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thesynapse

THE MEDICAL PROFESSIONALS' NETWORK

Skin Microbiota and Body Malodour

The Non-Invasive Vital Signs Monitoring Project

Transposable Elements in Human Cancer

Management of Incidentally Noted Adnexal Cystic Lesions

As a strong complement
to a maximally tolerated statin[1]

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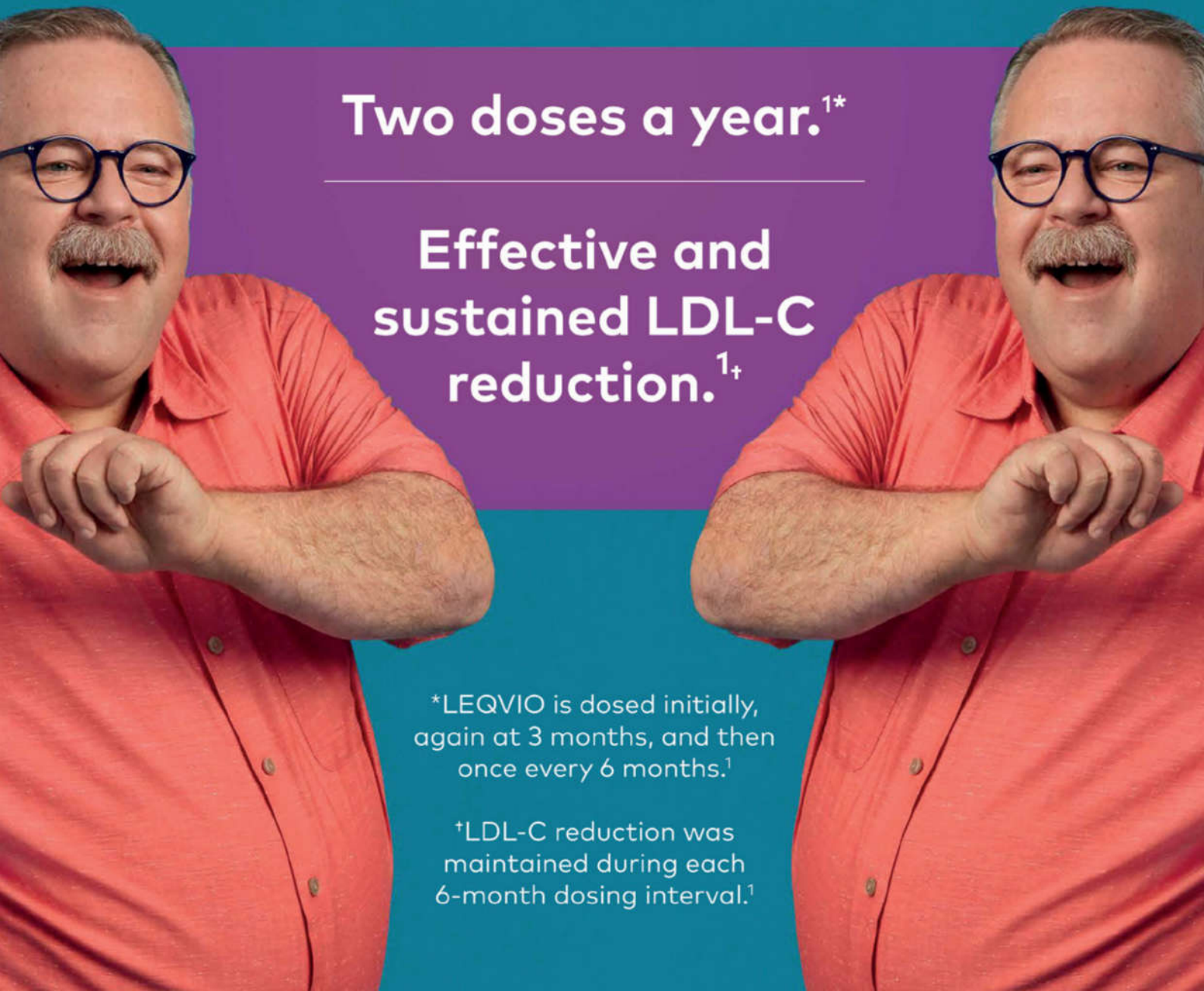
(inclisiran)



Two doses a year.[1*]

Effective and sustained LDL-C reduction.[1†]

*LEQVIO is dosed initially, again at 3 months, and then once every 6 months.[1]

†LDL-C reduction was maintained during each 6-month dosing interval.[1]

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TWO DOSES A YEAR[1*]

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EFFECTIVE AND SUSTAINED LDL-C REDUCTION[1†]

†LDL-C reduction was maintained during each 6-month dosing interval.[1]

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This medicinal product is subject to additional monitoring. This will allow quick identification of new safety information. Healthcare professionals are asked to report any suspected adverse reactions. See section 4.8 for how to report adverse reactions.

PRESENTATION: Leqvio 284 mg solution for injection in pre filled syringe. Each pre-filled syringe contains inclisiran sodium equivalent to 284 mg inclisiran in 1.5 ml solution. Each ml contains inclisiran sodium equivalent to 189 mg inclisiran.

INDICATION: Leqvio is indicated in adults with primary hypercholesterolaemia (heterozygous familial and non-familial) or mixed dyslipidaemia, as an adjunct to diet: in combination with a statin or statin with other lipid-lowering therapies in patients unable to reach LDL-C goals with the maximum tolerated dose of a statin, or alone or in combination with other lipid-lowering therapies in patients who are statin-intolerant, or for whom a statin is contraindicated.

DOSAGE: The recommended dose is 284 mg inclisiran administered as a single subcutaneous injection: initially, again at 3 months, followed by every 6 months. ♦*Missed doses:* If a planned dose is missed by less than 3 months, inclisiran should be administered and dosing continued according to the patient's original schedule. If a planned dose is missed by more than 3 months, a new dosing schedule should be started – inclisiran should be administered initially, again at 3 months, followed by every 6 months. ♦*Treatment transition from monoclonal antibody PCSK9 inhibitors:* Inclisiran can be administered immediately after the last dose of a monoclonal antibody PCSK9 inhibitor. To maintain LDL-C lowering it is recommended that inclisiran is administered within 2 weeks after the last dose of a monoclonal antibody PCSK9 inhibitor. ♦*Elderly, hepatic impairment, renal impairment:* no dose adjustment is necessary. Inclisiran should be used with caution in patients with hepatic and renal impairment.♦*Paediatric population:* The safety and efficacy of inclisiran in children aged less than 18 years have not yet been established. ♦*Method of administration:* Inclisiran is intended for administration by a healthcare professional via subcutaneous route. Each pre-filled syringe is for single use only.

CONTRAINDICATIONS: Hypersensitivity to the active substance or to any of the excipients listed in the SmPC.

WARNINGS/ PRECAUTIONS: ♦*Haemodialysis*: The effect of haemodialysis on inclisiran pharmacokinetics has not been studied. Considering that inclisiran is eliminated renally, haemodialysis should not be performed for at least 72 hours after inclisiran dosing. ♦*Sodium content:* This medicinal product contains less than 1 mmol sodium (23 mg) per dose, that is to say essentially "sodium-free".

INTERACTIONS: Inclisiran is not an inhibitor or inducer of cytochrome P450 enzymes or common drug transporters. Therefore, inclisiran is not expected to have clinically significant interactions with other medicinal products. Based on the limited data available, clinically meaningful interactions with atorvastatin, rosuvastatin or other statins are not expected.

PREGNANCY, LACTATION AND FERTILITY: ♦There are no or limited amount of data from the use of inclisiran in pregnant women. As a precautionary measure, it is preferable to avoid the use of inclisiran during pregnancy. ♦It is unknown whether inclisiran is excreted in human milk. A risk to newborns/ infants cannot be excluded. A decision must be made whether to discontinue breast-feeding or to discontinue/abstain from inclisiran therapy, taking into account the benefit of breast-feeding for the child and the benefit of therapy for the woman. ♦ No data on the effect of inclisiran on human fertility are available.

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LEGAL CATEGORY: POM

PACK SIZE: One pre-filled syringe.

MARKETING AUTHORISATION HOLDER: Novartis Europharm Limited, Vista Building, Elm Park, Merrion Road, Dublin 4, Ireland.

MARKETING AUTHORISATION NUMBER: EU/1/20/1494/001

Please refer to Summary of Product Characteristics (SmPC) before prescribing. Full prescribing information is available on request from Novartis Pharma Services Inc, Representative Office Malta, P.O. Box 4, Marsa MRS 1000 Malta. Tel +356 21222872.

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References: 1. Novartis Europharm Ltd. Leqvio Summary of Product Characteristics.

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Issue Guide

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17 years

Whilst I was starting Junior College, in October 1996, The Synapse magazine was being simultaneously launched by Dr Wilfred Galea. Little would I have imagined that, in November 2005, I would take the helm of this publication.

Back in 2005 The Synapse Magazine was an 8-pager which would pale in front of the current publication, be it in terms of quality or print run. We have invested much, especially time, to deliver this magazine to the doorstep of our 3,500 readers on a bimonthly basis. The ultimate aim has always been to continually improve the quality of the publication. I still remember the 8pm till 1am meetings at Dr Galea's Dingli office to discuss the design of the publications, nibbling sandwiches with bloodshot eyes ...

During these past 17 years I tried to reciprocate Dr Galea's trust. I strongly believe that we have managed to position the publication well amongst local healthcare professionals. Everybody is familiar with 'The Synapse' and cme30.eu which is our online continuous medical education portal.

Apart from the run-of-the-mill editorial work, I particularly enjoyed two aspects. The first was penning the editorials. I have written practically about everything, always in relation to health. Topics have included micro- and macro-economics, socio-politics, procurement, etc. I was also privileged to have published guest editorials from various local members of parliaments and even recently, from Dr Denis Horgan who is the Executive Director of the European Alliance for Personalised Medicine (EAPM). This was my way to garner views possibly, outside the box.

Secondly there were the interviews, which I felt were needed in this publication, in 2007. I wanted to insert the interviews because I strongly believe in camaraderie, even across different professions. I wanted to convey the message that we should all strive to reduce the silo mentality which at times, erodes our soul. In 2019 I completely took over the interviews with a view to improve the quality. Indeed, each interview which I made took me an average of 20 hours, and this involved fact-checking, meeting up with the interviewee and then writing it. I also managed to conduct interviews with Maltese trailblazers residing abroad including Prof. Sandro Galea, the late Prof. Edward de Bono and Prof. Dame Clare Gerada.

Nonetheless, as weeks rolled into months and months turned into 17 years, I came to realise that the greatest wealth is time and health. Alias, these invariably have an expiry date. Every our decision needs to be gauged against these two things. What is the opportunity cost?

But then again, what will our legacy be?

Ian Ellul

DR ETIENNE GRECH, DR GEORGIANA FARRUGIA BONNICI, DR MARTINA HÖHMANN

Skin Microbiota and Body Malodour

KEYWORDS
Bacterial skin commensals, body malodour suppression, hyperhidrosis, bromhidrosis, skin microbiome

ABSTRACT
Chronic body malodours are often considered to be universal triggers of distress for many patients. Various human body malodours relate to the bacterial transformation of compounds conveyed by sweat on the skin surfaces and axillae. There is skin microbiome variation between individuals which typically results in a change in one's sweat constitution and ultimately influences the final odour produced. Body malodour may be managed through good personal hygiene, the use of antiperspirants, deodorants, zinc emulsions, antifungals, glycopyrronium tosylate, triclosan body soaps and povidone-iodine. Moreover, certain lifestyle modifications, the use of oral probiotics and prebiotics, along with more invasive medical and surgical procedures, such as axillary laser therapy, iontophoresis, Botulinum toxin type A, suction-curettage, elliptical axillary skin excision as well as transthoracic sympathectomy may also result in body malodour suppression. In this article, the relationship between bacterial skin commensals and body malodour will be discussed.

INTRODUCTION

Chronic body malodours are often considered to be universal triggers of distress for many patients.[1] They may result in significant negative psychological, social and sexual outcomes, such as low self-esteem, anxiety, impaired quality of life, social exclusion as well as decreased intimacy.[2] There are various odours which are emitted by the human body, including but not limited to the oral, reproductive, dermatological, urinary, apocrine and endocrine systems.[2] Phages, archaea, mites, viruses, fungi and bacteria may all form part of the skin microbiome, some of which have been documented to affect various skin properties, immune responses, pathogen growth and wound healing.[3-5] Human body malodours relating to the bacterial transformation of compounds conveyed by sweat in the skin surfaces and axillae, will be discussed in greater detail.

THE ANATOMY AND PHYSIOLOGY OF HUMAN SKIN AND SWEAT PRODUCTION

The largest organ in the human body is the skin, which has a number of important functions.[3] This includes its ability to act as a physical barrier and offer protection against insults from the external environment.[3] The skin also supports a diverse microbiota, including bacteria. Although bacteria are primarily associated with infection, one must bear in mind that not all bacteria are harmful to the human body. On the contrary, certain bacteria are beneficial to humans. This may be appreciated by considering the variety of bacteria that are known to colonise the human skin, which ultimately may contribute to protecting humans from pathogens.[4,5]

The skin is divided into two parts; the superficial epidermis, consisting of a stratified epithelium, and the deeper dermis, which is made up of a dense connective tissue containing blood vessels, lymphatic vessels and nerves.[6] The dermis is also connected to the deep fascia by the subcutaneous tissue. The skin appendages are made up of the nails, hair follicles, sebaceous glands and sweat glands.[6] Sebaceous glands pour sebum onto the hair shafts, whilst sweat glands are long, spiral, tubular glands which are distributed over several body surfaces, except for the margins of the lips, the nail beds, the glans penis and the clitoris.[6] There are three types of human sweat glands. The eccrine sweat glands are mainly responsible for thermoregulation,

APOCRINE SWEAT GLANDS	ECCRINE SWEAT GLANDS
Concentrated in the axillae, groin and mammary glands, become active at puberty	Found all over the skin surfaces
Ducts in the axillae and groin secrete viscous fluid into a hair follicle	Ducts open to a skin surface
Ductal epithelium made up of only one lobule layer of cuboidal cells	Ductal epithelium made up of several layers of cuboidal cells
Measure 80-100 µm in diameter	Measure 30-40 µm in diameter
Secretions are oily and viscid, consisting mostly of proteins, steroids and fatty acids	Secretions are watery, consisting mostly of water and solutes
Secretions are mainly responsible for body odour due to bacterial decomposition	Secretions are mainly responsible for thermoregulation and partly for body odour

Table 1: The major differences between the apocrine sweat glands and the eccrine sweat glands.

which is carried out by secreting water-rich sweat over various body surfaces. Contrastingly, the apocrine sweat glands are restricted to the axillae, mammary glands and groin, which initially produce an odourless, oily, opaque secretion which then gains its characteristic odour from bacterial decomposition.[6] It is known that the axillary anatomy provides a favourable environment for bacteria to flourish.[7] The third type, the apo-eccrine glands, are also identified in the same areas of the apocrine glands. Similarly, the apo-eccrine glands also secrete watery substances.[6] The various differences between the two major types of sweat glands, the apocrine glands and the eccrine glands, are outlined in Table 1 and Figure 1.

On average, humans have two to four million sweat glands. It is also estimated that in adults, the maximum sweat rate is up to 10 to 14 litres daily.[6] The components of sweat are primarily water, with small amounts of solutes and trace minerals, such as sodium, potassium, calcium, lactate, urea, magnesium, zinc, copper, iron, chromium, nickel and lead.[6] Sweat has a moderately acidic to neutral pH, typically ranging between 4.5 and 7.[6] Certain chemical compounds are also found in sweat, such as thioalcohols, carboxylic acids, fatty acids, ketones, ammonia, sulphides, aldehydes, esters, androstene steroids as well as hydrocarbons.[7-11] Even though human sweat is initially odourless, the enzymatic action of resident skin bacteria then transforms these odourless precursors to volatile chemical compounds. Over time, a distinct body malodour is produced.[7,9-11]

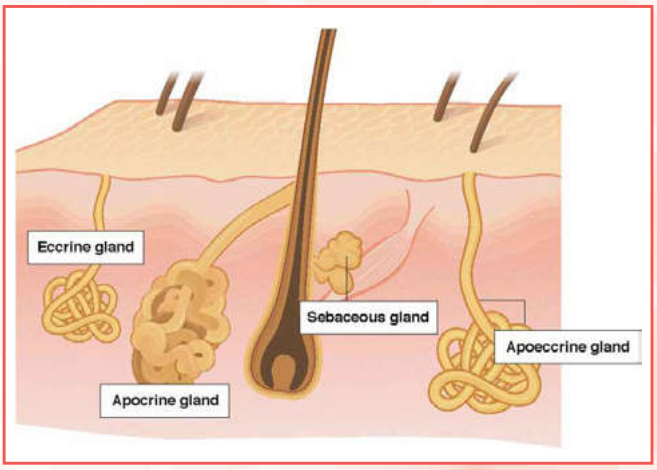

Figure 1: An illustration of the three different types of human sweat glands: eccrine, apocrine, and apo-eccrine glands.

There is skin microbiome variation between individuals which may be attributed to a number of different factors. These include the living environment, the working environment, lifestyle including diet, hygiene, ethnicity, gender, the health status of the host, age, as well as antibiotic use, cosmetics and textiles.[3,12] This variation in skin microbiota typically results in a change in one's sweat constitution, and ultimately influences the final odour produced.[2,7] Interestingly, it is thought that humans may have been given an adaptive evolutionary advantage due to the specific biosynthesis and secretion of odourants.[7] Various kinds of societal information and messages in mammals are thought to be sent via these volatile odours.[8] Moreover, this may have an impact on mating selection as humans are highly sensitive to odourants.[7] Furthermore, it has been

proposed that both odour-producing bacteria and human beings have evolved in conjunction with one another.[8]

BACTERIAL SKIN COMMENSALS AND OTHER CAUSES OF BODY MALODOUR

The commonest bacteria that have been previously identified on the skin include Gram positive bacteria, such as Staphylococci, Streptococci, Micrococci, Cutibacteria, Corynebacteria, Anaerococci and the *Peptoniphilus* species, as well as Gram negative bacteria such as *Acinetobacter* species.[5,10,12] In particular, Corynebacteria produce carboxylic acids and androgen steroids which contribute to a pungent axillary odour.[7,9,11] Contrastingly, an acidic and sulphur-like odour is produced by Staphylococci through the synthesis of short-chain fatty acids and thio-alcohols.[7,9,11] As has been previously discussed, the composition of body odours is mostly determined by genetics and ethnicity.[9,12,13] However, it may be influenced through other factors such as age, temperature, diseases, skin pH, physiological and emotional states, diet and bacterial metabolic activity in the gut, as well as personal hygiene.[2,5,9] Moreover, it is known that body odours are also discriminable by gender. Male axillary odours tend to be more intense than female axillary odours.[9,13] This could result from the fact that men have Coryneform-dominant microflora.[9] On the other hand, the production of androstenes is sexually dimorphic. Women tend to secrete lesser quantities of androstenone than men, whilst estratetraenol is mainly produced during pregnancy.[13] Furthermore, body odour is also related to intestinal, thyroid, cardiac, liver and renal function.[2,7,11] In fact, the body odour of sick individuals is more aversive than that of healthy persons.[9] Human malodour may be associated with conditions such as obesity, menopause, anxiety, toxin ingestion, bromhidrosis, halitosis, hyperhidrosis, phenylketonuria, methionine malabsorption syndrome, hypermethioninaemia, trimethylaminuria, maple syrup disease, liver failure, end-stage renal failure, bacterial vaginosis, urinary incontinence and diabetic ketoacidosis.[2,6,7]

MANAGEMENT OF SKIN MALODOUR

The first step in reducing body malodour is maintaining good personal hygiene, which includes daily bathing with low pH soaps as well as the regular washing of clothes and linen.[14] Shaving, waxing, or axillary laser hair removal may also reduce body malodour by decreasing the amounts of bacterial skin microflora.[14] Most anti-perspirants contain aluminium salts, which diminish perspiration by blocking the sweat gland excretory ducts, hence reducing bacterial proliferation.[14] The use of an anti-perspirant at night will block excretory channels by day-time. Unlike antiperspirants, deodorants mask body malodours with perfume. Zinc emulsions applied to the under-arms, groins, and various other body folds absorb malodours.[14] Moreover, antifungal creams such as clotrimazole, miconazole and ketoconazole have also been shown to reduce body malodour, especially if preceded by dilute hydrogen peroxide cleansing.[14] The application of topical 2.4% glycopyrronium tosylate on a pre-moistened cloth to the axillae decreases sweat.[15,16] Triclosan-based body soaps have been used for decades, due to the lack of resistance of malodour-forming bacteria.[2] In addition, the antibacterial compound povidone-iodine also reduces the malodorous scent which is typically created by androstenone in sweat.[2]

Some dietary substrates which are metabolised by bacteria in the stomach and intestines may eventually end up in body fluids, including sweat, thereby causing body malodour.[2] In such cases, one needs to ensure dietary modifications, as well as the avoidance of particular foods, such as garlic, curry, onions and alcohol.[2] Oral probiotics and prebiotics may also help in favourably altering the gut bacterial flora.[2] Frequent bowel movements will speed up the passage time and hence, decrease unwanted gut bacterial metabolism and metabolite absorption. Furthermore, increasing water consumption also increases the urinary excretion of metabolites, which in turn decreases body malodour.[2]

More invasive treatment may be undertaken to reduce axillary or palmar hyperhidrosis or bromhidrosis, depending on the severity of each particular case. Treatment with an 800-nm diode or a 1064-nm Nd-Yag lasers may permanently destroy axillary sweat glands by heating them, without injuring the surrounding tissues.[14] Such treatment usually takes less than one hour.[14] Moreover, microwave treatment may fibrose both the eccrine and the apocrine glands in order to alleviate axillary hyperhidrosis. Such treatment is best conducted with tumescent anaesthesia and microneedle radiofrequency.[14,16] Nevertheless, such methods require further research. Iontophoresis, on the other hand, involves a complicated mechanism of ion transport via the skin that blocks the sympathetic innervation of sweat glands in the axillae, the palms of the hands or feet. Iontophoresis employs a local electric current to attract tap water or anti-cholinergic drug ions into the axillary subdermal tissues.[16,17]

Botulinum toxin type A may also be injected in various parts of the axillae to reduce body malodour, by blocking the sympathetic innervation that releases sweat. This method is safe and efficient, with its effect typically lasting around 6 to 8 months.[14,15,17] Another minimally invasive procedure for treating axillary hyperhidrosis, known as suction-curettage partly takes out the deep skin layer that contains sweat glands.[18] Malodour may also be significantly reduced by an elliptical axillary skin excision.[16,17] Axillary and palmar hyperhidrosis can also be treated through the removal or disconnection of the T2-T4 thoracic ganglia via endoscopic, transthoracic sympathectomy.[16-18] However,

due to the significant risks involved, such treatment is not recommended as first-line.

CONCLUSION

Medical science is still evolving when it comes to developing new targeted therapies for body malodour. This can be seen in the potential niche that is currently lacking in the development of therapy targeted towards the skin microbiota.[5] An advanced understanding of the molecular, biochemical and bacterial pathways involved in body malodour may encourage the development of new microbial therapeutics for various skin conditions which would ultimately benefit many individuals.[19] An example of which could possibly include therapy geared towards the inhibition of peptide transporters involved in thioalcohol recognition and transport.[8] Even though several theories and hypotheses have been put forward, there is a need for more research and evidence-based medicine in this field.

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None.

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The Non-Invasive Vital Signs Monitoring Project

Engineering Meets Healthcare Innovation

ABSTRACT

In recent years, the concept of contactless and remote monitoring of patients has gained momentum due to its multiple advantages over traditional contact monitoring involving leads and wires. The Non-Invasive Vital Signs Monitoring Project (NIVS) aims to extract heart rate data from both healthy volunteers as well as patients in real-world hospital scenarios using normal red-green-blue (RGB) and thermal imaging cameras. NIVS aims to overcome the limitations which exist with current contact systems. This article describes the underlying scientific principles, the execution and the potential benefits of such a system in the local healthcare setting.

KEYWORDS

Contactless Monitoring, RGB camera, Vital Signs, Thermal Imaging Camera, Clinical Monitoring

AIM AND INTRODUCTION

NIVS is a collaborative project between the University of Malta's Centre for Biomedical Cybernetics, the Faculty of Medicine and Surgery and Mater Dei Hospital. The project is funded by the Malta Council for Science &Technology (MCST), for and on behalf of the Foundation for Science and Technology, through the Fusion: R&I Technology Development Programme. Ethical approval was obtained from the Faculty Research Ethics Committee of Medicine and Surgery.

The aim of the project is to design a system capable of measuring heart rate in real-time in clinical settings, without modifications to patient care. Red-green-blue (RGB) cameras and thermal imaging cameras are used for this purpose.

The underlying principle behind the extraction of cardiovascular data from RGB videos is remote photoplethysmography (rPPG). This principle is based on the absorption of specific wavelengths of visible light by haemoglobin in the blood perfusing the skin.[1] This principle is the same as that used in pulse oximetry, but visible ambient light is used instead of specific wavelengths.[2] Algorithms can be used to detect the light reflected in a pulsatile manner from the skin of various body parts, most commonly the face, neck and hands. These body parts are used as a region of interest (ROI) for rPPG. From this, the rate and rhythm of the heart beat can be deduced.[3-5]

Many algorithms exist to extract heart rate; for this project, a type of algorithm called a convolutional neural network (CNN) is used. This is an algorithm constructed to recognise certain aspects and objects in a video and can be trained to apply this knowledge to new videos.[6-8] In the case of the NIVS project, a set of videos were set up where the subject's heart rates are fed to the CNN so that it can learn to extract heart rate data from the RGB videos.

CNN models have proved to be reasonably accurate for medical purposes in estimating heart rates from videos of patients' faces; apart from the legal age of consent, no exclusion criteria were applied for the purposes of this project. Nonetheless, a number of limiting factors exist with current models. Their performance is often negatively impacted by changes in ambient light and motion of the subject and other persons in the patient's immediate surroundings. Videos processed via CNNs have so far been quite short, and few studies have obtained real-time measurements.[9-11] Our study aims to overcome some of these limitations and test out a CNN based system in a real-world hospital setting.

METHODOLOGY

The project was designed in two stages. For the first stage, twenty-seven healthy volunteers with no documented cardiovascular medical pathologies were recruited and their consent was obtained. Data collection involved a setup where the person is lying on a couch and cameras (a digital camera, Canon Legria HF G25, and a smartphone camera, (Xiaomi Redmi 9A) are positioned at a two-metre distance from the subject's face. Ground truth heart rate was collected using electrocardiogram electrodes and pulse oximetry which link the participant to the data acquisition equipment (BIOPAC MP150). Video clips were recorded with varying conditions, such as bright light, minimal light, and with the cameras which were then moved to four metres away from the subject's face. Another video clip also captured random movements of the head, arms and legs of subjects in order to assess the effect of the movements to the accuracy of results. These videos were

Figure 1: Setup for data collection in the laboratory.

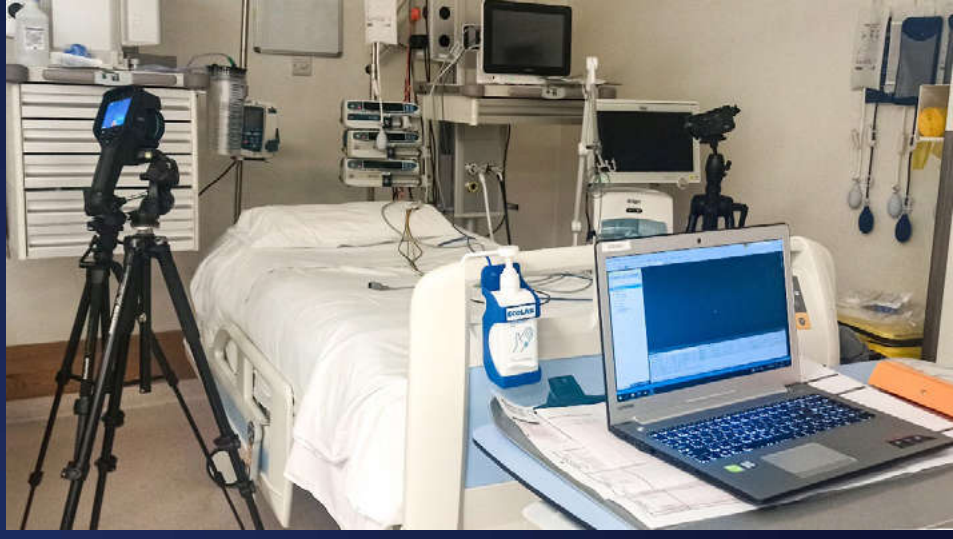

Figure 2: Setup for data collection in ITU.

used for training the CNN to recognise heart rates from videos while comparing them to the ground truth data available.

The second part of the study took place in the Intensive Therapy Unit (ITU) at Mater Dei Hospital. This stage involved taking videos of thirty-five consenting patients with no specific exclusion criteria, other than that all participants must be above eighteen years of age. The aim was to collect real-world data with normal ongoing patient care, while patients move freely, with other individuals visible around them. The cameras were set up in a practical manner around the patient's bed and ten-minute video clips were taken of patients at rest, during physiotherapy sessions if applicable, during small procedures such as venous catheterisation or bloodletting, as well as during night-time when overhead lighting was dimmed or switched off. Ground truth data in this case was obtained using a software programme (VS Capture) to obtain real-time readings from the Philips Intellivue monitors used in the ITU, and converted to a Microsoft Excel file.

The videos obtained from ITU were used for testing purposes to assess the accuracy of the developed CNN compared to standard Phillips Intellivue monitors when video frames from an ITU environment are fed into it to extract heart rate data.

RESULTS

Models Description

The deep learning models that were being used in this study for the estimation of the heart rate were two, i.e. the spatio-temporal CNNs C3D and the 3D-DenseNet.[12-14] This deep network model was originally employed for video classification task and, as reported in Du Tran et al, it has powerful spatio-temporal feature extraction capability.[12] This is due to its design and architecture which allows it to model both appearance and motion features in the video.

Datasets

As mentioned in previous sections, data were collected from volunteers in a laboratory environment simulating the various conditions which would later be encountered in hospital. We collected videos which were then split into 30-second clips. The extracted frames from the video clips were resized to images of size 64x64 pixels. Although the frame size may seem too small, the deep net models displayed good estimation results after they have been trained. For this current stage, we used over 500 short clips, which include different lighting and motion conditions. This, therefore, can help our models to be robust against different quality of videos.

Training

70% of the processed data was then used to train the deep net models from scratch, using different parameters such as different learning rates, batch sizes, and number of epochs. Finally, the model that achieved the best results was selected to be tested on new videos in order to estimate the corresponding heart rate.

Models' Evaluation

Once the model training converged, they were tested on the remaining 30% of the videos. As data from more subjects are acquired, the deep net models could be trained with more varied data which is known to improve the generalization capability of the networks in estimating the heart rate. The models were evaluated by computing metrics such as the Mean Absolute Error (MAE). Experimentally, it was found that the C3D model was capable of reaching a MAE of 4.87. On the other hand, the 3D-DenseNet performed slightly better with a MAE of 3.7. This means that the values obtained by these models differed from those obtained by gold standard methods by 4.87 beats per minute and 3.7 beats per minute respectively.

In view of the relatively small sample number of subjects used for training the models, these results may be considered satisfactory in terms of accuracy for general medical monitoring. It is expected that with more data and more varied scenarios, model performance will improve.

A number of challenges have been encountered during the second stage of the project at the ITU, which are important learning points to consider if this system is to be developed for more widespread use. Placement of cameras to obtain a good view of the patient's face without causing obstruction for members of staff can be an issue. In the ITU, patients may have part or all of the face covered by tube ties, masks and sterile sheets during certain procedures such as tracheostomy insertion and central venous catheterisation. Therefore, having

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Brintellix is indicated for the treatment of major depressive episodes in adults.[1]
1. Brintellix Summary of Product Characteristics.

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a video frame that also includes other portions of skin such as the hands and neck is helpful in these situations, but this is not always feasible due to the patient position relative to the cameras, as well as bandages or clinical equipment that may be obscuring the view of these body parts. Members of staff and relatives moving around the patient can easily obscure the camera's field of vision and accidentally move the cameras, therefore the cameras would be better placed overhead.

CONCLUSION

This project has the potential to improve patient care and comfort. Patients are more able to move freely if less electrodes and wires are attached to them. This could also prove beneficial to the reduction of post-operative complications such as pneumonia and deep vein thrombosis since these conditions are worsened by immobility which will be less of an issue with contactless monitoring, as well as enhancing rehabilitation of patients who can feel more independent and be able to cooperate better during physiotherapy sessions.[15-18] In some categories of patients such as those with extensive burns and other skin pathologies, a non-contact monitoring system can solve the issue of difficult electrode placement due to poor skin quality.[19]

Another major advantage of a non-contact system is infection control, especially that of multidrug resistant strains (MDROs) which are unfortunately all too common inside Mater Dei hospital. This could stem from inadequately disinfected leads and through staff members' contact with patients, attempting to troubleshoot monitoring leads. WHO describes the issue of MDRO spread as one of the main health crises of our times.[20]

In the case of patients who require isolation, such as those with infectious diseases, including COVID-19, and those who are immunosuppressed and require protection themselves, a contactless system can reduce the number of times staff members need to enter the room, which can also contribute to less spread of infection and better health economics in terms of personal protective equipment wastage. This scenario has become a reality in the past two years with multiple waves of the COVID-19 pandemic.[21-23]

This project is therefore very relevant to the current local situation and may contribute to scientific innovation even within the international community. Although many obstacles need to be overcome in terms of the technology available and the data it is able to process, so far we have obtained promising results and we hope to strive for a practical operational system which can concretely improve upon current patient care.

COMPETING INTERESTS

There are no competing interests in relation to this article.

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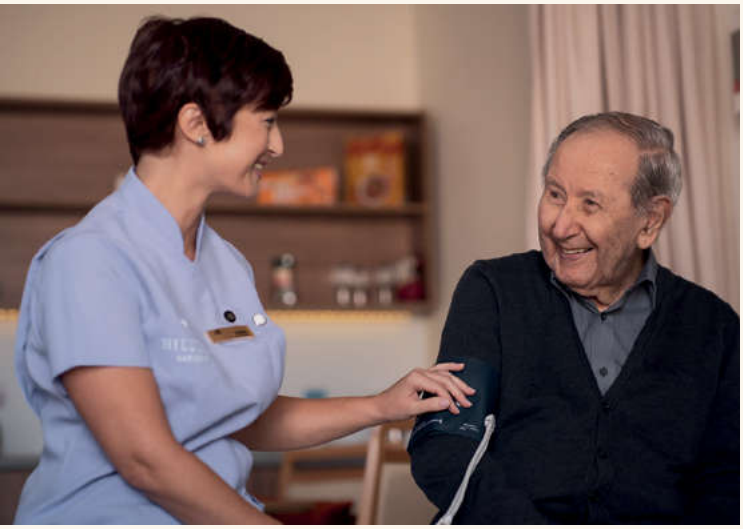

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DR ALFRED GRECH & DR STEPHEN WEST

Transposable Elements in Human Cancer

ABSTRACT

Transposable elements are repetitive DNA sequences consisting of RNA transposons, DNA transposons, and endogenous retroviruses. Repetitive sequences cover more than two-thirds of the genome in humans and transposable elements comprise the majority of the repetitive sequences (approximately 50% of the human genome).

Transposable elements can transpose (i.e. can jump) and cause havoc. Indeed, research is showing that their deregulation impinges on the stability of the genome and on the regulation of transcription and that of non-coding RNA. This leads to carcinogenesis and to cancer progression. These insights are furthering their research to discover novel targets for theranostic applications in cancer.

INTRODUCTION

A 'transposable element' (TE) is a DNA sequence that can move and change its position (transpose) within the same chromosome or from one chromosome to another. These mobile DNA sequences are also called 'mobile elements'. It was the geneticist Barbara McClintock back in 1948 that discovered these elements in her seminal work on the mosaic colouration in maize. Her theory met a lot of scepticism in the field, and had to wait years before her work was recognized. Indeed, it was in 1983 when she was awarded the Nobel prize in Physiology/Medicine. Since then, similar mobile elements have been discovered in mammalian genomes, including that of humans. Moreover, they have also been found in almost all living species, including bacteria. The presence of active mobile DNAs in humans was only appreciated in 1988, when research showed that a TE was responsible for Haemophilia A. Since then, several genetic diseases have been discovered to be mediated by TEs.

DNA transposons and retrotransposons (also called retro-elements) are the two main classes of transposable elements. This classification is based on whether an RNA intermediate is involved during transposition. DNA TE hop by a "cut-and-paste" mechanism, while retrotransposons replicate via a "copy-and-paste" mechanism. This essay will focus on retrotransposons and specifically, on LINE-1 (long interspersed nuclear element-1, also called L1), which are the most well-studied retrotransposons.

The human genome harbours over 500,000 copies of LINE-1 elements and it is estimated that they represent 18% of the genetic code. In the human somatic cell, the majority of LINE-1 elements are not active. In fact, it has been estimated that there are only 80 to 100 LINE-1 elements that are 'hot', so defined because they are capable of propagation ('retrotransposon competent' L1). Scientists call these subsets of LINE-1 elements as 'genetic parasites'.[1]

STRUCTURE OF LINE-1 ELEMENTS

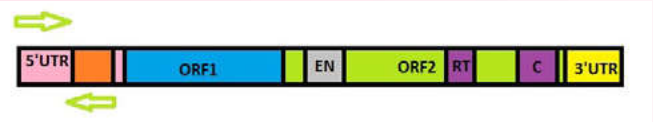

Figure 1. Structure of LINE-1. 5'UTR (five prime untranslated region); ORF1 (open reading frame 1); EN (endonuclease segment); ORF2 (open reading frame 2), RT (reverse transcriptase segment); C (carboxy-terminal segment; 3'UTR (three prime untranslated region).

LINE-1 elements are approximately 6 –7 kilobases (kb) in length. Simplistically, a LINE-1 element consists of the following parts:

(i) a 5'-UTR (five prime untranslated region) which is rich in cytosine-phosphate-guanine (CpG) – this region has two internal promoters, one is sense and the other is antisense;
(ii) two open reading frames (ORFs) which do not overlap, with ORF1 being 1 kb long and ORF2 being 3.8 kb long;
(iii) a 3'-UTR (206 nucleotides) with a poly(A) tail.

THE PROCESS OF L-1 RETROTRANSPOSITION

Briefly, the process of L-1 retrotransposition consists of the following stages:

(i) A cycle starts in the nucleus with the disassembly of the nucleosomal and remodeling deacetylase (NuRD) multiprotein complex from the L1 promoter. NuRD is a repressor complex, which together with epigenetic repressor marks (e.g. histone-3 lysine-9 trimethylations (H3K9me3) and histone-3 lysine-20 trimethylations (H3K20me3)) cause L1 promoter to be in a heterochromatic state, the latter being a compact condensed state of the DNA making it inaccessible for transcription. Thus, removal of NuRD and the epigenetic repressor marks, shifts the L1 promoter from its heterochromatic state to a euchromatic one, which is open and uncompacted chromatin, allowing transcription.

(ii) The L-1 DNA sequence is transcribed by RNA polymerase II into the L1 mRNA. The RNA polymerase binds to the sense promoter and starts transcription in a 5′ to 3′ manner.

(iii) L1 mRNA exports to the cytoplasm.

(iv) In the cytoplasm, L1 mRNA is translated. ORF1 frame encodes ORF1 protein (ORF1p) which is 40kDa and has an RNA recognition motif. ORF2 frame encodes ORF2 protein (ORF2p) which is 150kDa and has endonuclease and reverse transcriptase activities.

(v) In the majority of cases ORF1p and ORF2p proteins preferentially assemble with their own L-1 transcript forming L1 ribonucleoprotein complex (L1 RNP). This preference binding is called *cis* preference. However, a minority of them show *trans*-preference. Indeed, they can bind to SINEs (short interspersed nuclear elements) and other mRNAs to form RNPs.

(vi) From the cytoplasm the L1 RNP complex imports into the nucleus.

(vii) Reverse transcription of the L1 mRNA occurs producing the L1 complementary DNA (L1 cDNA). The endonuclease activity of ORFp targets a DNA sequence (5=-TTTTAA-3=), nicks it and the L1 element is inserted. This process is called 'target-site primed reverse transcription' (TPRT).[2]

MECHANISMS THAT SILENCE TEs

It is obvious that L1 elements like other TEs can cause havoc when unleashed. That is why the host response to limit the harmful effects of these TEs is a multilayered one directed at the various stages of their life cycle. Below are some of the known processes that the mammalian somatic cell has developed to repress their activity. Many of the processes 'cross talk' with each other and form complicated networks involving several stages and molecules. These repressive mechanisms have been found to be deregulated in cancer cells or in cells that are exposed to environmental carcinogens, thus allowing L1 retrotransposition and propagation.

One of the somatic mechanisms that the host cell uses to inhibit the expression of L1 and its transposition is 'epigenetic repression'. Epigenetics refers to reversible chemical mechanisms that affect gene expression without altering the DNA sequence. Some of these epigenetic mechanisms affect the L1 5'UTR and notably include DNA hypermethylation, repressive histone modifications (H3K9me3, H3K20me3) and recruitment of the nucleosome remodeling and deacetylase (NuRD) complex. These changes cause the chromatin structure to condense into the 'inaccessible' heterochromatin and so transcription cannot occur. Another epigenetic repressive mechanism is that by micro-RNAs. Micro-RNA-induced L1 silencing is mediated by the micro-RNA binding directly to the L1 mRNA, which is then degraded. Specifically one such micro-RNA is microRNA-128.[3]

Besides micro-RNAs, L1 mRNA is also degraded or suppressed by small interfering RNAs (siRNAs) and piwi-interacting RNAs (piRNAs).[4]

Another system that is triggered when L1 is activated is that involving the Apolipoprotein B (apo B)-editing catalytic (APOBEC) gene family. Its action is to catalyse the deamination of cytosine to uracil in L1 mRNA.[5] These cytosine-to-uracil point mutations are then recognized and L1 mRNA is degraded by endonucleases or RNA degrading enzymes.

Another mechanism is the preferential insertions of L1 sequences to gene-poor regions like those found in chromosome 13.[6] Chromosome 13 (like chromosome 18, 21, and Y) is a gene-poor chromosome. Thus, new insertions of L1s will unlikely disrupt any gene loci.

L1 mRNA and its proteins can also be sequestered into storage centres called stress granules (SG). These granules are cytosolic membraneless compartments where stalled translation complexes are stored. Being thus sequestered, the RNP is prevented from entering into the nucleus and complete the retrotransposition cycle.[7] Subsequently, the L1 mRNA and its proteins are degraded in cytoplasmic membraneless P-bodies (PB) or autophagosomes. PBs and SGs then 'dock' and shuttle the LI mRNA and its proteins.[8,9]

If by any chance the above mechanisms fail and LI RNP manages to enter into the nucleus, other inhibitory mechanisms come into play. An important one involves the highly conserved ERCC1/XPF complex (excision repair cross-complementation group 1/ xeroderma pigmentosum complementation group F), which has endonuclease activity and is involved in various DNA repair pathways to keep the genome stable. Specifically, here, during the TPRT process the complex recognizes the cDNA and removes it, restoring the DNA sequence.[10]

LINE-1 MOBILISATION AND TUMORIGENESIS

Transposable elements shaped and still are shaping our genome. However, their jumping around can cause genomic instability and cause disease, including cancer.

Several mechanisms have been proposed for the role of L1 in tumorigenesis. L1 can be involved directly or indirectly. Briefly, when they insert their sequence through retrotransposition, L1 directly can disrupt or dysregulate the structure and function of genes by the following mechanisms (Table 1).

Mechanism	Reference
Introduction of an early stop codon	(11)
Polyadenylation	(12)
Deletions	(13)
DNA CpG island and histone methylation	(14)
Aberrant splicing	(15)
Insertion of new promoter sequences	(16)
Alteration in gene regulatory networks	(17)

Table 1. Mechanisms relating to the disruption or dysregulation of genes.

Indirectly, L1 mRNAs and its proteins can be used in cellular functions other than those used for the retrotransposition, favouring tumour growth. Table 2 shows some of the mechanisms involved.

Mechanism	Reference
L1 chimeric transcripts	(18)
L1 miRNA sequestering miRNA by competing with cellular endogenous RNAs (ceRNA)	(19)
L1 mediation in Epithelial-to-Mesenchymal Transition (EMT)	(20)

Table 2. Role of L1 mRNAs and its proteins in tumour growth.

LINE-1 AS BIOMARKERS IN CANCER

A biomarker is of benefit if it can provide useful information in the diagnosis (especially early detection) and/or prognosis and/or monitoring of the effect of treatment. It can also help in the stratification of patients and hence in their tailored treatment. The extensive accumulating research on L1 is showing that it has the potential to be such a biomarker in cancer. Specifically, the following are being proposed as L1 biomarkers: L1-ORF1, L1-ORF2, L1-mRNA, L1-methylation status and L1-DNA.

Various cancers release cancer cells into the circulation. Circulating L1-DNA from these circulating cancer cells has been proposed by Sunami et al.[21] as a biomarker in breast cancer. They found that L1-DNA was higher in breast cancer patients and could be useful in detecting early-stage breast cancer. Also, they observed a correlation between LI-DNA copy number and tumour size. Another study[22] also showed that circulating L1-DNA level was associated with tumour size.

Other feasible L1-biomarkers are L1-mRNA and its proteins (L1-ORF1 and L1-ORF2). L1-mRNA can be profiled in tumours. More importantly, using tissue specific markers, L1-mRNA can be traced back to the tissue releasing L1,[23] thus serving as an indirect approach to determine the tissue of origin. Over-expression of L1-ORF2 protein was reported in colon, lung, breast and prostate cancer of epithelial phenotypes.[24] Similarly, expression of L1-ORF1 protein was found in several invasive tumours[25] and was associated with a poor prognosis.

It has been shown that L1-hypomethylation is linked with a poor prognosis. For example L1-hypomethylation in tissue samples of ovarian cancer,[26] colon cancer,[27] glioblastoma multiforme[28] and hepatocellular carcinoma[29] was associated with a lower 5-year survival rate. L1-hypomethylation can also be used to stratify patients to treatment.[30]

LINE-1 AS A POTENTIAL THERAPEUTIC TARGET IN CANCER

L1 life cycle offers a plethora of molecules that can be targeted to inhibit its propagation and thus inhibit L1-mediated mutagenesis.

Line-1's ORF2 reading frame encodes the protein ORF2p which has endonuclease and reverse transcriptase activities. L-1 inhibition by reverse transcriptase inhibitors can thus be a potential treatment in cancer by targeting reverse transcriptase activity.

In 2005, Sciamana et al.[31] used two reverse transcriptase inhibitors (nevirapine and efavirenz) to slow down the growth proliferation of cells in cell lines of prostatic carcinoma and malignant melanoma.

In 2010, Carini et al.[32] also showed the effectiveness of another reverse transcriptase inhibitor, abacavir, on the proliferative growth of prostatic cancer.

In 2014, the FAVE clinical study[33] showed the efficacy of efavirenz in metastatic castration-resistant cancer of the prostate. In a follow-up phase 1 clinical trial (Identifier: NCT01878890) called ESCALE, efavirenz was tested on patients with solid tumours and Non-Hodgkin lymphoma in higher doses than those used in the FAVE study. This was done to test doses above 600mg daily in order to establish the maximum tolerated therapeutic dose.

Translational medical researchers have always looked into photochemicals for their therapeutic use. This is because they function in numerous biological pathways and besides, these usually have a low toxic profile. Nishikawa et al.[34] screened various phytochemicals and found that capsaicin has reverse transcriptase inhibition properties and it suppresses L1 retrotransposition. They thus propose that one way in which capsaicin may defeat the progression of tumorigenesis is by inhibiting L1-mediated mutagenesis. They also propose further studies to investigate capsaicin and related compounds called capsaicinoids in the quest to prevent and treat cancer.

RNA-based treatments are also gaining momentum. Another pathway of treating cancer based on L1 silencing might be the use of micro-RNA mimics. It has already been

pointed out that miR-128 represses L1 activity. Idica et al.[35] found that miR-128 binds directly to L1 ORF2 RNA thus repressing its transposition.

CONCLUSION

The ubiquitous transposable elements have been underappreciated because they have been labelled as unimportant and also, because of their sheer high number of copies and variants, created technical challenges.

These obstacles have now been overturned. Unfaltering research and new methodical platforms are showing that dysregulated expression of TE is a characteristic of cancer and possibly plays an important role in tumour initiation and progression. As more ongoing translational research on TEs unfolds, one hopes that it will soon be applied in oncology clinical practice in the field of diagnostic and prognostic biomarkers and also, as potential therapeutic targets.

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All this was delivered between March 2022 and May 2022 thanks to a great team. Thank you all for your participation.

DR PIERRE VASSALLO

Management of Incidentally Noted Adnexal Cystic Lesions

Adnexal cysts are a very common incidental finding on all cross-sectional imaging modalities, specifically ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI). They may cause unnecessary concern and lead to unnecessary imaging and possibly also to surgical interventions.

In one study, 15% of patients undergoing routine pelvic US were referred for follow-up of an incidentally noted adnexal cyst.[1] In another study that evaluated almost 11,600 women undergoing surgery for adnexal masses, 21% were benign serous cysts and 15% were functional cysts;[2] surgery would have been avoided in most of these cases had a standardised clinical/imaging-based classification been employed.

A large majority of adnexal cysts are benign and can be managed with imaging or no follow-up. Management decisions are based on the patient's age and pre/post-menopausal status, and on imaging features detected on US.

CLASSIFICATION OF ADNEXAL CYSTS

There are three main consensus articles published between 2019 and 2020 that can be combined to classify cystic lesions based on clinical and imaging findings;[3-5] these documents recommend further management based on lesion classification. In spite of some size differences between the three documents, a combined classification has been recommended and may be applied in clinical practice for patients who are not pregnant and who have an average risk for ovarian cancer.[6] Average risk is a rather vague classification, but it usually refers to those women who have no genetic predisposing conditions and no personal or family history of cancer.

The Society of Radiologists in Ultrasound (SRU) (3) recommends that premenopausal 5-10cm simple cysts are optimally re-evaluated after 6-12 months, whereas the Ovarian-Adnexal Imaging-Reporting-Data System (O-RADS)[4] advises that they are re-evaluated after 8-12

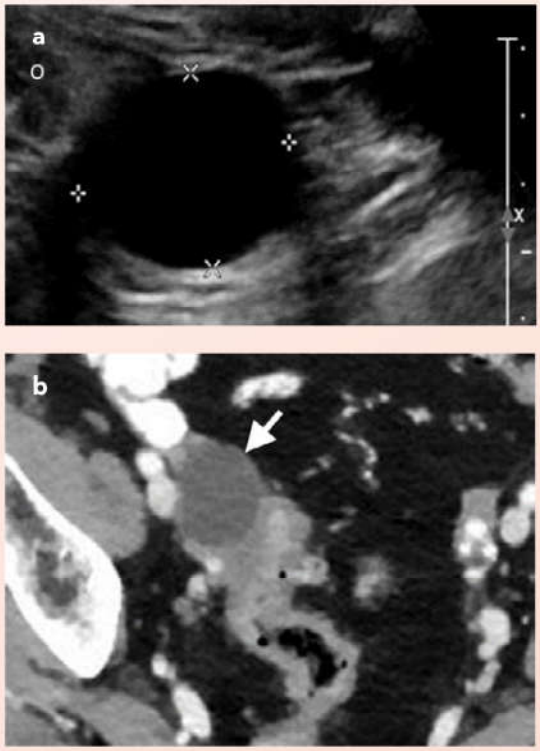

Figure 1.a. Transvaginal US image in a post-menopausal woman showing a 3cm simple cyst (between callipers) with thin walls and no internal structure. Ovary (O). b. CT scan image showing an incidental 3.5cm cyst (arrow) in a post-menopausal woman that had not changed from a CT scan taken 5 years earlier.

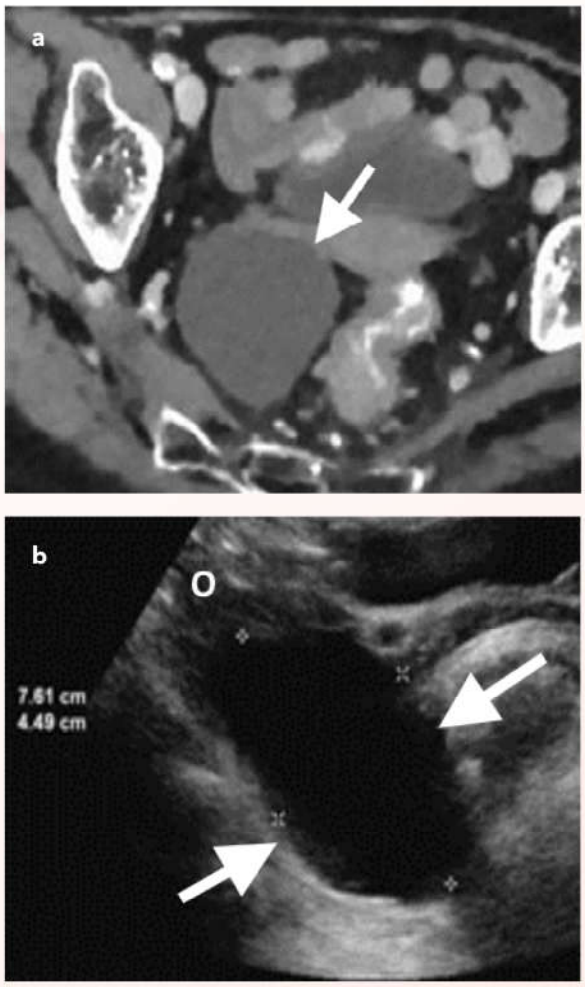

Figure 2.a. Incidentally noted 7cm cyst (arrow) on a CT scan in a post-menopausal woman. b. US follow-up performed after 12 weeks confirms the simple nature of the cyst with no change in size. The lesion was excised at the patient's request. Histological analysis confirmed a cystadenoma, which is a benign tumor.

weeks. SRU advises short-interval follow-ups (6-12 weeks), or an MRI when an adnexal cyst of any size regardless of menopausal status is not adequately visualised with US, whereas the American College of Radiology Incidental Findings Committee (ACR-IFC)[5] recommends that a simple-appearing cyst with limited assessment at CT or MRI does not require further imaging when under 5cm in a pre-menopausal patient or under 3cm in a post-menopausal patient.

It is difficult to implement recommendations from all three aforementioned sources in clinical practice. The mission of the radiologist who detects an adnexal lesion is to classify the lesion according to the following four categories:[7]

a. Very likely non-neoplastic: no further follow-up required;
b. Most likely benign even if neoplastic: imaging follow-up required;
c. Cannot be confirmed benign but low likelihood of malignancy: further cross-sectional imaging and gynaecologist consultation;
d. Moderate-to-high likelihood of malignancy; further cross-sectional imaging and surgical/ oncologic intervention.

A unilocular cyst with thin smooth walls containing simple fluid and no solid or vascular components is almost always benign; such lesions are called simple adnexal cysts (Fig 1). Such a lesion, if it is smaller than 5cm in a pre-menopausal woman or smaller than 3cm in a post-menopausal woman, is characterised as very likely non-neoplastic, and no further imaging or follow-up is required.

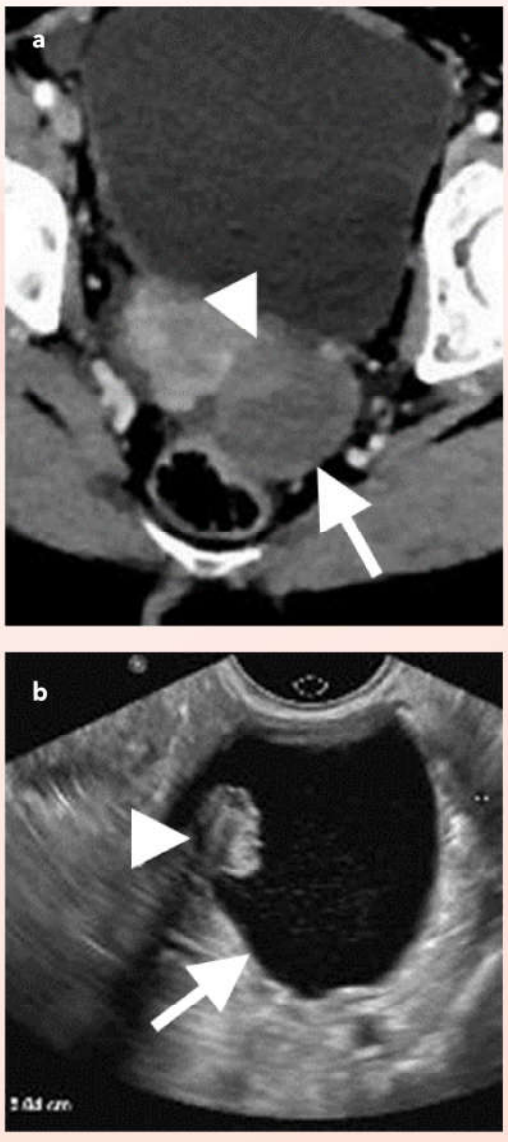

Figure 3.a. Incidentally noted 5cm non-simple cyst (arrow) on CT containing a mural solid component (arrowhead) in a post-menopausal woman. Such a lesion should be imaged further with transvaginal US or MRI. b. US image shows a unilocular cyst (arrow) with a mural nodule (arrowhead).

Larger unilocular cysts (>5cm in pre-menopausal and >3cm in post-menopausal women) that exhibit thin walls and contain no solid or vascular components should be classified as most likely benign even if neoplastic. Such lesions require imaging follow-up with transvaginal US being the imaging modality of choice (Fig 2).

Cystic lesions that show solid components, irrespective of size, should be classified as having a low likelihood of malignancy, but one cannot exclude it. While many of these are benign, the possibility of malignancy mandates further imaging and in most cases surgical intervention (Fig 3).

Cystic lesions showing large solid and hypervascular components particularly in the presence of ascites should be considered as having moderate-to-high likelihood of malignancy irrespective of menopausal state (Fig 4).

Any lesions that fall within categories b and c listed above should be followed up by the imaging radiologist; this will benefit the patient through detection of any changes that alter the lesion's category, even though this may be rare. In addition, it is beneficial to the radiologist's personal education as it will improve his/her ability to characterise such lesions. Lesions showing features that suggest a specific diagnosis such as hemorrhagic cysts, hydrosalpinges, peritoneal inclusion cysts, endometriomas, and dermoids may change on follow-up scans and require a revised diagnosis.

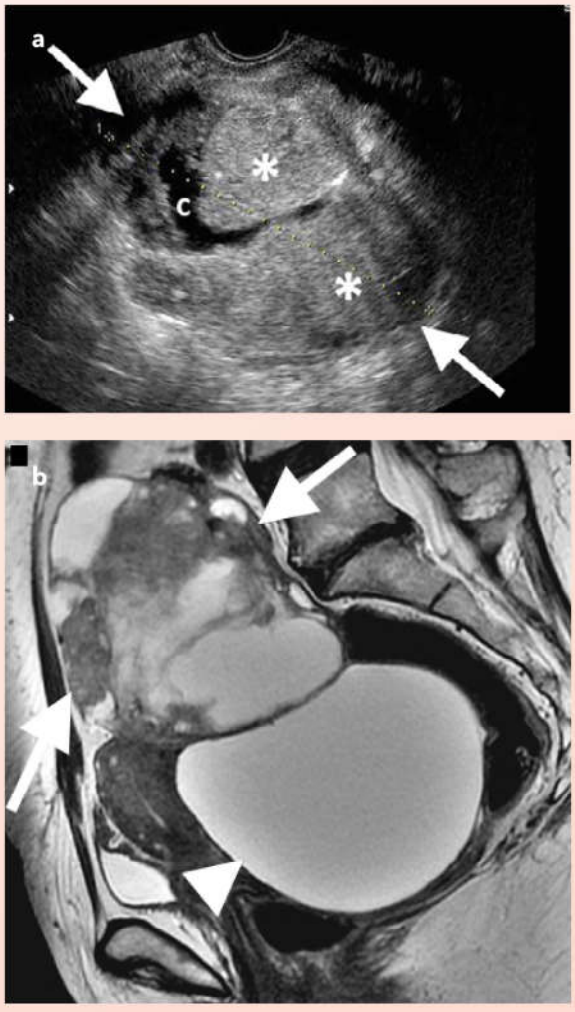

Figure 4.a. US showing an ovarian mass that is mostly solid (*) with cystic component (c) in a pre-menopausal woman; solid components were vascular on Colour Doppler US (not shown here). b. Sagittal T2-weighted MR image in a different post-menopausal patient showing a large complex ovarian mass (arrows) above the bladder (arrowhead). Both lesions a and b were confirmed to be ovarian cancers.

CONCLUSION

Based on a large volume of imaging research studies, we now know that a simple adnexal cyst does not significantly increase a woman's likelihood of ovarian malignancy regardless of menopausal status. Considerable progress has been made over the past 50 years through advances in diagnostic imaging from the time when all palpable postmenopausal ovaries went to surgery. Simple cysts measuring <5cm in pre-menopausal and <3cm in post-menopausal women do not require further follow-up. Larger lesions and those showing complex features require further imaging, consultation and/or surgical intervention based on the criteria described above.

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Contraindications: Hypersensitivity to the active substances or to any of the excipients. Concomitant use with ACE inhibitors. Do not administer until 36 hours after discontinuing ACE inhibitor therapy. Known history of angioedema related to previous ACE inhibitor or ARB therapy. Hereditary or idiopathic angioedema. Concomitant use with aliskiren-containing medicinal products in patients with diabetes mellitus or in patients with renal impairment (eGFR <60 ml/min/1.73 m²). Severe hepatic impairment, biliary cirrhosis and cholestasis. Second and third trimester of pregnancy.

Warnings/Precautions: Dual blockade of the renin angiotensin-aldosterone system (RAAS): Combination with an ACE inhibitor is contraindicated due to the increased risk of angioedema. Sacubitril/valsartan must not be initiated until 36 hours after taking the last dose of ACE inhibitor therapy. If treatment with sacubitril/valsartan is stopped, ACE inhibitor therapy must not be initiated until 36 hours after the last dose of sacubitril/valsartan. Combination of Entresto with direct renin inhibitors such as aliskiren is not recommended. Entresto should not be co administered with another ARB containing medicinal product. Hypotension: Treatment should not be initiated unless SBP is ≥100 mmHg. Patients with SBP <100 mmHg were not studied. Cases of symptomatic hypotension have been reported in patients treated with sacubitril/valsartan during clinical studies, especially in patients ≥65 years old, patients with renal disease and patients with low SBP (<112 mmHg). Blood pressure should be monitored routinely when initiating or during dose titration with sacubitril/valsartan. If hypotension occurs, temporary down-titration or discontinuation of sacubitril/valsartan is recommended. Impaired or worsening renal function: Limited clinical experience in patients with severe renal impairment (estimated GFR <30 ml/min/1.73m²). There is no experience in patients with end-stage renal disease and use of sacubitril/valsartan is not recommended. Use of sacubitril/valsartan may be associated with decreased renal function, and down-titration should be considered in these patients. Impaired renal function: Patients with mild-moderate renal function are more at risk of developing hypotension while patients with severe renal impairment may be at a greater risk of hypotension. sacubitril/valsartan is not recommended in patients with end-stage renal disease. Hyperkalaemia: Treatment should not be initiated if the serum potassium level is >5.4 mmol/l. Monitoring of serum potassium is recommended, especially in patients who have risk factors such as renal impairment, diabetes mellitus or hypoaldosteronism or who are on a high potassium diet or on mineralocorticoid antagonists. If clinically significant hyperkalaemia occurs, consider adjustment of concomitant medicinal products or temporary down-titration or discontinuation . If serum potassium level is >5.4 mmol/l discontinuation should be considered. Angioedema: Angioedema has been reported with sacubitril/valsartan. If angioedema occurs, discontinue sacubitril/valsartan immediately and provide appropriate therapy and monitoring until complete and sustained resolution of signs and symptoms has occurred. It must not be re administered. Patients with a prior history of angioedema were not studied. As they may be at higher risk for angioedema, caution is recommended if Entresto is used in these patients. Black patients have an increased susceptibility to develop angioedema. Patients with renal artery stenosis: Caution is required and monitoring of renal function is recommended. Patients with NYHA functional classification IV: Caution should be exercised due to limited clinical experience in this population. Patients with hepatic impairment: There is limited clinical experience in patients with moderate hepatic impairment (Child Pugh B classification) or with AST/ALT values more than twice the upper limit of the normal range. Caution is therefore recommended in these patients. B-type natriuretic peptide (BNP): BNP is not a suitable biomarker of heart failure in patients treated with sacubitril/valsartan because it is a neprilysin substrate. Psychiatric disorders: Psychiatric events such as hallucinations, paranoia and sleep disorders, in context of psychotic events, have been associated with sacubitril/valsartan use. If a patient experiences such events, discontinuation of sacubitril/valsartan treatment should be considered.

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Undesirable effects: *Very common (≥1/10)*: Hyperkalaemia, hypotension, renal impairment. *Common (≥1/100 to <1/10)*: Anaemia, hypokalaemia, hypoglycaemia, dizziness, headache, syncope, vertigo, orthostatic hypotension, cough, diarrhoea, nausea, gastritis, renal failure, acute renal failure, fatigue, asthenia. *Uncommon (≥1/1,000 to <1/100)*: Hypersensitivity, postural dizziness, pruritis, rash, angioedema.

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