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About the Masterclass

The International Masterclass in Diabetes Management will consist of a series of 13 on demand elearning sessions and ten live webinars. The planned launch date will be Quarter 4 of 2021. All sessions will be delivered by leading diabetologists and specialists in their respective field. In this way, the Master Class will consist of:

- **Thirteen eLearning sessions**: these will take the form of pre-recorded video lectures with completion of Multiple-Choice Questions: participants will be invited to watch the session on their own time and to answer a set of MCQs. Completion of any sessions will earn 1 CME credit each. The eLearning sessions currently scheduled are listed below.
- **Ten live webinars** including a Question-and-Answer session: these webinars will take the form of ninety-minute live sessions with faculty and participants having a direct discussion. Participation in these webinars will earn 1.5 credits each. The titles of the webinars are yet to be confirmed we are actively seeking the participation of international faculty in the masterclass to broaden the mix of inputs.

Scientific Programme:

Programme Launch 18 October 2021 Pre recorded eLearning sessions on cme30.eu

Identification & Management of Pre-Diabetes	**Bio:** Dr Mark Gruppetta
Managing Overweight & Obesity in Type 2 diabetes	**Bio:** Dr Rachel Agius
Guidelines for the Management of Type 2 Diabetes	**Bio:** Dr Sandro Vella
Adult Type 1 diabetes in Primary Care	**Bio:** Dr Alexia Abela
Initiation of insulin in Type 2 Diabetes	**Bio:** Prof Josanne Vassallo
Reducing Cardiovascular Burden in Diabetes – Risk Factor Control	**Bio:** Prof Stephen Fava
Reducing Cardiovascular Burden in Diabetes 2 – Use of novel drugs	**Bio:** Prof Stephen Fava
Nephroprotection in Diabetes	**Bio:** Prof Stephen Fava
Diabetic eye disease	**Bio:** Dr Maria Agius
Prevention & Management of Diabetic Foot Problems	**Bio:** Dr Mario Cachia
Diabetic Neuropathy	**Bio:** Dr David Coppini
Planning Pregnancy in Patients with Diabetes	**Bio:** Dr Katia Vella
Gestational Diabetes	**Bio:** Dr Johann Craus

Testimonials

Prof. Francesco Carelli
Professor Family Medicine University of Milan, Italy
"CME30.eu Webinars and Courses see a lot of participants at every session, because are very useful, well managed, with good choices for expert speakers, and participants are greatly involved, putting series of questions and open discussions."

Dr Tonio Piscopo
Consultant in Internal Medicine and Infectious Diseases Foundation School Director

"As Foundation School Director, I have seen CME on thesynapse.net flourish from sporadic but ambitious beginnings into a serious e-learning platform led by a medical doctor who has been on the forefront of online medical education from the very start of the net. It is delivered by high level specialists and educators who are at the forefront of their respective fields of expertise. The short, targeted videos of CME30 will not disappoint students, trainees or indeed specialists in other fields, who wish to improve their learning or keep updated in the respective topics."

Funding

The Master Class will be free to participants and the production will be supported by an unrestricted grant by Bioton and PAC3 Ltd. The scientific content will be completely independent and in no way influenced by the sponsors. The funds will only be used to cover the cost of the production and to provide a token honorarium to the speakers.

More information

Details and registration for updates for this masterclass are available on **www.diabetesmasterclass2021.com**

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Authors

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Lorna Muscat Baron B.Sc.(Hons)(Melit.) graduated in medical biochemistry in 2020 and is currently a second year medical student at the University of Malta.

Dr Byron Baron B.Sc.(Hons)(Melit.) PhD is a researcher and lecturer at the University of Malta. His research area is functional proteomics, with particular focus on methylation of non-histone proteins and the methyltransferase enzymes responsible for such changes.

Dr Alfred Grech MD graduated from the University of Malta in 1985. He has been working in Primary Health for these last 30 years. His special interests are molecular biology and epigenetics. As a pastime he cultivates bonsai trees and plays his sax alto. The co-authors of the article are Dr Stephen West and Dr Ramon Tonna.

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Issue Guide

TheSynapse

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ENTRESTO®(sacubitril/valsartan)

Presentation: Each film-coated tablet of Entresto 24 mg/26 mg, 49 mg/51 mg and 97 mg/103 mg contains sacubitril and valsartan respectively (as sacubitril valsartan sodium salt complex).

Indications: In adult patients for treatment of symptomatic chronic heart failure with reduced ejection fraction.

Dosage & administration: The recommended starting dose of Entresto is one tablet of 49 mg/51 mg twice daily, doubled at 2-4 weeks to the target dose of one tablet of 97 mg/103 mg twice daily, as tolerated by the patient. In patients not currently taking an ACE inhibitor or an ARB, or taking low doses of these medicinal products, a starting dose of 24 mg/26 mg twice daily and slow dose titration (doubling every 3 - 4 weeks) are recommended. A starting dose of 24 mg/26 mg twice daily should be considered for patients with SBP ≥100 to 110 mmHg, moderate or severe renal impairment (use with caution in severe renal impairment) and moderate hepatic impairment. Do not co-administer with an ACE inhibitor or an ARB. Do not start treatment for at least 36 hours after discontinuing ACE inhibitor therapy. Entresto may be administered with or without food. The tablets must be swallowed with a glass of water. Splitting or crushing of the tablets is not recommended.

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 m^2). 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/1.73m^2). 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.

Interactions: Contraindicated with ACE inhibitors, 36 hours washout is required. Use with aliskiren contraindicated in patients with diabetes mellitus or in patients with renal impairment (eGFR <60 ml/min/1.73 m^2). Should not be coadministered with another ARB. Use with caution when co-administering sacubitril/valsartan with statins or PDE5 inhibitors. No clinically relevant interaction was observed when simvastatin and sacubitril/valsartan were co-administered. Monitoring serum potassium is recommended if sacubitril/valsartan is co-administered with potassium-sparing diuretics or substances containing potassium (such as heparin). Monitoring renal function is recommended when initiating or modifying treatment in patients on sacubitril/valsartan who are taking NSAIDs concomitantly. Reversible increases in serum lithium concentrations and toxicity have been reported during concomitant administration of lithium with ACE inhibitors or angiotensin II receptor antagonists including sacubitril/valsartan. Therefore, this combination is not recommended. If the combination proves necessary, careful monitoring of serum lithium levels is recommended. Co-administration of sacubitril/valsartan and furosemide reduced C_{max} and AUC of furosemide by 50% and 28%, respectively, with reduced urinary excretion of sodium. Co-administration of nitroglycerin and sacubitril/valsartan was associated with a treatment difference of 5 bpm in heart rate compared to the administration of nitroglycerine alone, no dose adjustment is required. Co administration of sacubitril/valsartan with inhibitors of OATP1B1, OATP1B3, OAT3 (e.g. rifampicin, ciclosporin), OAT1 (e.g. tenofovir, cidofovir) or MRP2 (e.g. ritonavir) may increase the systemic exposure of LBQ657 or valsartan. Appropriate care should be exercised. Co-administration of sacubitril/valsartan with metformin reduced both C_{max} and AUC of metformin by 23%. When initiating therapy with sacubitril/valsartan in patients receiving metformin, the clinical status of the patient should be evaluated.

Fertility, pregnancy and lactation: The use of sacubitril/valsartan is not recommended during the first trimester of pregnancy and is contraindicated during the second and third trimesters of pregnancy. It is not known whether sacubitril/valsartan is excreted in human milk, but components were excreted in the milk of rats. Entresto is not recommended during breastfeeding. A decision should be made whether to abstain from breast feeding or to discontinue Entresto while breast feeding, taking into account the importance of sacubitril/valsartan to the mother.

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.

Packs sizes: Entresto 24 mg/26 mg – x28 tablets; Entresto 49 mg/51 mg – x28 tablets; Entresto 97 mg/103 mg - x28 & x56 tablets.

Legal classification: POM.

Marketing Authorisation Holder: Novartis Europharm Ltd, Vista Building, Elm Park, Merrion Road, Dublin 4, Ireland.

Marketing Authorisation Numbers: Entresto 24 mg/26 mg film coated tablets EU/1/15/1058/001; Entresto 49 mg/51 mg film coated tablets EU/1/15/1058/002-004; Entresto 97 mg/103 mg film coated tablets EU/1/15/1058/005-007.

Please refer to the 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.

REFERENCES: 1. Maddox TM, Januzzi JL Jr, Allen LA, et al. 2021 update to the 2017 ACC Expert Consensus Decision Pathway for optimization of heart failure treatment: answers to 10 pivotal issues about heart failure with reduced ejection fraction: a report of the American College of Cardiology Solution Set Oversight Committee. J Am Coll Cardiol. 2021;77(6):772–810. 2. Claggett B, Packer M, McMurray JJV, et al; for the PARADIGM-HF Investigators. Estimating the long-term treatment benefits of sacubitril–valsartan. N Engl J Med. 2015;373(23):2289-2290. 3. Lewis EF, Claggett BL, McMurray JJV, et al. Health-related quality of life outcomes in PARADIGM-HF. Circ Heart Fail. 2017;10(8):e003430. 4. ENTRESTO Summary of product characteristics. European Medicines Agency website. http://www.ema.europa.eu. Accessed June 2021.

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Entresto® sacubitril/valsartan

Headwinds
A Catalyst for Change?

Change does not necessarily translate in improvement. But there can be no improvement without change. It is very apt to pen this on this page during this month since we need to celebrate two anniversaries. The first is the 25th anniversary of The Synapse. This was closely followed by the launch of this magazine in 2001 … two decades ago. I joined as editor of the magazine in 2005; what a journey it has been! I still remember the 6 pager in black and white printed back then.

Why am I mentioning all this? I confess that I am very much inclined to walk memory lanes. Alone. I seem to seek solace in the warmth of the sun on my face at St Francis School in Msida when I was young or later on, at the Archbishop's Seminary secondary school; in the strong embrace of my loved ones when I was older; and so many other seemingly small things which still make all the difference to a bruised soul … self-anointed to heal through my life events.

I am mentioning this since nursing one's abrasions and lacerations, although important, is only the first step to good health. One needs to recognise the challenges, understand one's weakness and act proactively. The Synapse was created through such SWOT analysis to precisely overcome the challenges faced by doctors back then, in 1996. These are the same challenges still present today but to a different degree. These relate primarily to lack of timely communication, or miscommunication, between different stakeholders within the medical profession. The Synapse was a game changer through its networking service; in parallel, it seeded camaraderie. Logically, along the years it needed to adapt and develop a different skill-set to embrace other technologies to support this philosophy. I am specifically referring to CME30.eu.

CME30.eu needs no introduction. In the organisation of medical education, CME30.eu delivers the best speakers, addresses the key topics and attracts the right audience. Indeed, this year we have launched numerous CME sessions with audiences at times even reaching 650 participants.

To commemorate the 100 years of the discovery of insulin, CME30.eu liaised with the Diabetes Department of Mater Dei Hospital, Faculty of Medicine & Surgery at the University of Malta as well as the Malta Association of Physicians to develop an international masterclass in Diabetes Management. This online masterclass, accredited by MAM and MCFD, is free of charge. It is currently being launched.

At this stage I wish to comment on the evolution of our eLearning modules. CME30.eu was fortuitously launched a couple of weeks before the pandemic started. I consider CME30.eu as a seminal online platform which has been specifically developed as an eLearning repository for medical doctors, as well as other healthcare professionals. Nonetheless, CME30.eu traces its origin to 2012 when The Synapse launched a series of eLearning modules in collaboration with the Malta Foundation Programme.

Our raison d'etre has always been to provide high quality online medical education for doctors, including CME sessions. We have always believed that eLearning is key for the busy healthcare professional since it eliminates commuting and is thus more effective to deliver the module. It also drastically reduces the logistics and associated costs of organising a CME in some room in a hotel. Obviously, our trailblazing meant a heavy investment from our end in IT infrastructure, training, as well as a modern studio to deliver the online eLearning modules. But who would have imagined that a few weeks after its launch, the paradigm shift created by COVID-19 would have catapulted CME30.eu to the forefront of online medical education in Malta?

We look forward to meeting you during in the free online international diabetes masterclass. The future is here for all to embrace. Will you join?

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

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LEQVIO®

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.

ADVERSE REACTIONS: *Common:* Adverse reactions at the injection site.

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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LEQVIO® inclisiran

GLORIA MONTEBELLO & PROF. KEVIN CASSAR

Vascular Surgery

The Importance of Multidisciplinary Care in Diabetic Foot Amputations

ABSTRACT

In Malta, 10% of the population over 18 years of age is estimated to be living with diabetes. This chronic condition can lead to complications which heavily impact our patients' quality of life. In this case study, complications such as peripheral artery disease which can lead to ulceration and limb amputations are discussed. Providing care in an interprofessional team allows the sharing of best practice and expertise in order to achieve optimal results and ultimately save limbs.

KEY WORDS

Diabetes, Diabetic foot, Amputation, Skin graft, Multidisciplinary care

A 55-year-old Maltese male patient who was diagnosed with DM in 2016 after developing chronic kidney disease 5 years before is now in end stage renal failure (CKD stage V) and is being managed with haemodialysis through a left radiocephalic fistula. The patient has a family history of diabetes in his family. He is a non-smoker and he is a social drinker. Other comorbidities include diabetic nephropathy, controlled hypertension, morbid obesity and hyperlipidaemia, all of which are being treated.

In 2019, the patient was referred to vascular surgery as he was approaching end stage renal failure. A left radiocephalic fistula at the distal forearm was fashioned. All arteries including the radial and ulnar were heavily calcified but the waveforms in both arteries at the wrist were triphasic. On needling, the venous pressures in the fistula were elevated. Left upper limb fistulography and central venography revealed a left subclavian vein stenosis that was successfully angioplastied.

In January 2021, he complained of a heavy, painful left foot with restricted movements. The second toe was dusky, all toes felt cold and he noted pus discharging from the 2nd toe. He was hospitalised by the vascular surgery team, for investigation and treatment. On assessment significant peripheral arterial disease was diagnosed with absent peripheral pulses and monophasic continuous waveforms at the ankles. Emergency transmetatarsal amputation of the left foot, and left superficial femoral, proximal posterior tibial and anterior tibial artery angioplasty was performed.

The gross diabetic foot infection was treated with IV 4g/0.5g piperacillin/tazobactam every 12 hours and teicoplanin IV 200mg daily. Bone cultures grew *Morganella morganii* which required IV antibiotic treatment for at least 6 weeks and a peripherally inserted ventral venous catheter (PICC line) was inserted in his right arm. The selection of antibiotic treatment was based on advice received from the microbiology team.

The transmetatarsal amputation site was treated with negative pressure treatment and after adequate granulation of the amputated site was achieved, a split skin graft was applied by the plastic surgery team to achieve skin cover.

Podiatric assessment and appropriate education about footcare were provided. Review by the orthotist was performed and he was measured for provision of footwear in an effort to prevent new ulceration. Daily sessions with the physiotherapy team were held to ensure that the patient retained good muscle tone and was able to bear weight safely. Blood glucose and blood pressure control was managed by the diabetologists. The tissue viability team reviewed the patient regularly and provided negative pressure treatment and dressed the surgical wounds appropriately.

The skin graft was healthy and complete skin cover of the amputated foot was obtained. Discharge from hospital was successfully planned with appropriate social support, close monitoring by the community podiatrists and regular physician review with regard to diabetes and blood pressure control. He will continue to be dialysed at

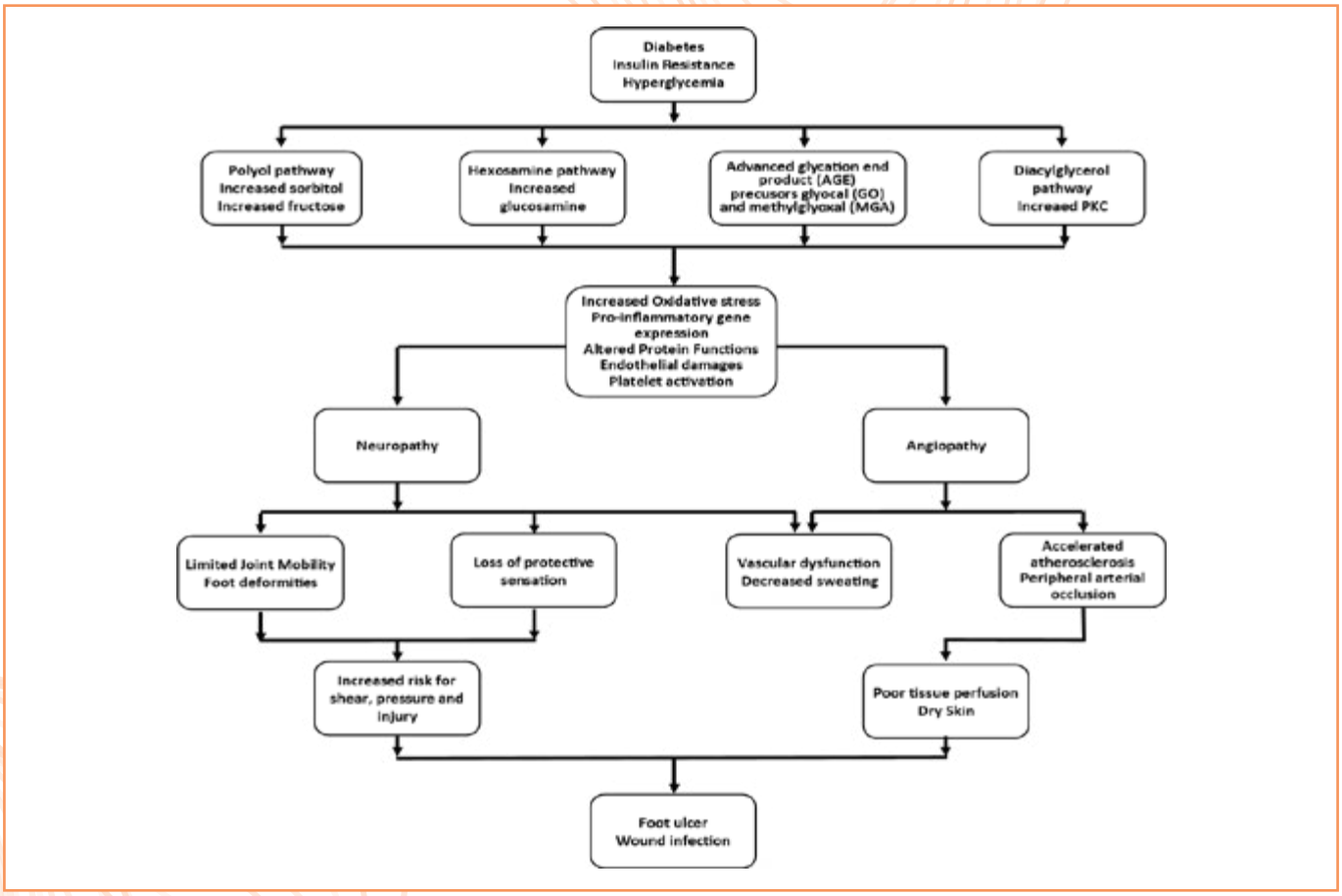


Figure 1: Pathophysiology of diabetic foot ulcers.[1]

the renal unit and his renal failure managed by the renal physicians.

Diabetic ulcers are a common cause of lower extremity amputation. Up to 25% of patients with DM will develop diabetic foot ulcers. It is reported that more than 50% of DM patients are unaware of the disease (as classified by the WHO).[1] Common risk factors for amputation include diabetic neuropathy, structural foot deformity and peripheral arterial occlusive disease.[2]

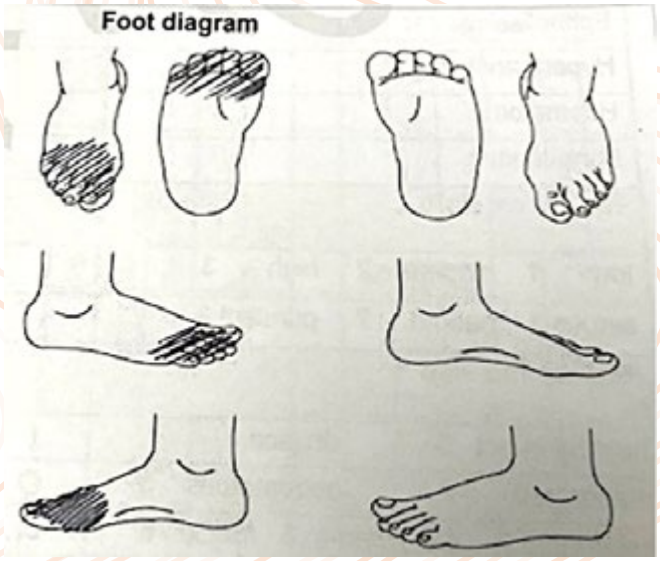


Figure 2: Wound assessment tool of the left foot.

The pathophysiology of the diabetic foot is characterised by hyperglycaemia which drives neuropathy and vascular foot changes and can cause diabetic foot ulcers. Hyperglycaemia inhibits the production of nitric oxide (NO) by limiting endothelial NO synthase activation which can lead to superoxide formation. Enzymatically, superoxide is converted to hydrogen peroxide by superoxide dismutase. The superoxide anion binds to NO to produce peroxynitrite which limits the availability of a potent endothelium-derived vasodilator.

This disruption in endothelial function affects vasoconstriction, and causes platelet aggregation, abnormal intimal growth, inflammation and atherothrombosis formation. Atherogenesis may form due to the vessel wall characteristics with the interaction of inflammatory cytokines following glycoxidation and lipoxidation of vascular wall structural proteins. This atherogenesis of small vessels that supply peripheral nerves contribute to neuropathy.[3]

Vascular disease is the result of micro and macrovascular disease. The inflammatory response in the microcirculation leads to thickening of capillary basement membranes with arteriolar hyalinization. This comprises the normal transfer of nutrients and activated leukocytes between the capillary lumen and the interstitium. The inelasticity of capillary walls leaves a limited capacity for vasodilation in response to local injury and leads to functional ischaemia.[4]

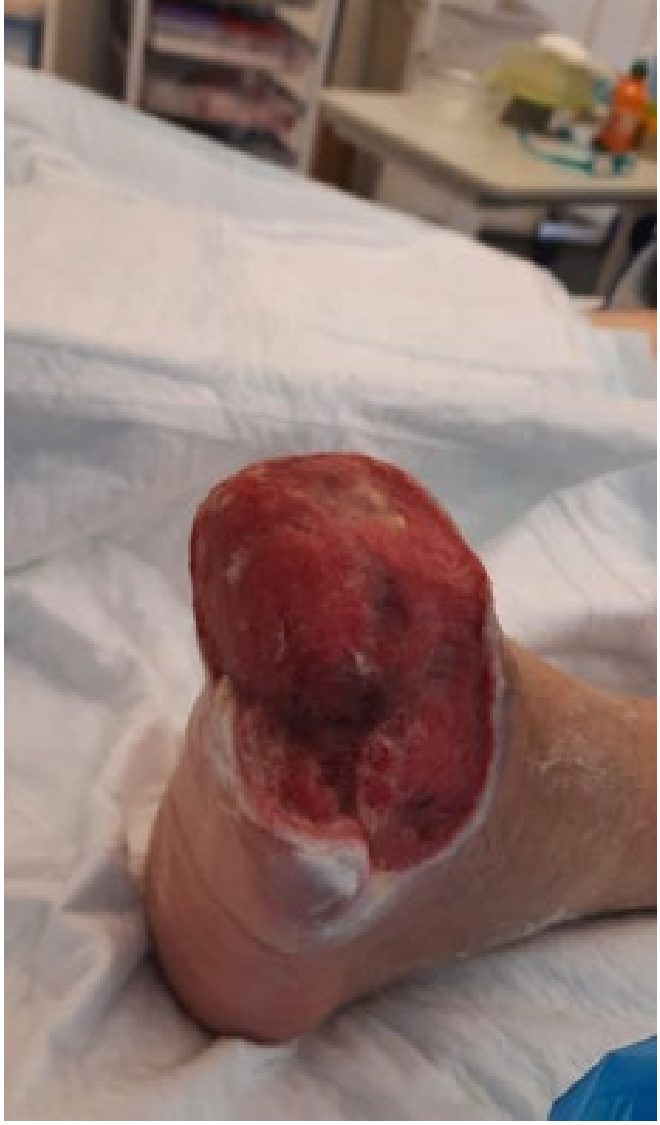
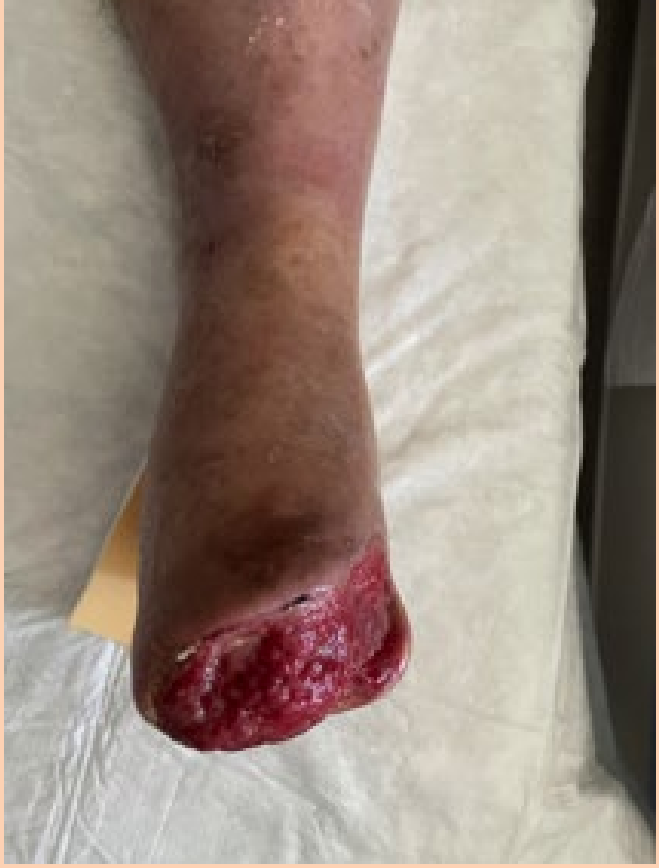


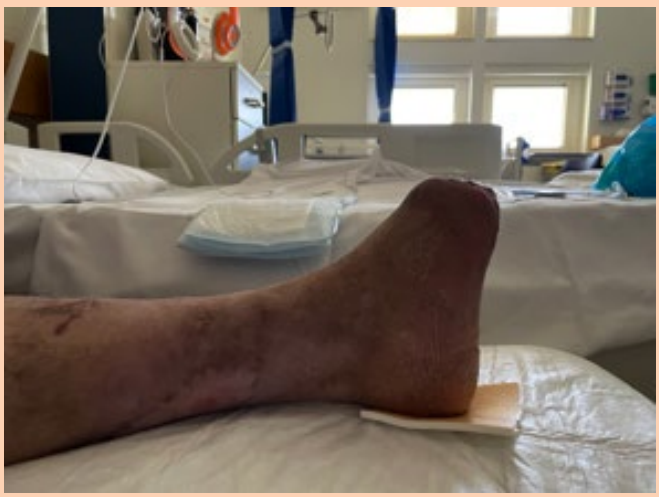
Photo taken by patient after transmetatarsal amputation.

The patient's main concerns were related to his mobility as he had recently lost his job due to the COVID-19 pandemic that contributed to his anxiety and affected his wellbeing. The patient was compliant to physiotherapy with balance exercises since his centre of gravity was altered. Factors that may delay wound healing include respiratory problems, immobility, diabetes, anti-coagulant therapy and any co-existent wound infection. Since the patient was allergic to penicillin, he was given teicoplanin.

The patient also received dietary advice to improve his blood glucose control and weight. Through social services, the patient was installing a stair lift in his home.

Multidisciplinary care is essential in the management of the diabetic foot. Routine foot examination to identify high-risk feet is part of all international recommendations for the care of patients with diabetes. Once an ulcer develops, continuous assessment of adequate perfusion, neurology and any potential infection is of utmost importance. Most ulcers require specialist care to prevent the deterioration and achieve resolution. A gap of interprofessional care may be due to busy schedules, lack of knowledge, lack of routine practice procedures and possible health care organizational barriers.[5]





Photos taken 3 days prior to skin graft operation, at the MDH Diabetic Foot Ward.

This case report concluded that the patient did not recall any previous DM testing and there were no records of any pre-diabetes records. This window of opportunity for lifestyle change and early intervention for this patient have been missed. The management of diabetes and diabetic foot ulcers required interprofessional care from specialised nurses, vascular surgeons, diabetologists, podiatrists, orthotists, physiotherapists and occupational therapists. Moreover, the GP was also part of the team and should be a key player in the community to pick up pre-diabetic cases for early action and referral. Such a team-based framework is essential within our healthcare system in order to utilise best expertise to achieve optimal results and save limbs and function.

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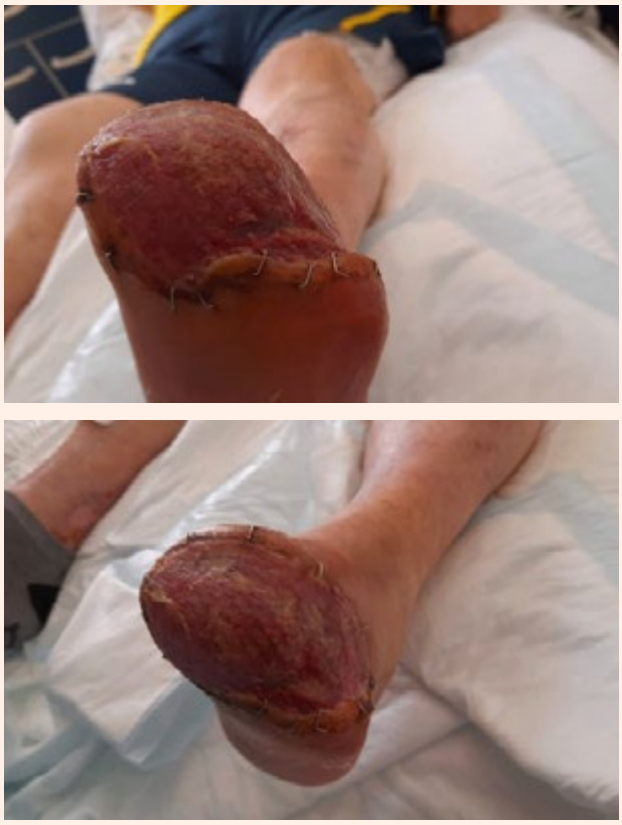

Photos illustrating the result of the skin graft operation on the left foot

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* Cesaroni MR, Belcaro G, Di Renzo A, et al. Prevention of influenza episodes with colostrum compared with vaccination in healthy and high-risk cardiovascular subjects: the epidemiologic study in San Valentino

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DR ALFRED GRECH, DR STEPHEN WEST, DR RAMON TONNA

Transcriptome Profiling in Health and Disease

ABSTRACT

The transcriptome is the collection of RNAs transcribed by a cell or tissue. Transcriptomics is an integral component with other -omics technology. Specifically, it is being used with them in translational research to understand health and disease at different levels, be it single-cell resolution, a tissue or a patient. The knowledge gained will and is being translated in the prevention and management of human diseases, including Covid-19.

INTRODUCTION

The term transcriptome was coined in 1996 by Charles Auffrey, a distinguished researcher at the French National Centre for Scientific Research. The transcriptome is the collection of RNAs transcribed and that includes tRNA (transfer RNA), rRNA (ribosomal RNA), mRNA (messenger RNA) and ncRNA (noncoding RNA). The transcriptome is dynamic and changes temporally and spatially in various cells of the body. Its profiling with the help of bioinformatics is and will provide knowledge of its functionality in human health and disease, including Covid-19. With other –omic technology (genomics, epigenomics, proteomics, metabolomics, microbiomics), transcriptomic profiling is being applied to understand normalcy and pathology, be that of a single-cell, a tissue or a patient. Indeed, transcriptomic profiling is becoming instrumental for stratification of patients, for the selection of better targeted therapies (personalized medicine), and for biomarkers in diagnosis and prognosis. Moreover transcriptomic data is guiding drug repositioning, saving resources and time, and thus speeding the development of drugs.[1]

METHODS USED

The first method to profile the human transcriptome was 'EST (Expressed Sequence Tags) sequencing'. Later on, other methods included 'SAGE' (Serial Analysis of Gene Expression) and 'microarray'. Today, NGS (Next Generation Sequencing)-based RNA-Seq is the method of choice. Notably, the most commonly method used is Illumina sequencing.

RNA-Seq is able to detect the expression of all the genes and also the different species of the RNA world. This yields huge amounts of data that might hold back its use clinically because of costs and feasibility. A practical solution is available in targeted RNA-Seq methods like 'AmpliSeq-RNA panels' that are made to study a relatively small number of gene sets (usually 150 to 900 genes).[2]

Of course, in order to cater for the explosion of data coming from transcriptomics and other –omic platforms, there is constantly the need to find the best bioinformatic pipelines.

CANCER TRANSCRIPTOMICS

There is a lot of research worldwide to understand the molecular mechanisms involved in carcinogenesis. It has now been well-established that mutations in genomic regions that do not code for proteins are also responsible for carcinogenesis. These regions are still transcribed into ncRNAs and their aberrant expression is linked with different types of cancer.

Amongst the thousands of ncRNAs, research is showing that two classes of non-coding RNAs, (i) long non-coding RNAs (lncRNAs) and (ii) small nucleolar RNAs (snoRNAs) co-ordinate metastasis. Creaet al.[3] propose that some snoRNAs and lncRNAs synergistically interact with protein-coding genes, generating a metastatic cellular phenotype. Moreover, they point out that lncRNAs and snoRNAs expression profiles can be potential biomarkers and be of use in the diagnosis and prognosis in cancer. And since the silencing of these lncRNAs and snoRNAs in models causes the death of cancerous cells and their metastasis, Creaet al. propose that they can be potential new targets for cancer treatment as well.

A specific example of a lncRNA implicated in carcinogenesis and the integrated application of –omics (proteomics, genomics and transcriptomics) is that done by Wuet al.[4] The lncRNA is called HOTAIR and stands for HOXTranscript Antisense Intergenic RNA. The study showed that HOTAIR is dysregulated in hepatocellular carcinoma (HCC). Specifically, HOTAIR inhibition was found to be associated with dysregulation of several transcripts and proteins. Functional bioinformatic studies of the data collected showed that these transcripts and proteins relate to biological circuits in cancer. Furthermore, the study showed that HOTAIR caused cell proliferation

partly by its regulation of OGFr expression (opioid growth factor receptor), the latter being known to have a negative regulation on cell proliferation in HCC.

HOTAIR is also dysregulated (specifically it is overexpressed) in breast cancer. This over-expression is responsible in metastasis through HOTAIR recruitment of another complex molecule called Polycomb Repressive Complex 2 (PRC2), which then silences additional genes, besides the HOXD gene cluster. In 2016, Meredith et al.[5] carried out a proteomic analysis and found that other proteins are associated with HOTAIR's action. One such significant interaction is that between HOTAIR and hnRNP (heterogeneous nuclear ribonucleoprotein) A2/B1. This interaction is central to chromatin structure regulation in cells of breast cancer. Indeed, the authors found that knocking down A2/B1 reduced PRC2 activity and also, cell invasion.

Also regarding HOTAIR, Tan et al.[6] from their study propose that the lncRNA HOTAIR can be used as a new biomarker in the prognosis and diagnosis for glioblastoma multiforme (GBM), a primary brain tumour that is very aggressive in adults. In fact, they found that HOTAIR expression is associated with high-grade brain tumours. Several other studies are showing HOTAIR as a potential clinical biomarker in oesophageal squamous cell carcinoma, gastric cancer, thyroid cancer, and colorectal cancer amongst others.

There are other lncRNs besides HOTAIR that are being studied in cancer, like the lncRNA EBLN3P that promotes liver cancer progression[7] and several other lncrNAs (A2M-AS1, DLEU2, LINC01133, LINC00675, MIR155HG, SLC25A25-AS1, LINC01857, LOC642852 (LINC00205), ITGB2-AS1, TSPOAP1-AS1 and PSMB8-AS1) implicated in pancreatic cancer.[8]

Other studies are showing that snoRNAs also have apivotal role in tumorigenesis and can be used as biomarkers.[9] They have been found to be dysregulated in multiple cancers like those of the lung, digestive tract and urologic tract where they participate in tumour cell proliferation and metastasis.[10]

TRANSCRIPTOMICS AND MENTAL DISEASES

Schizophrenia and bipolar disorder are two mental illnesses affecting more than 2% of adults. Their pathophysiology remains unclear but transcriptomic studies are starting to reveal some of the mechanisms involved, implicating dysfunctions in GABA, glutamate and immunological pathways.[11]

In their study, Labontéet al.[12] analysed the known sexual dimorphism in major depressive disorder (MDD). They analysed transcriptomically six brain regions in patients with MDD. They also compared the human transcriptome profiles with that of a mouse model. They characterised sex-specific gene networks, like the female-specific hub gene Dusp6 (Dual Specificity Phosphatase 6) which is downregulated by increased ERK (extracellular signal-regulated kinase) signalling and increased pyramidal neuron excitability. They propose that their findings are further investigated in order to guide treatments for MDD based on its sexual dimorphism.

Also, Gandalet al.[13] profiled the transcriptome of patients with schizophrenia, depression, bipolar disorder, autism and alcoholism with a control of healthy people. They found that there are transcriptional perturbations that are shared, implicating that these neuropsychiatric disorders have molecular pathways that are convergent.

In posttraumatic stress disorder (PTSD), Girgentiet al.[14] have used RNAseq to study transcriptome signatures of PTSD patients. They found aberrant expression of genes in the brain and in peripheral blood cells. These findings are starting to reveal the molecular and cellular mechanisms that underlie PTSD and so will help in its management.

TRANSCRIPTOMICS AND AUTISM SPECTRUM DISORDER (ASD)

Using transcriptomic profiling, Tran et al.[15] analysed post-mortem brains in patients with ASD to understand the culprit molecular mechanisms involved. Specifically they profiled 'adenosine-to-inosine (A-to-I) editing', the latter being the commonest type of RNA editing and is a mechanism that produces RNA and protein diversity, which is not coded from the genome directly. Adenosine-to-inosine (A-to-I) editing is done by RNA-editing enzymes called ADAR (adenosine deaminase acting on RNA) proteins. Their study showed global hypo-editing in ASD brains involving many synaptic genes. Thus, they propose that dysregulation of RNA-editing in ASD might be one of the pathomechanisms involved.

Certain cancers are a co-morbidity in patients with ASD. Forés-Martos et al.[16] carried out a transcriptomic analysis of ASD brains to support this. They found that 4 cancer types (out of the 22 studied), specifically thyroid, kidney, brain and pancreatic cancers, have transcriptomic dysregulation as occurs in ASD. In particular, the study revealed that both diseases have impairments of the immune system, of oxidative phosphorylation and ATP synthesis. Also, the analysis involving the brain and kidney cancers showed dysregulation in the PI3K/AKT/MTOR axis, which is also found in ASD.

TRANSCRIPTOMICS AND AUTO-IMMUNE DISEASE

Studies of the transcriptome profiles of auto-immune disease are showing the potential in identifying biomarkers for their early diagnosis. Also, they will definitely help in the identification of new therapies, and may even help prevent specific autoimmune diseases.

For example in rheumatoid arthritis (RA), Li et al.[17] studied the role of the transcript MEG3 (maternally expressed gene 3), which is a lncRNA. They showed that MEG3 is protective in RA by inhibiting the over-expression of miR-141 and inactivating AKT/mTOR signalling pathway.

Continues on page 18

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What is HPV?

The Human Papilloma Virus (HPV) is a group of more than 150 viruses of small size related to each other, with around 40 having oncogenic potential.

It is transmitted by sexual contact to both men and women and is the most common sexually transmitted infection. It can be found in the cervix, vagina, anal canal, throat, mouth, penis and scrotum.

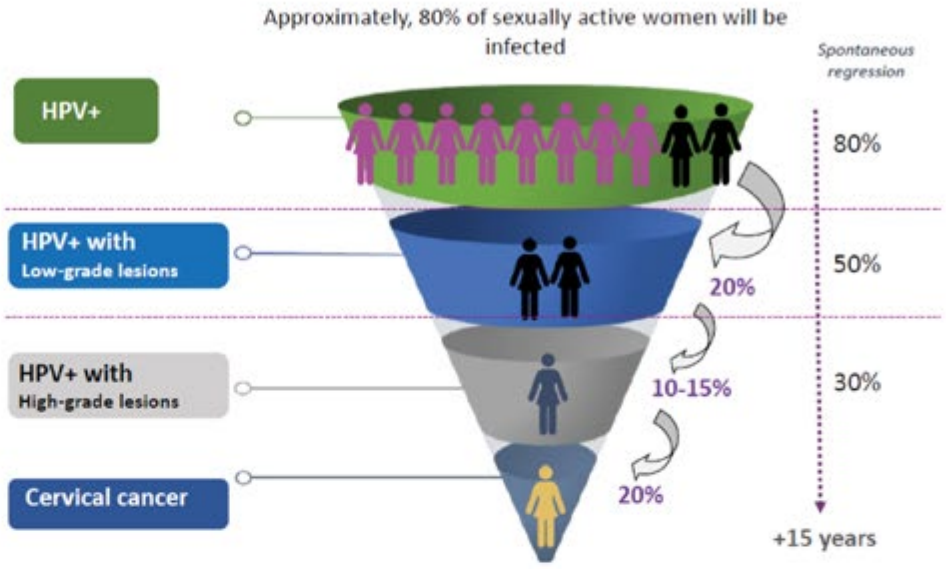
HPV infection is one of the most common sexually transmitted infections causing around 500,000 cases of cervical cancer and 300,000 cervical cancer deaths per year. Cervical cancer is the 2nd most common female cancer in women aged 15 to 44 in Europe. Every two minutes, somewhere in the world, a woman dies from cervical cancer.

VIVIAN

[1]Low grade lesions: ASCUS/LSIL

The Natural History of HPV infection

HPV infects the basal cells of the organs' lining. As the infected cells divide, the virus begins to make copies of itself. For most people, the immune system can destroy the infected cells along with the virus within 2 years whilst in others, the immune system is not able to destroy the virus, leading to persistent infection. If the HPV is high risk, it may damage the cells genetic material, causing the cells to become precancerous.



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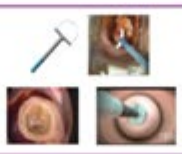
HPV Management depending on the situation

No infection: ➡ Primary prevention: Avoid infection

HPV+ without lesions: ➡ Secondary prevention: Prevent Lesions

HPV + with lesions : ➡ Tertiary Prevention: Prevent Lesion Progression = Lesion treatment



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The authors propose that MEG3 holds great potential in managing RA.

In systemic lupus erythematosus (SLE), Panousiset al.[18] identified transcriptome signatures for SLE susceptibility and severity. Specifically they showed three signatures, (i) a 'susceptibility signature' in clinical remission, (ii) an 'activity signature' associated with regulation of immune cell metabolism and (iii) a 'severe signature' especially in patients with nephritis. They propose that their findings could be used in the diagnosis and stratification of patients and hence assist in personalised management given the vast heterogeneity in SLE. To tackle this heterogeneity, Rai et al.[19] had also done a transcriptomic analysis to stratify SLE patients based on autoantibody specificities. Again they identified SLE subgroups implicating specific immunological pathomechanisms that could assist in theranostics of SLE patients.

TRANSCRIPTOMICS AND THE HUMAN MICROBIOME

Transcriptomics together with genomics, proteomics and metabolomics is being used to identify the human microbiome and its gene products.[20] It is being shown that each individual has a dynamic microbiome that changes temporally and spatially in the body and that it has a pivotal role in health and disease. Again such information is being translated in the clinical setting for personalized theranostics. For example, in HBV-associated hepatocellular carcinoma, gut microbiome-transcriptome signature studies have shown that patients' gut microbes have the potential to be used as prognostic biomarkers. In inflammatory bowel diseases, similar gut microbiome-transcriptome studies during inflammatory bowel disease activity showed functional dysbiosis of the gut microbiome. Such signatures have the potential to monitor the inflammation.

TRANSCRIPTOME STUDIES IN COVID-19

The ongoing COVID-19 pandemic is causing overwhelming mortality world-wide. In order to study the molecular mechanisms of the immune response towards this infectious disease, Xiong et al.[21] transcriptomically analysed bronchoalveolar lavage fluid (BALF) and peripheral blood mononuclear cells from patients with Covid-19. They found out a disproportionate cytokine release of CCL2/MCP-1, CXCL10/IP-10, CCL3/MIP-1A, and CCL4/MIP1B. Moreover, their transcriptomic signatures showed that the lymphopenia seen in COVID-19 patients might be due to activation of apoptosis and P53 signalling pathway in lymphocytes. They also propose that their transcriptomic data can be further investigated to guide anti-inflammatory therapy.

Along similar transcriptomic pipelines, Fagone et al.[22] analysed the pathomechanisms brought about by SARS-CoV-2. Their transcriptomic profiles indicated potential therapeutic drugs like mitogen-activated protein kinase (MEK), serine-threonine kinase (AKT), mammalian target of rapamycin (mTOR) and I kappa B kinase (IKK) inhibitors.

Gardinassiet al.[23] also used transcriptomic platforms to analyse the pathophysiology of this new complex infectious disease. They found amongst other things that there is an over-expression of genes connected to oxidative phosphorylation in BALF and peripheral mononuclear leukocytes. This implicates mitochondrial activity during SARS-CoV-2 infection. They also found increased gene expression associated with the biosynthesis of heme, which is known to be a mechanism that offers protection against oxidative stress. It is also known that hypoxia alters gene expression that code for proteins important for heme biosynthesis. Knowing all this and together with their findings, Gardinassiet al. thus propose that the excessive accumulation of heme might be intensifying the production of pro-inflammatory cytokines or cause the observed increased coagulation inside blood vessels in COVID-19.

TRANSCRIPTOME-GUIDED DRUG REPOSITIONING

Drug repositioning (also called drug repurposing)[24] investigates existing drugs for new therapy. It facilitates discovering new mode of actions of old drugs as well as the discovery of new medicines.[25] In this field, transcriptomic data of diseases is being harnessed to understand the pathological pathways and drug mode of action. All this is guiding drug repositioning, saving resources and time and thus speeding up the development of drugs. For example, Arakelyan et al.[1] used transcriptome-guided analysis to reposition infliximab and brodalumab, two already approved biologics. Specifically they found that the former has potential therapeutic action for ulcerative colitis and Crohn's disease whilst the latter has potential for psoriasis.

Serafin et al.[24] also point out that drug repositioning studies have pointed to potential drugs that can be used against SARS-CoV-2 and the treatment of COVID-19 disease.

TRANSCRIPTOMICS AS PART OF INTEGRATED -OMICS APPROACHES

Surely, the knowledge gained from the integration of transcriptomics with other –omic platforms (including single cell –omics) will be harnessed to understand the organisation and control of different tissues and of single cells. The data gathered and analysed will also reveal the perturbations/aberrations which are specific to pathological states and this will ultimately provide better patient stratification and targeted theranostics.

Such integrated studies are already being done. For example, in 2019, Schüssler-Fiorenza Rose et al.[26] used –omics (specifically, immunomics, transcriptomics, proteomics, metabolomics and microbiomics) together with wearable monitoring devices to study molecular and physiological profiles at the individual level. They targeted

type 2 DM individuals and revealed several associated cardiovascular and oncologic pathophysiological pathways.

Integrated –omics, including transcriptomic analysis has also been applied to understand systemic autoimmunity. Fujio et al.[27] carried out a study on SLE, the classic prototype of systemic autoimmune disease, and identified several immunological pathways linked to autoimmunity. Moreover they could stratify SLE patients based on their immunological and clinical characteristics. Amongst other things they found that 'CD8 exhaustion' is a favourable prognostic feature while 'IFN score' is prognostic towards developing systemic autoimmune diseases.

An interesting study is that performed by Hou et al.[28] Here they used what they called 'single-cell triple omics sequencing' (scTrio-seq), whereby they concurrently did transcriptomic, DNA methylomic, and genomic CNVs (copy-number variations) profiling on a single mammalian cell. They also used scTrio-seq on 25 single human hepatocellular cancer cells and found two subpopulations. They propose that the method applied can be a tool to understand the intricate heterogeneous states of cancer cells.

SINGLE-CELL TRANSCRIPTOME PROFILING

Single-cell RNA sequencing (scRNA-seq) is a promising platform when it comes to studying the transcriptome of individual cells. Surely, understanding the transcript landscape that make a specific cell will aid further our understanding of the functions of organs better. In fact it is believed that these single-cell molecular profiling will help researchers to reveal novel biological discoveries[29] that are being missed with traditional analytical methods that use bulk populations of cells. Hence, single-cell transcriptome profiling will be another tool to understand health and disease.

Studies are already being done to understand cancer, autoimmune diseases, diabetes mellitus, the immune response, tissue regeneration, stem cells, embryogenesis, and much more.

Single cell transcriptomic platforms are still being discovered.[30] Indeed, one interesting method called 'spatially resolved transcriptomics' is where tissues are cryo-sectioned into thin slices and subsequently each slice is profiled transcriptomically. Thus spatially resolved transcriptomics has the potential to reveal subcellular localization or spatial information of the tissue.

CONCLUSION

Transcriptome profiling, now even at a single cell resolution, is another valuable supply of knowledge that will lead to a deeper understanding of cell biology and also of the pathophysiology of disease. Together with other –omics, it is already being proposed and in some instances applied in focused personalized theranostics.

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LORNA MUSCAT BARON AND DR BYRON BARON

Generation of 3D Cell Cultures

from Patients for Colorectal Cancer Research

ABSTRACT

Traditionally the growth of human cells for cancer research is in two-dimensions (2D). However, three-dimension (3D) culturing has been shown to better recapitulate the cellular behaviours and interactions, offering great advantages. 3D cultures retain the benefits of 2D cultures whilst mimicking the structure and interactions of tumours in vivo, possibly bridging the research gap between in vitro and in vivo studies. The 3D cultures, called organoids, can be seeded with cells from many sources, including healthy tissue or a primary tumour. However, barriers are often faced in using 3D cultures for research, such as a lack of biopsy availability which is of acceptable quality.

KEYWORDS

Colorectal cancer, Organoid, Methylation, Biomarkers.

INTRODUCTION

The culturing of human cells in the lab is an essential component of all biomedical research, with the cell cultures produced being used to represent both the healthy and diseased state. Most human diseases are modelled for research purposes using cells that have become accustomed to growing on a plastic surface, making these two-dimensional (2D) cell lines. These cell lines, in most cases are homogeneous cultures composed of the cells which grow fastest on plastic, outcompeting any other cells. In cancer research, such cell cultures have been used extensively to perform experiments to characterise cancer biology and the impact of novel drugs and small molecules for personalised therapeutics.

This type of cell culture however does not truly represent cancer progression within the human body, since tumours are not homogenous, single cell layers. In the body, once a cell mutates and starts dividing rapidly, the daughter cells formed undergo different additional changes to reprogram themselves and overcome the barriers set by the cell fate programming of their organ of origin, adding heterogeneity to the growing tumour. These tumour cells then have to interact with numerous cell types both within the organ itself and the immune system.

ORGANOID GROWTH

The revolution in cell culture came around a decade ago, when cells started being cultured in three-dimensions (3D) with the addition of various growth factors and small signalling molecules. These 3D cell clusters, can be seeded either from commercially established immortalised cell lines or from cells extracted from a primary tumour. Whilst the latter are preferable, barriers are often faced in their preparation, such as a lack of available patient material.[1] Those 3D cultures obtained from cell lines, which usually produce solid spheres, are called 'spheroids' whilst those containing all the cell types representing the organ from which they are grown, usually forming hollow spheres, are referred to as 'organoids'.

The use of multicellular tumour organoids is rapidly becoming a popular method of studying cancer biology, to analyse various aspects of tumour biology such as genetics, proteomics, and novel drug efficacy. These organoids are typically generated from a single cell

suspension in a culturing medium, within plasticware or a gel matrix which prevents adherence and thus within a few days the cells form clusters and then develop into organoids. Thus, an entire 3D culture is produced from the small number of initial cells available that develop into numerous organoids.[2]

Whilst immortalised cell lines have been one of the most popular resources in the initial stages of research in various fields, such as cancer research including drug studies and genetic research, they have significant drawbacks.[3] There are significant differences in the biochemical profiles of cells derived from patient material and those found in commercial cell lines. Cells taken directly from patient samples produce cultures which retain many of the phenotypic characteristics of the original tissues, whilst providing for the most translational results, with the cost benefits of in vitro conditions.

Culturing cells in 3D is a step forward towards the *in vivo* condition as compared to the traditional 2D cell culturing because such cultures have been confirmed to resemble the structure and interactions observed in tumours.[4] The initiation of organoids from primary tumours allows researchers to study the exact make-up of the tumour found within a patient in a laboratory setting, providing the most ideal comparison to the *in vivo* environment without requiring animal studies.[1] Organoid production thus enables researchers to gain some of the benefits of *in vivo* condition, whilst retaining the benefits of 2D cultures, such as quick and easy access to the biochemical modifications to the cells, whilst not requiring the labour involved in *in vivo* research.[5]

OPTIMISATION OF ORGANOID GROWTH

The technique of extracting and culturing cells in 3D has been optimised so that organoids can now be initiated from the cells of most organs including stomach, large intestine, liver, bladder and even brain. Organoids made from either intestine or stomach have been colloquially described as 'mini-guts'.[6] Intestinal organoids start off as solid cell clusters that eventually form a hollow cavity surrounded by a simplified intestinal lining, having nutrient-absorbing protrusions. Intestinal organoids can be generated from just a few cells extracted from a biopsy because the intestinal epithelium has Lgr5+ stem cells at the bottoms of small-intestinal crypts.[7] These stem cells then differentiate into all the different cell types of the intestine through the creation of a Wnt gradient which decreases as the cells divide and distance themselves from the stem cell niche.[8]

PATIENT PARTICIPATION IN ORGANOID RESEARCH

In order to generate colorectal cancer organoids, a patient must first be approached by the surgeon in order to participate in a research study. Once the patient consents, a biopsy is collected, generally during a tumour resection or a colonoscopy. The sample is stored in antibiotic medium and transported immediately to the lab where the extracellular matrix is digested, the cells are extracted and incubated with a mixture of growth factors and cell medium, providing the signals and nutrients for the various cell types present in the biopsy to grow.[9] The initial cluster of cells forming each organoid has a diameter of just 10-30 µm which, over a period of two weeks, may grow to around 1-5mm in diameter.

ADVANTAGES OF 3D CULTURE

The use of 3D cultures for the study of colorectal cancer has multiple advantages over the use of the traditional 2D culture system. Among these are an increased cellular heterogeneity, a better representation of the patient intestinal structure, as well as the possibility to interrogate and follow cell-to-cell communication and interactions.[5] These organoids mimic both the protein expression and the cellular organisation of the intestine, allowing for an improved modelling of both colorectal cancers and various forms of intestinal inflammatory diseases. Thus, the utilisation of 3D cultures is of great benefit to the study of the multistep process involved in colorectal cancer progression, from a benign polyp up to the development of a malignant adenocarcinoma.[5] This in turn allows for more physiologically relevant screening for drug efficacy or toxicity, improving patient therapies, whilst reducing the need for animal testing.

EPIGENETIC MARKERS IN COLORECTAL CANCER

Epigenetic markers in colorectal cancer have been noted to change depending on the type of tumour microenvironment the cells experience, which is known as epigenetic plasticity. Complex cell-to-cell interactions enabled by the 3D culture system have been detected, influencing tumour markers for cell adhesion. Epigenetic markers were found to be silenced in 2D culture and then re-expressed when utilising 3D cell culture of colorectal cancer.[10] This is also expected to be the case at the protein level, where chemical modifications called post-translational modifications such as methylations,

acetylations or phosphorylations alter protein properties.[11] Such modifications if validated would thus make ideal diagnostic or prognostic markers.[12]

Colorectal cancer organoids have the potential to be grown alongside non-cancerous colorectal organoids, allowing for the comparison of their proteomes. This is a possibility which is not possible on 2D culture. The lack of the intestinal structure in 2D culture means that inherently some of the proteome and the biochemical intricacies are lost, however, in 3D culture, the growth of organoids allows for an accurate replication in vitro with the added benefit of potentially growing healthy and cancerous cells together allows the study of their interactions.[13] Advanced proteomics on colorectal cancer has already been demonstrated to be feasible with clear results, allowing for the further investigation into the post-translational changes in the proteome.[14] The difference in the signalling cascades pertinent to cancer development are noted between 2D and 3D cultures, showing the need to shift to 3D culture to ensure that the results are reflective of the cell proteome in a patient.[15]

The use of organoids to study cancer is a new and highly promising field, with the potential of finding nuances previously lost to the 2D studies, especially when using unmodified patient samples, creating the most accurate image of the patient's biochemical environment. Finding methylation biomarkers could potentially be used to show the progression of the colorectal cancer, the type of cancer, and possibly the types of drugs it may respond to. Finding clinically relevant biomarkers for cancer utilising the *in vitro* techniques which are most translatable to the clinical environment may enable early diagnosis and more accurate treatment for the specific cancer subtype a patient has.

At the University of Malta, such colorectal cancer organoids are being applied to the study of protein methylations throughout the various stages of cancer progression, in order to identify potential diagnostic and prognostic markers. This research is being led by Dr Byron Baron and is supported by surgeons at Mater Dei Hospital and custom software developed by Incredible Web Ltd. Project Kme-CRC is financed by the Malta Council for Science & Technology, for and on behalf of the Foundation for Science and Technology, through the FUSION: R&I Technology Development Programme.

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DR PIERRE VASSALLO

Breast Cancer Screening in 2021

October is Breast Cancer Awareness month, which is an annual worldwide campaign aimed to increase awareness of the disease and to stress the importance of screening, early diagnosis, and prompt treatment.

Why is screening for breast cancer so important? Primarily because after skin cancer, breast cancer is the most common cancer in women affecting 1 in 8 individuals. It is therefore the cause of much suffering particularly amongst women and may cause early death. Breast cancer screening detects cancer early allowing more effective management with less aggressive therapy. Early and effective treatment results in a better quality of life and less stress for family members and loved ones. Screening of family members of women suffering from breast cancer particularly siblings and twins, is recommended to detect early disease in these individuals and to prevent complications.

The currently available breast cancer screening tests are Ultrasound, Mammography and MRI. However, each of these modalities consists of a number of different technologies. In the following paragraphs, we will describe each technology and list its advantages and limitations.

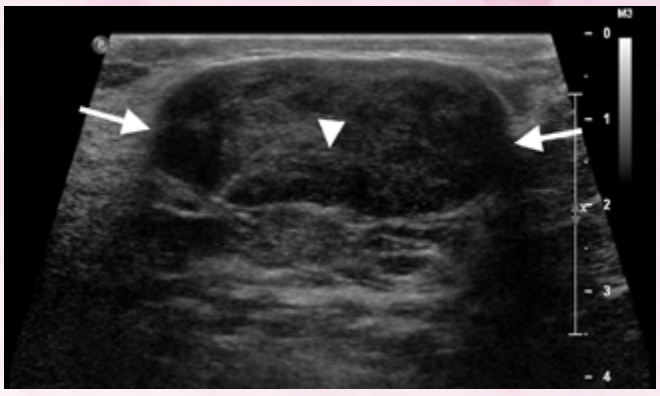

Figure 1. HHBUS showing a flat oval well-defined nodule (arrows) with a central echogenic line (arrowhead) in a 28 year old woman; all features are indicative of a fibroadenoma.

1. ULTRASOUND

There are two types of breast ultrasound, Handheld Breast Ultrasound (HHBUS) and Automated Breast Ultrasound (ABUS).

HHBUS

HHBUS is the standard screening test for women less than 40 years of age. It is also used as a supplemental exam with mammography for those who are 40 years and older, particularly for those women who have dense breasts.[1] Combining HHBUS with mammography results has significant advantages for breast cancer detection, which will be discussed in more detail below.

If a lesion is found with HHBUS, it can be further evaluated with Ultrasound Elastography. Shear Wave Elastography has been shown to be valuable in distinguishing benign from cancerous nodules.[2] However, irrespective of findings on Elastography, one must proceed to ultrasound-guided biopsy for more reliable confirmation.

HHBUS is operator-dependent and achieves greater accuracy in the hands of an experienced breast radiologist. For patients presenting with a palpable nodule, HHBUS is the best evaluation tool, allowing for rapid treatment decisions on whether biopsy is required (Fig 1).

ABUS

ABUS was conceived specifically to address the operator dependence of HHBUS. As its name states, it is an ultrasound machine that obtains a 3D volumetric image of the breast, which can later be reviewed by the radiologist. ABUS lacks the flexibility of targeted analysis with free selection of scan plane that is possible with HHBUS in the hands of an experienced radiologist. ABUS has other limitations particularly for covering large breasts, where supplemental HHBUS may be required. It is associated with long interpretation times and results in an increased number of recalls for reassessment with HHBUS. ABUS has lower sensitivity but higher specificity than HHBUS, but overall it has no demonstrable statistical advantage over HHBUS.[1]

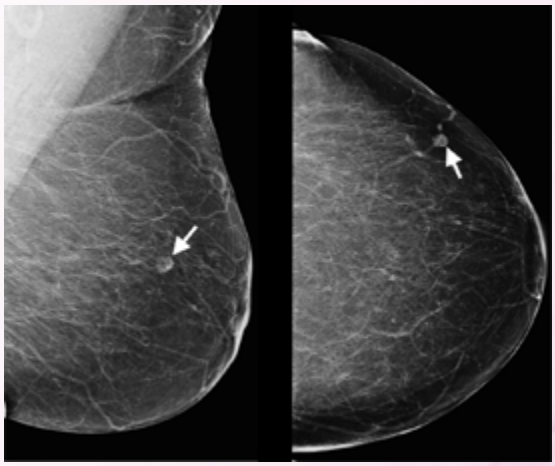

Figure 2. DM showing a small lesion in the lateral portion of the left breast that contains central hilar fat; the fatty hilum was also noted on HHBUS confirming the lesion to be an intramammary lymph node. Note the high quality of the images delivered by DM that cover the whole breast in each view.

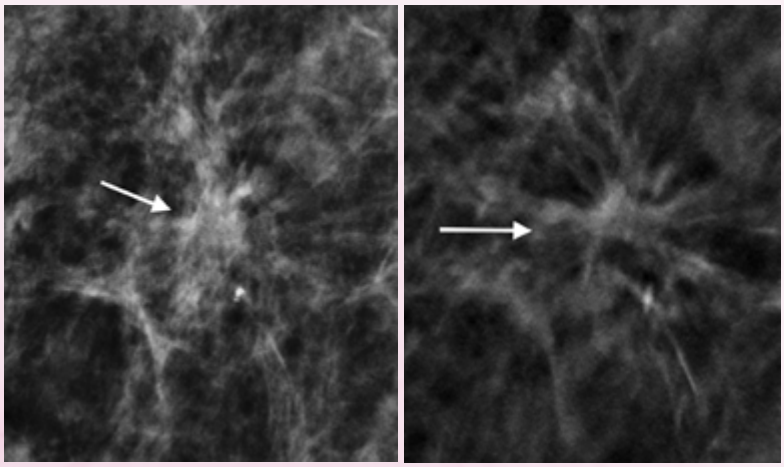

Figure 3. (a) Invasive ductal cancer detected on DM; however, margins of the lesion are better detected on DBT (b).

2. MAMMOGRAPHY

There are different mammographic technologies available on the market, which differ in their diagnostic accuracy. We will discuss Computed Mammography (CM), Digital Mammography (DM), Digital Breast Tomosynthesis (DBT) and contrast-enhanced Mammography (CEM).

Computed Mammography

CM, also known as CR (computed radiographic) mammography, is old technology that is not truly digital. It uses a CR plate to take a breast image that is then read through a dedicated scanner. It is listed here because it is still widely available in breast cancer screening clinics. It is inferior to DM and DBT mammography for the detection of breast cancer. CM has been shown to be 21% less effective in detecting breast cancers than DM.[3] In fact, some authors go as far as recommending that patients should be advised before performing CM that the test has a lower cancer detection rate.[4]

Digital Mammography

DM (sometimes called 2D mammography) is a well-establish accurate technology that is the mainstay for breast cancer screening programs. Images of the breast are obtained through a special solid-state detector and transferred to a diagnostic workstation, where they are reviewed by a radiologist (Fig 2). DM is frequently adequate alone as a screening tool for breast cancer. With equivocal findings or in case of dense breasts, supplemental exams such as HHBUS or DBT may be required.

Digital Breast Tomosynthesis

DBT (sometimes incorrectly referred to as 3D mammography) is a technological enhancement of DM. DBT delivers 1mm thick slices through the breast, the number of which varies depending on breast thickness. Thus, rather than having one image in each plane as with DM, as many as 85 images per plane may be delivered by DBT. Scrolling through such a stack of images is like flipping through the pages of a book; it allows detailed analysis at different depths of the breast. This is particularly useful with dense breasts, where overlying dense glands may hide a small breast cancer.

DBT has the added advantage of reducing the requirement for additional views such as magnified and spot compression views, thus reducing radiation exposure.

Synthesised Mammograms (SM) are single images of the breast generated from a DBT dataset. While they may appear similar to DM, they are not a replacement from an accuracy standpoint. SMs must be used as a rapid overview of the breast before proceeding with more detailed evaluation of the DBT images.

DBT has the advantage of detecting the architectural distortion, which is an indicator of invasive cancers. It therefore preferentially improves the detection rate of invasive ductal and lobular cancers without increasing that of in-situ cancers.[5] DBT therefore maximises the detection of biologically significant disease and prevents over-diagnosis. DBT is particularly valuable for detecting invasive lobular cancers, which are normally known to be elusive or occult on DM. DBT improves conspicuity of lesion margins (Fig 3).

It is important to note, that not all architectural distortions are due to breast cancer. In fact, 33.2% of architectural distortions seen on DBT are radial scars.[6] However, even biopsy-confirmed radial scars are best excised since analysis of the excised lesion may upgrade it to cancer.

The fact that DBT improves cancer detection rate over DM has been demonstrated in a number of large studies (Fig 4). In one large population-based study, cancer

detection rates in women who underwent consecutive biennial screening with DM after DM, DBT after DM, and DBT after DBT were 4.6, 9.9, and 8.3 per 1000 examinations, respectively.[7]

Detection rate of breast cancer is highest on the first DBT; the statistical reason for this is that women coming for follow-up mammograms have already been previously screened for breast cancer.

Follow-up DBT detects interval cancers; these are cancers that develop in the interval between one mammogram and the next. These are aggressive cancers, because their rate of growth has outpaced the frequency of screening mammograms (recommended yearly). Their early detection prevents progression to advanced cancers; these are defined as those with metastases, positive lymph nodes, invasive tumours >2cm in diameter, and oestrogen/progesterone receptor negative or Herceptin receptor-positive invasive cancers >1cm in diameter.[8]

Contrast Enhanced Mammography

CEM is a new technology that is still under investigation. It uses images taken with two X-ray energies to obtain mammograms that are sensitive to injected contrast medium. In some respect, it is similar to contrast-enhanced MRI since cancer detection is based on uptake of intravenously injected contrast material. Early reports are suggesting that it has similar accuracy. CEM may be combined with DBT technology to improve lesion conspicuity.[9]

3. MRI

Breast MRI is the most sensitive technology for the detection of breast cancer with a sensitivity (up to 96%) reported as being higher than that of mammography and ultrasound (Fig 5). It is specifically recommended for women with a high risk for breast cancer, such as those with inherited genetic mutation and those who have had mantle radiation to their breasts before 30 years of age.[10]

Breast MRI is based on functional imaging that detects breast cancer based on its increased vascularity and contrast enhancement. This modality detects invasive and high-grade cancers.[11]

Breast MRI using the standard protocol is not practical as a screening tool, because of the long exam times. It is best used as a supplemental exam for DBT or DM, when findings on these modalities are equivocal.

The development of an abbreviated MRI protocol has been discussed in recent years and appears to be gaining interest. However, the exam still takes longer to perform than DBT or DM. A recent comparison between Abbreviated Breast MRI and DBT has shown that MRI is more sensitive, but DBT is more specific for breast cancer detection.[12]

MULTIMODALITY SCREENING

When evaluating the accuracy of a screening service, it is important to appreciate that combining modalities increases the rate of cancer detection and has an impact on treatment outcome.

Supplemental HHBUS has been shown to improve the sensitivity for breast cancer detection in women with extremely dense breasts[13] (Fig 6). This is particularly important since women with extremely dense breasts have a 4-6 times higher risk of developing breast cancer.[14]

Studies have shown that supplementing DM with HHBUS increases cancer detection rates. There is also an incremental gain in cancer detection rate with each year of screening.[13] Cancers detected with DM with supplemental HHBUS tended to be small, invasive and node negative.[13] Supplemental HHBUS has been shown to lower the interval cancer detection rate.[15]

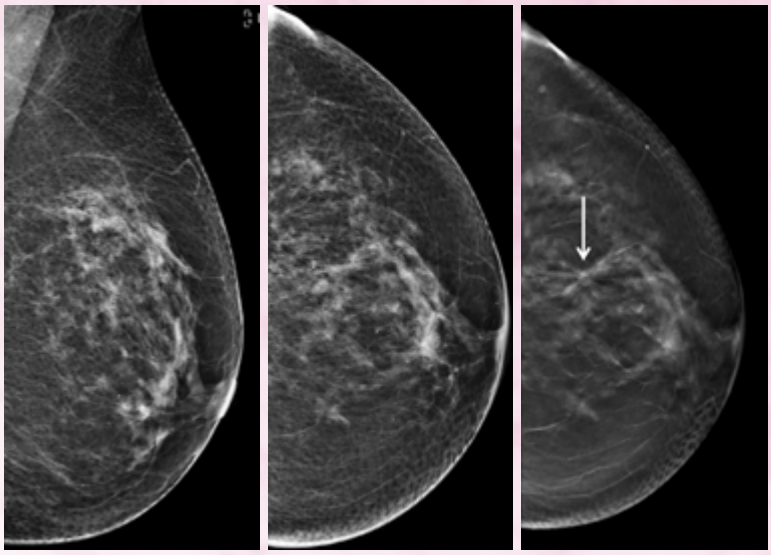

Figure 4. Occult invasive ductal cancer on mediolateral oblique (a) and bilateral craniocaudal (b) DMs, but visible on bilateral craniocaudal DBT (c).

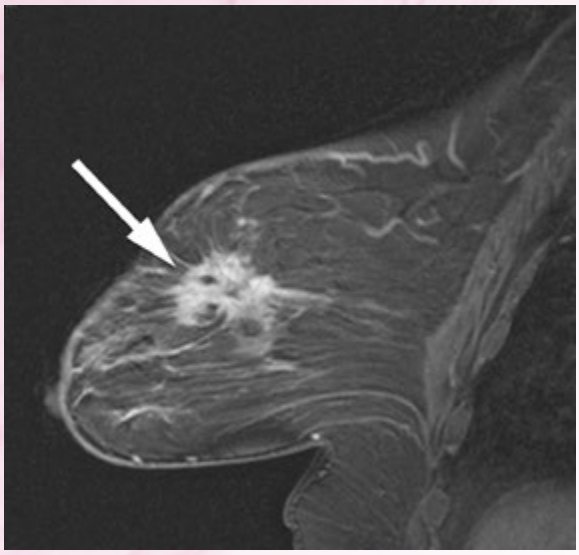

Figure 5. Sagittal contrast enhanced T1-w breast MRI scan showing an invasive ductal cancer (arrow).

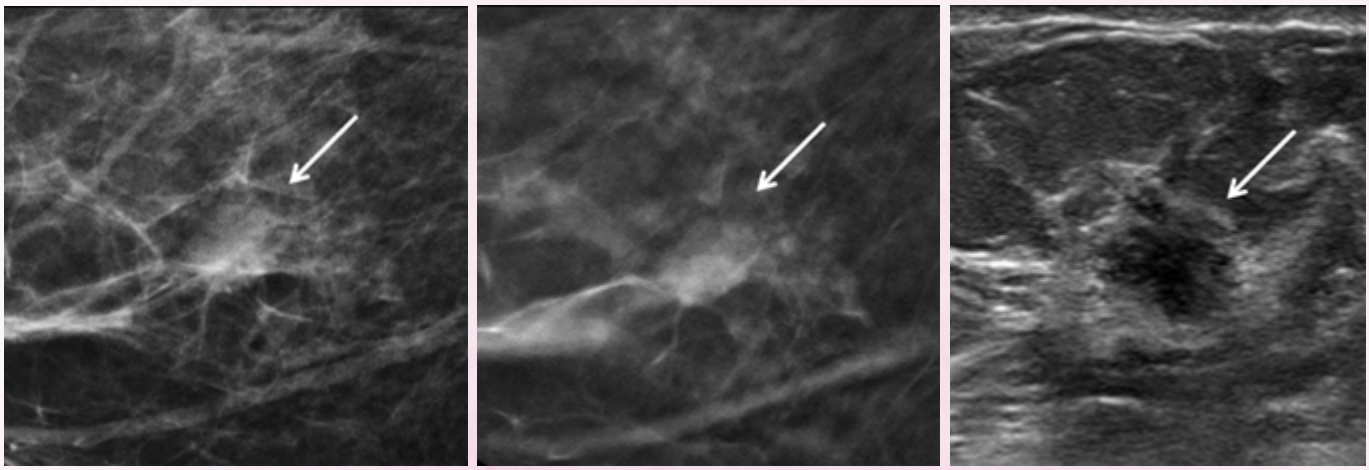

Figure 6. Invasive ductal cancer (arrow) seen on DM (a) and in-plane DBT (b). HHBUS confirmed a spiculated lesion (arrow) at the site and subsequently helped guide core biopsy.

HHBUS diminishes the false positive recall rate; this is the rate at which patients who are called back for further imaging turn out to be disease negative.

One question that remains to be answered is whether DBT can fully replace the combination of HHBUS and DM. In clinical practice, using DBT results in less need for supplemental HHBUS.

Supplemental breast MRI detected additional cancers when performed after DM and HHBUS.[13] In extremely dense breasts, a number of studies are recommending proceeding directly to supplemental MRI rather than performing HHBUS.

In case of extremely dense breasts, even with DBT, supplemental evaluation with HHBUS and MRI may still be required. Combining these modalities has been shown to increase the detection rate of invasive cancer.

The complexity of current breast cancer screening options is daunting. Studies are constantly revealing new knowledge that helps us establish a standard protocol. However, it is becoming increasingly clear that this protocol will need to be tailored to patient needs based on cancer risk and to biological subtype.

CURRENT STANDARD PROTOCOL

1. Women 40 years and over should have yearly mammograms (DBT preferred over DM) and supplemental imaging as deemed necessary by the breast radiologist.
2. Between the ages of 30 and 40 years, women should have a yearly HHBUS if they have a strong family history of any cancer especially breast and ovarian cancers.
3. Positive breast cancer gene (BRACA1/2) women should have yearly breast MRI scans especially when they have dense breasts.

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