinical dilemmas in CRC that would benefit from a molecular classification approach: a) who might develop metastases even though they have a favourable pathological stage? b) who needs adjuvant therapy even if there is no nodal involvement? c) who is likely to best respond to specific chemotherapy or targeted therapy? To this end, there have now been a number of efforts to develop a better molecular classification of colorectal cancer. Over the past decade, a number of complex classification studies producing variable results have been published. Although the different cohorts, methods, group sizes, and clinical information have meant that these classification systems have seemed different, in fact there is emerging commonality between them. In this brief review, we highlight six of the most recent classifications that together form the basis of the recently published consensus on molecular classification of CRC developed by the CRC Subtyping Consortium (CRCSC).

### Classification based on mesenchymal or epithelial expression signature

In 2012, Schlicker *et al.* stratified and pharmacologically characterised a panel of 74 different CRC cell lines.<sup>16</sup> Furthermore, using iterative clustering, this analysis revealed five CRC subtypes (1.1, 1.2, 1.3, 2.1, 2.2) that were successfully validated on over 1600 CRC tumour samples from publicly available gene expression datasets.

Type 1 tumours exhibited a mesenchymal expression signature, had a poor prognosis, and similar number of MSI and MSS tumours (out of the annotated cohorts (n = 229), 58 Type 1 tumours exhibited MSI and 59 exhibited MSS). Type 2 tumours exhibited an epithelial expression signature, had a good prognosis, and were enriched with MSS tumours. Further subtyping of Type 1 tumours revealed that subtype 1.1 was strongly mesenchymal, enriched in late-stage CRC, and the up-regulated genes were mainly involved in Ca-signalling and SRF-targeted. Moreover, subtype 1.1 was characterized by pathways involved in angiogenesis, inflammation, and proliferation. Subtype 1.2

contained more female than male patients, were mostly MSI enriched, and activated similar pathways to 1.1 but also strongly activated the JAK-STAT signalling pathway. Furthermore, the main up-regulated genes were immune-system related. Subtype 1.3 exhibited high expression of transporter genes and were mainly MSS.

Additional subtyping of type 2 tumours resulted in two further subtypes – subtype 2.1 and subtype 2.2. In the former, pathways related to inflammation, angiogenesis and proliferation were activated. Moreover, stress response and immune system-related genes were up-regulated. In subtype 2.2, a number of genes that were up-regulated were involved in cell cycle and amino acid synthesis. Some other genes up-regulated in subtype 2.2 were located on a number of cytobands in chromosome 20q and 13q.

The authors also adopted a comprehensive cell line model to investigate the relationship between the CRC subtypes and cell signalling and, additionally, associate the molecular features with drug responses. They showed that CRC cell lines classified as subtype 1.2 were highly sensitive to glycogen synthase kinase, Src, and Wnt signalling inhibitors. On the other hand, CRC cell lines designated as type 2 when compared to those classified as type 1 were considerably more sensitive to aurora kinase inhibitors.

### Classification based on CIN subtype, MSI/CIMP positive subtype and KRAS/BRAF mutation subtype

In 2013, two colorectal classification papers were published in Nature Medicine. In the first article De Sousa E Melo F *et al.* described three molecularly distinct CRC subtypes (CCS1, CCS2 and CCS3), where one of the subtypes (CCS3) was characterized for the first time.<sup>17</sup> An integral part of this study was the analysis of the gene expression data of 1,164 CRC patients using an unsupervised classification strategy. All the CRC samples, xenografts, cell lines, and precursor lesions were classified into the three subtypes using a 146-gene classifier.

Colon Cancer Subtype 1 (CCS1) mainly consisted of chromosomal instable (CIN) cancers – the majority of the samples in this group generally had *KRAS* and/or *TP53* mutations. Moreover, the tumours in this group were principally located on the left side of the colon. From a metastasis point of view, the authors concluded that Wnt target genes were highly expressed in CCS1 and that they metastasize less frequently compared to CCS3 tumours.

Colon Cancer Subtype 2 (CCS2) mainly consisted of MSI/CIMP-positive tumours located on the right side of the colon. The authors focused principally on CCS3 since it was the least well characterized subtype and, furthermore, compared it mainly with CCS1 tumours; hence, there is relatively little information on CCS2.

Colon Cancer Subtype 3 (CCS3) was not enriched with either CIN or MSI tumours but contained a relatively large number of patients with *KRAS* or *BRAF* mutations. The tumours were distributed throughout the colon and tended to be poorly differentiated. Based on the microsatellite stable and CIMP+ status, together with relative over-representation of *BRAF* mutants, the authors hypothesised that these tumours can arise from pre-neoplastic lesions associated with the serrated pathway. This was also confirmed using Principal Component Analysis on the patient set, classifier, and independent cohorts. Furthermore, when comparing CCS1 with CCS3 using Gene Set Enrichment Analysis (GSEA), it appeared that in the CCS3 subtype the up-regulated genes were involved in epithelial to mesenchymal transition, matrix remodelling, cell migration, and transforming growth factor  $\beta$  signalling. The authors concluded that the least characterized subtype (CCS3) is highly malignant when compared to CCS1 and CCS2.

Disease-free survival (DFS) was statistically significantly lower in CCS3 compared to CCS1 and CCS2 tumours. Furthermore, over half of the CCS3 patients had a recurrence within two years and, overall, the patients in this subgroup had a poorer DFS. In addition to the proposed classification, the authors examined the responses of the different subtypes to targeted therapy. The authors inferred that CCS3 metastatic colorectal cancer patients were resistant to cetuximab, independent of the *KRAS* mutational status. This difference in response was also observed *in vitro* when comparing CCS1 cell lines versus CCS3 cell lines.<sup>17</sup>

### Classification based on intestinal stem cells and their respective differentiated cells

In the second paper, Sadanandam *et al* described six CRC subtypes (stem-like, inflammatory, transit-amplifying cetuximab resistant, transit-amplifying cetuximab sensitive, goblet-like, enterocyte) together with their response to cetuximab, standard of care chemotherapy, and DFS.<sup>18</sup> The CRC

subtypes were generated using consensus-based unsupervised clustering of the gene expression profiles of 1,290 CRC samples.

The least differentiated stem-like subtype phenotype was associated with the base of the colon crypt and had a poor DFS. Patients with this subtype had higher expression of Wnt signalling, stem cell, myoepithelial, and mesenchymal genes while having lower expression of differentiation markers. This group was predicted to benefit from chemotherapy (preferably FOLFIRI – a chemotherapy regimen containing leucovorin calcium, 5-fluorouracil and irinotecan) – both as an adjuvant treatment and also in the metastatic setting.

The second subtype was referred to as inflammatory and was not associated with crypt-top or base phenotypes. The main upregulated genes in this group were interferon-related and chemokines. With regards to treatment, it was suggested that patients in the adjuvant setting should ideally be treated with chemotherapy, more specifically with FOLFIRI. On the other hand, in the metastatic setting, both chemotherapy and cetuximab could be ineffective. This subtype also had an intermediate DFS.

The goblet-like subtype was equated with the top part of the colon crypt and had a good DFS. This subtype exhibited high mRNA expression of goblet-specific MUC2 and TFF3. The patients in this subtype would possibly be unresponsive to treatment in the adjuvant setting and hence it was suggested that watchful surveillance should instead be implemented following surgical resection. On the other hand, the authors advised that, in the metastatic stage, chemotherapy (preferably FOLFIRI) or another therapy (that still to be determined) should be administered.

The enterocyte-like sub-type was characterized by high expression of enterocyte-specific genes. Patients assigned to this group had an intermediate DFS. Based on the gene signature of the enterocyte-like sub-type, it was determined that these tumours identify with the colon-crypt top phenotype. Furthermore, when compared to the stem or progenitor cell phenotype, the enterocyte-like sub-type had a more differentiated phenotype. As a result of these association studies, the authors recommended that patients receiving adjuvant treatment in this subgroup should be treated with chemotherapy (preferably FOLFIRI) or other therapy (excluding cetuximab or c-MET therapy).

On the other hand, for metastatic disease, the authors did not recommend treatment with chemotherapy, cetuximab, or a c-MET inhibitor.

The final sub-type described was the transit-amplifying (TA) sub-type which, to a certain extent, was considered a heterogeneous group since both the stem cells and Wnt target genes were irregularly expressed. Furthermore, this subtype also exhibited a mixed phenotype, since 59% of these tumours had a crypt top signature with low expression of Wnt signalling targets. On the other hand, the remainder of the TA subtype were significantly associated with crypt base and over-expressed stem and progenitor markers such as *LGR5* and *ASCL2*. In this subtype, the authors recommended that both adjuvant chemotherapy and chemoradiotherapy should be avoided, since they identified a trend that patients in this subtype had a lower DFS when treated. Following a series of proliferation assays to monitor drug responses, this subtype was further divided into another two sub-subtypes – cetuximab-sensitive transit-amplifying (CS-TA) and cetuximab-resistant transit-amplifying (CR-TA). CS-TA exhibited statistically significant higher expression of *EREG* and *AREG* compared to CR-TA. In contrast, CR-TA demonstrated higher expression of filamin A, which is involved in c-MET regulation. In effect, this subtype (CR-TA) was sensitive to *in vitro* treatment with a c-MET inhibitor. The authors hence concluded that, in the metastatic setting, CS-TA patients should be treated with cetuximab while CR-TA should be treated with a c-MET inhibitor.

### Classification based on clinicopathological variables and commonly used DNA markers

In a third paper in 2013, Marisa *et al.* published in PLoS Medicine a gene expression classification of six molecular subtypes based on clinicopathological variables and commonly used DNA markers.<sup>10</sup> The relevance of this classification is that the subtypes were associated with different prognoses. The study was performed using a discovery set of 443 patients and a validation set of 1,029 patients. In this classification, the subtypes were named in accordance with their biological characteristics as described below.

The first subtype C1, termed CIN<sub>Immune-Down</sub>, shared a lot of similarities with C5<sub>CINWntUp</sub>. Both subtypes fell under the

conventional precursor neoplasia pathway, with very low frequencies of mismatch repair deficient genes (dMMR) and CIMP in contrast to very high frequencies of CIN. Furthermore, in both subtypes, the majority were located in the distal colon. With respect to mutational status, both C1-CIN<sub>Immune-Down</sub> and C5<sub>CINWntUp</sub> exhibited intermediate *KRAS* and *TP53* mutation frequency but very low *BRAF* mutation frequency. When comparing resemblance to supervised gene expression signatures, both subtypes had minimal characteristics with *BRAF* mutant-like supervised signatures and exhibited intermediate likeness to a “normal-like” supervised signature. One of the main differences between the two subtypes was that C5<sub>CINWntUp</sub> demonstrated an intermediate frequency of tumours with stem-cell phenotype-like gene expression profiles, while C1-CIN<sub>Immune-Down</sub> exhibited a very low frequency. The other main difference between these two subtypes was in respect of deregulated signalling pathways. In C1-CIN<sub>Immune-Down</sub>, most signalling pathways were downregulated, especially cell communication and immune pathways. On the other hand, in C5<sub>CINWntUp</sub>, cell communication, Wnt and metabolism pathways were upregulated.

The other four subtypes, namely, C2 dMMR, C3 *KRAS*m, C4 CSC and C6 CIN<sub>normL</sub> were linked to the serrated precursor neoplasia pathway. The C2 dMMR subtype consisted mainly of dMMR (68%) and *BRAF* mutant tumours (40%) and were very frequently located in the proximal colon. Furthermore, a very high frequency of CIMP (59%) was recorded in this subtype. *KRAS* mutant tumours were also found at an intermediate frequency. With respect to supervised gene expression, the majority of the C2 dMMR exhibited a *BRAF*m-like signature and a serrated CC-like signature. Moreover, the immune system and cell growth pathways were found to be up-regulated in this subtype, whilst the Wnt pathway was found to be deregulated.

The C3 *KRAS*m subtype was also frequently located in the proximal colon and was mainly enriched for *KRAS* mutant tumours (87%). This subtype displayed an intermediate frequency of CIN+, and 18% of the tumour were CIMP+. Moreover, most signalling pathways in C3 *KRAS*m tumours were down-regulated.

The metastasis-enriched (31%) C4-CSC subtype was the only subtype with a reproducible association between poor

prognosis and the supervised stem-cell gene expression signature. This subtype was frequently located in the proximal colon, had intermediate frequency of CIN+, and 34% of the patients were CIMP+. Intermediate frequencies for *KRAS* and *TP53* mutations were reported in this subtype. The majority of the tumours in this subtype (91%) exhibited the stem cell phenotype-like gene expression signature. Besides the stem cell signature, this subtype also displayed the *BRAF*-mutant like gene expression profile and the serrated CC-like signature. The EMT/motility pathways were up-regulated, whilst the cell growth and death pathways were down-regulated.

The final subtype described in this study was C6 CIN<sub>normL</sub>. Although classified under the serrated precursor neoplasia category, this subtype was mainly CIN+ (86%), CIMP-, *TP53* mutant, and located in the distal colon. In contrast to the other CIN+ subtypes, this subtype exhibited very high frequencies of the normal-like gene expression signature and intermediate frequencies of the serrated CC-like gene expression profile. Moreover, C6 CIN<sub>normL</sub> showed down-regulation of the proliferation pathways and upregulation of the EMT/motility pathways.

Finally, the authors demonstrated that there was a statistically significant association between subtypes C4-CSC and C6 CIN<sub>normL</sub> and prognosis. In fact, Stage II/III patients with one of these two subtypes had a worse prognosis, with 5-year relapse-free survival rates of 52% and 61%, respectively.

#### **Classification based on biological motifs, morphology, common clinical variables, and molecular markers**

In July, 2013 Budinska and colleagues described an additional five subgroup classification using a discovery set of 1,113 CRC gene expression profiles and a validation set of 720 CRC transcriptomic profiles.<sup>19</sup> The authors used unsupervised clustering of genome-wide transcriptome analysis and moreover, described each subtype with respect to biological motifs, morphology, common clinical variables, and molecular markers. The five major subtypes characterized were: A – surface crypt-like; B – lower crypt-like; C – CIMP-H-like; D – mesenchymal-like and E – mixed. These subtypes do not replace classification using current clinicopathological variables or molecule markers but merely complement them.

The surface crypt-like subtype (A) was significantly enriched in *KRAS*-mutant tumours, having predominantly a papillary or serrated histopathology and a low percentage of  $\beta$ -catenin-positive nuclei at the invasive front. The authors also observed that tumours in this subtype were well differentiated and most comparable to normal colonic epithelium by gene expression profiling. Moreover, gene expression analysis showed up-regulation of genes involved in the top of the colonic crypt, secretory cells, and metallothioneins, whilst genes involved with EMT/stroma, Wnt, putative colon cancer stem cells (CSCs), Chr20q, and proliferation were down-regulated. In this subtype, survival after relapse (SAR) was 28.9 months.

The well-differentiated lower crypt-like subtype (B) displayed complex tubular morphology and had a high percentage of  $\beta$ -catenin-positive nuclei at the invasive front. A higher copy number gain/amplification was reported in Chr20q. Moreover, over-expression of genes involved in the top of the colonic crypt, proliferation (mainly *EREG*), and Wnt pathway was detected in the lower crypt-like subtype. In contrast, gene expression regulating the EMT/stroma, immune system, and secretory cells were down-regulated. From a clinical point of view, the tumours in this subtype were mainly located on the left side and were grade 2. The SAR was the highest when compared to the other subtypes (50.4 months). Furthermore, this subtype was significantly prognostic with respect to RFS, OS, and SAR.

Subtype C – CIMP-H-like was commonly MSI+ and *BRAF* mutant (87%) and histopathologically characterized by solid/trabecular or mucinous growth patterns. Commonly, tumours falling in this subtype were located on the right hand side and were high grade. At the invasive front, subtype C tumours did not show any  $\beta$ -catenin nuclear immunoreactivity, although transcriptomically they had high expression of immunity-associated genes, metallothioneins, and homeobox gene module. However, this subtype had low expression of gut development, top colon crypt, *EREG*, Chr20q genes, and genes differentially expressed in the CRC (GDC) gene module. The CIMP-H-like subtype was associated with a poor OS (SAR of only 6.9 months). The authors speculated that this subtype had a transcriptomic signature of a group of tumours that, once metastasized, would become resistant to chemotherapy.

Histopathological examination of the fourth CRC subtype (mesenchymal) revealed a desmoplastic pattern and immunohistochemistry (IHC) showed that only a low percentage of  $\beta$ -catenin-positive nuclei were present at the invasive front. Among other upregulated gene expression signatures, this subtype also had a high EMT/stroma gene expression. In contrast, lipid synthesis gene expression and the canonical Wnt signalling target signatures were among those down-regulated in the mesenchymal subtype. This subtype had a high frequency of *BRAF* mutant tumours and was correlated with poor OS, possibly as a result of the high EMT expression and low expression of proliferation-associated genes. Moreover, this subtype was also significantly prognostic with respect to RFS and SAR.

The final subtype described in this study was the mixed subtype (E), which was mainly located on the left side of the colon. Histopathologically, this subtype appeared as complex tubular, and IHC showed that a high percentage of  $\beta$ -catenin-positive nuclei were present at the invasive front. Among other upregulated gene expression signatures, this subtype also had high EMT/stroma gene expression, canonical Wnt signalling pathway target signatures, *EREG* gene module expression, and homeobox gene module expression.

### Classification based on three biological hallmarks of cancer

The last classification we review was published in July 2013 by Roepman *et al.*<sup>20</sup> This classification proposed three different intrinsic subtypes based on the three biological hallmarks of cancer – epithelial to mesenchymal transition, deficiency in mismatch repair genes, and cellular proliferation. This molecular CRC classification was derived by unsupervised clustering of multi-omic data generated from 188 stages I – IV CRC patients (discovery set). The validation set comprised of 320 Stage II and 223 Stage III CRC patients and was associated with prognosis and response to chemotherapy.

Thirty-five percent and 22% of patients were classified as MMR-deficient epithelial A-type in the discovery cohort and validation cohort, respectively. This subtype had a significantly higher percentage of MSI+ tumours (49%) and was also enriched for *BRAF* mutants. The authors speculated that this subtype may be more prevalent in early-stage cancers. Sixty-eight percent

of the patients in this cluster exhibited an MSI/dMMR expression profile. Differential gene expression analysis for mesenchymal and epithelial markers revealed that A-type tumours could be deemed epithelial. From a proliferative point of view, this subtype exhibited the highest expression of *MKI67*. When compared to the other two subtypes, the prognosis for A-type-MSI patients was very good (93% with 10-year distant metastasis-free survival). Response to chemotherapy was assessed in 222 Stage III patients, where patients in this group had a better OS from adjuvant chemotherapy with a hazard ratio (HR) of 0.39 ( $p = 0.18$ ).

The proliferative epithelial-like B subtype consisted of 52% of the discovery cohort patients and 62% of the validation cohort patients. This subtype encompassed mainly *BRAF* wild-type tumours (98%) and was almost entirely MSS and pMMR phenotype (99%). In this group, *CDH2*, *FGFR1*, and *TGFB1* (mesenchymal markers) were down-regulated, while four epithelial markers were up-regulated. This subtype was characterized as highly proliferative as a result of up-regulation of *MKI67* and even stronger with respect to *AURKA*. These patients had a relatively poor baseline prognosis but patients in this category would be expected to benefit from adjuvant chemotherapy with 5-fluorouracil (5-FU) (HR = 0.42,  $p = 0.014$ ).

The mesenchymal-like C subtype was the smallest subtype with 13% of the discovery cohort and 17% of the validation cohort. A small proportion of this subtype was *BRAF* mutant (16%) and 36% were dMMR. In this group, all the mesenchymal markers excluding *FLT1* were considerably up-regulated compared to *CDH*, *EGFR*, and *MET* (epithelial markers) that were down-regulated. Moreover, low proliferative activity was recorded in this group. These patients had a poor baseline prognosis and in addition, were resistant to chemotherapy with 5-FU. In the C-type-MSI patients, only 50% had 10-year distant metastasis-free survival.

### The consensus molecular subtypes of colorectal cancer

At the end of 2015, an international consortium published a consensus classification for CRC.<sup>21</sup> In this major collaborative study, the authors evaluated the results of the above CRC classifications and, by utilizing a network-based approach, four robust consensus molecular subtypes were identified. The eighteen datasets

utilized in the above characterization and subtyping studies amounted to a cohort of 4,151 patients. The authors divided the cohort into two equivalent groups for training and validation.

The four consensus subtyping clusters comprised 3,104 samples. Additionally, 858 samples did not fall into any of the subtypes and hence were described as unlabelled non-consensus samples. The clusters were first biologically characterized and associated with both clinical variables and prognostic values. The new taxonomy for CRC is described below.

#### Consensus Molecular Subtype 1

**(CMS1):** This subtype was referred to as microsatellite instability immune and comprised 14% of the cohort. CMS1 had an MSI+, CIMP-H, and hypermutated phenotype and was highly enriched for *BRAF* mutants. The MSI immune subtype was characterized by increased expression of genes associated with immune infiltrates together with strong activation of the immune evasion pathways. Clinically, these tumours were frequently diagnosed in females, located on the right side, and presented at later stages. Furthermore, when compared to the other subtypes, CMS1 had a worse survival after relapse.

#### Consensus Molecular Subtype 2

**(CMS2):** Thirty-seven per cent of the cohort fell in this canonical-epithelial subtype. CMS2 had the highest copy number gains and copy number losses when compared to the other subtypes. Additionally, this subtype had high expression of WNT and MYC downstream targets. Clinically these tumours commonly presented on the left side. In contrast to CMS1, patients in this subtype had better survival rates after relapse.

#### Consensus Molecular Subtype 3

**(CMS3):** Thirteen per cent of the cohort fell into this subtype. The authors referred to this as the metabolic subtype on account of the metabolic dysregulation that characterized the group. The majority of the tumours in this subtype were reported to be of mixed MSI status, mainly CIMP-L, and had a low frequency of copy number alterations. Moreover, *KRAS* mutations were common to this particular CMS.

#### Consensus Molecular Subtype 4

**(CMS4):** This subtype was characterised as having prominent transforming growth factor- $\beta$  activation, stromal invasion, and angiogenesis. As a consequence, this subgroup was also referred to as the mesenchymal subtype and comprised 23%

## Key points

- Colorectal cancer is a heterogeneous disease with several clinical, pathological, and molecular presentations
- Traditionally, cancers have been classified clinically and pathologically based on stage and grade
- To date there are still clinical dilemmas in colorectal cancer that would benefit from a molecular classification approach
- The integration of genomic, epigenomic, transcriptomic and proteomic data using applied bioinformatics and computational biology is one way of understanding better colorectal carcinogenesis and response to treatment
- Molecular classification is crucial for the discovery of actionable diagnostic, surveillance, prognostic and predictive biomarkers

of the cohort. CMS4 had a high frequency of copy number gains and losses. Clinically, tumours in this subtype tended to be diagnosed at a later stage and had the worst relapse free and overall survival.

**Samples with mixed features:** 13% of the samples did not stratify in any of the four consensus molecular subtypes. The authors have speculated that the samples in this group might be representative of either a transition phenotype or intratumoral heterogeneity.

## Conclusion

Molecular classification of colorectal cancer is of high significance since the colorectal subtypes are useful for generating correlations with a number of variables namely clinicopathological parameters, overall survival, progression free survival, and response to treatment and relapse. These correlations can facilitate our understanding of colorectal cancer biology and provide evidence regarding carcinogenesis.<sup>9</sup> Furthermore, these correlations can be used to discover new molecular subtypes. Moreover, association studies can be helpful in highlighting potential confounding factors and preventing incorrect associations.<sup>9</sup> Finally, robust molecular classification is of paramount importance for the discovery and clinical implementation of prognostic and predictive biomarkers - the precursors for successful implementation of point of care genomics and making personalised healthcare a reality.

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