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Health and Medical Research Fund

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Health and Medical Research Fund

Research Dissemination Reports

HEALTH SERVICES

INFECTION

Editorial

Dissemination reports are concise informative reports of health-related research supported by the Health and Medical Research Fund administered by the Health Bureau. In this edition, we present 11 dissemination reports of projects related to neurology, public health, cardiovascular disease, cancer, reproduction, health services, and infection. In particular, research findings of three projects may provide insights to enhance clinical practices and help inform health policy formulation in Hong Kong.

Alzheimer disease (AD) is a progressive incurable neurodegenerative condition. Current diagnostic tests are limited by their invasive nature, high cost, and limited resolution. There is a need for improved assays for biomarkers for AD that are sensitive, specific, reliable, non-invasive, accessible, and inexpensive. Cheung et al[1] assessed whether retinal vascular and neuronal changes could fulfil these requirements among 163 Chinese individuals who were patients with AD or amnestic mild cognitive impairment or healthy controls. They found that specific retinal microvascular abnormalities and retinal neuronal/axonal loss (measured using non-invasive retinal imaging technologies) could reflect cerebrovascular dysfunction and classic features of neuronal injury in the AD brain, were associated with AD, and were independently predictive of cognitive decline. Thus, retinal imaging measurements are potentially useful biomarkers for AD.

Breastfeeding is widely promoted worldwide and yet the mechanism of how breastfeeding exerts its long-term beneficial effects is unclear. One possibility is that breastfeeding mediates its effects by influencing lipid profiles in children and adolescents. Schooling et al[2] attempted to clarify the long-term effects of breastfeeding on apolipoprotein B (ApoB), which is emerging as the predominant lipid that causes ischaemic heart disease, by following up the 'Children of 1997' birth cohort. Overall, breastfeeding was associated with lower ApoB but not lower triglycerides or high-density lipoprotein cholesterol in older adolescents at ~17.6 years. From a public health perspective, this study gives further support to the promotion of breastfeeding given its known short-term benefits and potential lifelong benefits.

The Hong Kong SAR Government, in collaboration with the healthcare sector, facilitates and encourages women to have regular cervical screening to prevent cervical cancer. The Hong Kong SAR Government has also implemented human papillomavirus (HPV) vaccination for female adolescents. Starting from the 2019/2020 school year, schoolgirls in primary five and six (equivalent to age 11-12 years) can receive two doses of the nonavalent HPV vaccine without charge. Leung et al[3] aimed to evaluate the comparative cost-effectiveness of different applications of HPV testing in the local setting via modelling HPV vaccination and cervical cancer screening. They found that among cohorts without HPV vaccination programme, using HPV test results as stand-alone primary test or as a triage for cytology for atypical squamous cells of undetermined significance was likely a cost-effective cervical screening strategy to reduce deaths from cervical cancer when the willingness to pay threshold was one GDP per capita (US$46 615). Among cohorts who were able to receive nonavalent HPV vaccines via immunisation programme, cervical screening was less likely to be cost-effective if vaccine uptake was ≥75% and the routine screening interval was 5-yearly or shorter.

Supplement editors

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References

1. Cheung CY, Chan VTT, Au LWC, Tham CC, Kwok TCY, Mok CTV. Novel retinal imaging biomarkers for cognitive decline: abridged secondary publication. Hong Kong Med J 2023;29(Suppl 4):S4-7.
2. Schooling CM, Au Yeung SL, Kwok MK, Leung GM. Breastfeeding and late adolescent lipid subfraction: a Hong Kong birth cohort study (abridged secondary publication). Hong Kong Med J 2023;29(Suppl 4):S8-10.
3. Leung SMK, Wu J, Chan KKL, Jit M. Maximising the cost-effectiveness of human papillomavirus testing for cervical screening in the context of routine HPV vaccination in Hong Kong: abridged secondary publication. Hong Kong Med J 2023;29(Suppl 4):S11-5.

Novel retinal imaging biomarkers for cognitive decline: abridged secondary publication

CY Cheung *, VTT Chan, LWC Au, CC Tham, TCY Kwok, CTV Mok

KEY MESSAGES

1. There has been a search for Alzheimer disease (AD) biomarkers that facilitate early disease diagnosis while being non-invasive, widely available, and reliable. The retina, an extension of the central nervous system, offers a "window" for in vivo studies of the cerebral microvascular and neurodegenerative damage in AD.
2. In this prospective observational study, healthy controls and patients with AD or amnestic mild cognitive impairment underwent neuropsychological testing, retinal imaging, and neuroimaging to explore the associations of retinal changes with upstream pathological changes in AD and cognitive decline.
3. Compared with amyloid-β-negative individuals, amyloid-β-positive individuals had significantly thinner macular ganglion-cell inner plexiform layer thickness. Among amyloid-β-positive individuals, cognitively impaired individuals had significantly larger foveal avascular zone area, smaller fractal dimension, smaller skeleton density, larger vessel diameter index, and smaller inter-capillary area, compared with cognitively normal individuals. Global amyloid burden was associated with macular ganglion-cell inner plexiform layer thickness and foveal avascular zone area.
4. Central subfield thickness and mean cube thickness were associated with the progression of cognitive decline over 12 months.
5. Specific retinal microvascular abnormalities and retinal neuronal/axonal loss, measured using non-invasive retinal imaging technologies, may reflect cerebrovascular dysfunction and classic features of neuronal injury in the AD brain; moreover, they are associated with AD and can independently predict cognitive decline.

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Introduction

Alzheimer disease (AD) is an incurable and progressive neurodegenerative disorder. Its pathological features are extracellular amyloid-β plaques and neurofibrillary tangles composed of intracellular hyperphosphorylated tau protein (p-tau). Advances in detecting cerebral spinal fluid and neuroimaging markers (eg, the use of Pittsburgh Compound-B to detect cerebral amyloid-β) improve the accuracy of AD diagnosis. However, clinical applications of these novel biomarkers are restricted by standardisation issues and invasiveness and by high costs and limited availability. Additionally, although various indirect manifestations of cerebral small vessel disease (eg, white matter lesions, lacunar infarcts, and cerebral microbleeds) have been associated with cognitive decline, direct in vivo visualisation of changes in cerebral small vessels (ie, cerebral arteriolar narrowing or capillary microaneurysms) remains difficult to achieve by current neuroimaging technology. Thus, there is an ongoing search for high sensitivity and specificity biomarkers that are reliable, non-invasive, accessible, efficient, and inexpensive.

The retina is a central nervous system tissue and displays physiological properties and regulatory mechanisms similar to those within the brain. The retina can be visualised and offers an excellent "window" for direct, non-invasive evaluation of AD-related changes in the central nervous system and microvasculature.[1,2] The advantages of retinal imaging include lower cost, lower invasiveness, and greater accessibility, compared with neuroimaging and cerebral spinal fluid markers. Moreover, common age-related ophthalmic diseases share risk factors with AD; thus, patients with such ophthalmic diseases may benefit from additional screening.

We investigated associations between retinal vascular and neuronal changes in AD. Our hypothesis was that specific retinal microvascular and neuronal abnormalities, measured using new non-invasive retinal imaging technologies, would reflect features of cerebrovascular dysfunction in AD, and that these abnormalities would be independently predictive of cognitive decline.

Methods

In this prospective observational study, patients with AD or amnestic mild cognitive impairment (aMCI) were recruited from the dementia/memory clinic at Prince of Wales Hospital, Hong Kong, whereas age-matched cognitively normal controls without objective cognitive impairment on formal neuropsychological testing were recruited from a community-based study. Standardised inclusion and exclusion criteria were used to control for other conditions with potential effects on retinal neuronal thickness and microvasculature.

All recruited individuals underwent neuropsychiatric assessments, including the Hong Kong List Learning Test, Mini-Mental State Examination (MMSE), and the Hong Kong version of the Montreal Cognitive Assessment. Cognitive diagnoses were made by an experienced dementia specialist in accordance with the 2018 NIA-AA research framework.[3]

All participants were invited to undergo retinal imaging at 1, 6, and 12 months, then annually thereafter at the CUHK Ophthalmic Research Centre. Imaging modalities included optical coherence tomography (OCT), OCT-angiography, and ultra-wide field scanning laser ophthalmoscopy.

Retinal capillary network imaging was performed by OCT-angiography using a swept-source OCT device. Slabs of superficial capillary plexus were automated and segmented by the built-in software. En-face images of the included OCT-angiograms were exported in greyscale from the built-in software, then imported into a customised MATLAB program for image analysis (Fig 1). Retinal capillary network measurements included foveal avascular zone (FAZ) area, FAZ circularity index, vessel density, fractal dimension, non-perfusion area, vessel diameter index, and inter-capillary area.

Ultra-wide field fundus images were obtained using an ultra-wide field scanning laser ophthalmoscope and then graded semi-automatically using the Singapore I Vessel Assessment software. Various retinal vascular parameters including retinal vessel calibre, tortuosity, and fractal dimension were calculated.

Retinal neuronal imaging was performed using macular and optic disk cube scan protocols. The built-in CIRRUS analysis software provided automated measurements of macular ganglion cell-inner plexiform layer (GC-IPL) thickness and retinal nerve fibre layer (RNFL) thickness for assessment of retinal neuronal and axonal loss (Fig 2).

A subset of participants underwent ^{11}C-PIB and ^{18}F-T807 positron emission tomography (PET)/computed tomography to quantify deposition of beta-amyloid and tau, respectively.[4] They also underwent magnetic resonance imaging (MRI) in accordance with standard protocols.

Results

We initially recruited 163 individuals, including 57 healthy controls, 53 patients with aMCI, and 53

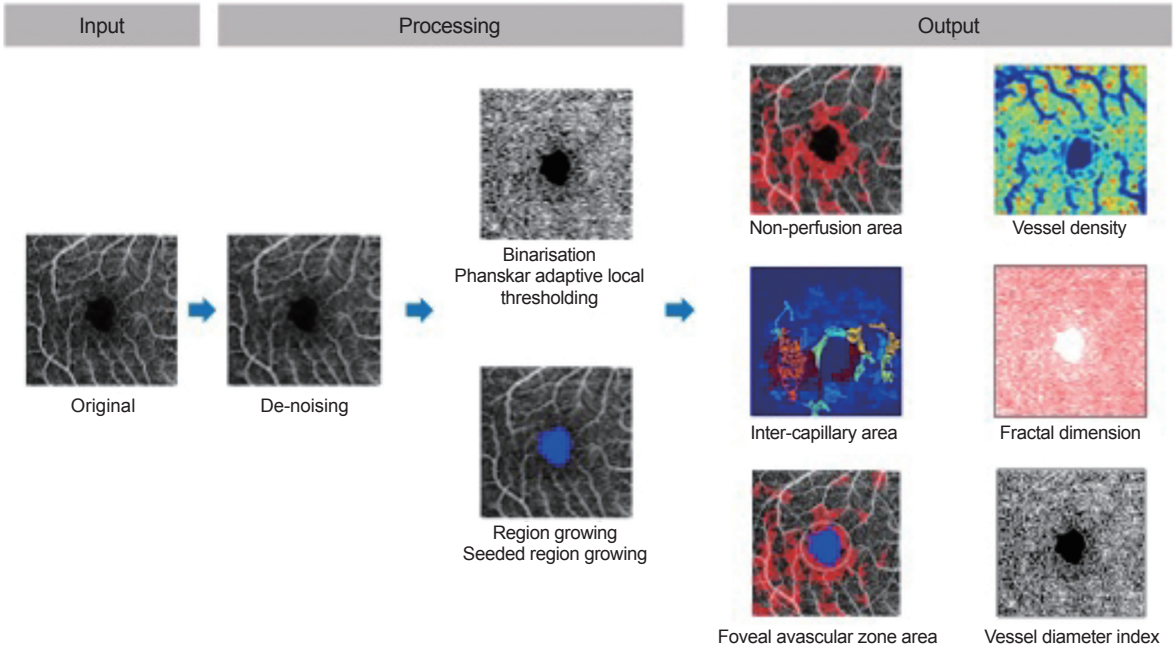

FIG 1. Retinal capillary network imaging via swept-source optical coherence tomography angiography: en-face images were exported in greyscale and then imported into a customised MATLAB program for image analysis.

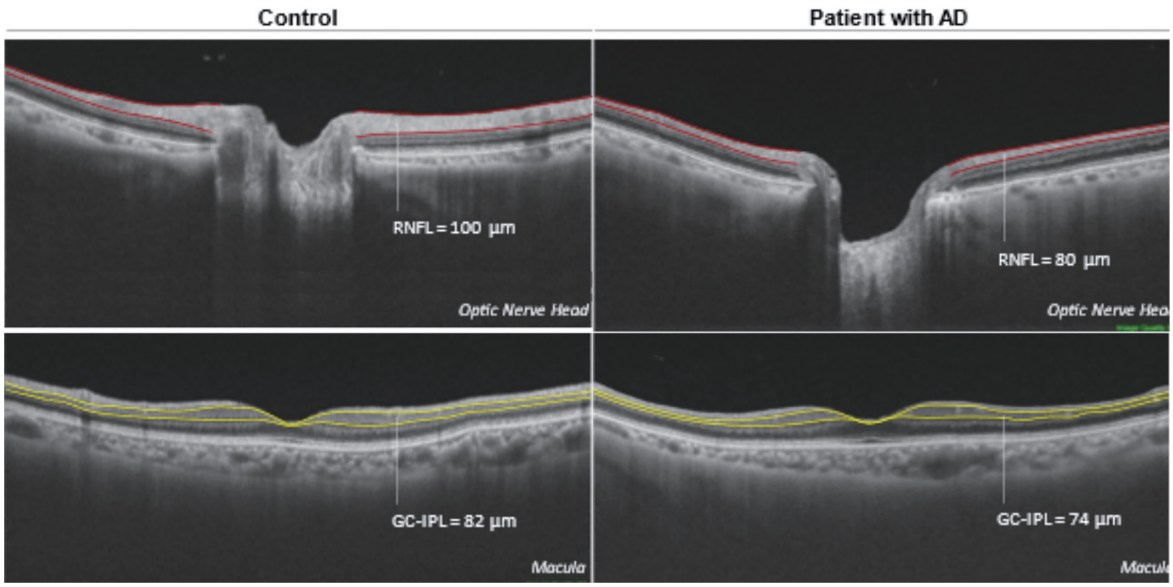

FIG 2. Spectral-domain optical coherence tomography: built-in CIRRUS analysis software provides automated measurements of macular ganglion-cell inner plexiform layer (GC-IPL) thickness and retinal nerve fibre layer (RNFL) thickness for assessment of retinal neuronal and axonal loss among healthy controls and patients with Alzheimer disease (AD).

patients with AD. During subsequent follow-up, diagnoses of some participants were revised and the distribution became 71 healthy controls, 40 patients with MCI, and 52 patients with AD. After recruitment, we excluded four healthy controls, six patients with MCI, and five patients with AD. Eventually, 148 participants were included in the analyses, including 67 healthy controls, 34 patients with aMCI, and 47 patients with AD. There were no significant differences among the groups, although there was a trend of retinal neuronal layer thinning, FAZ enlargement, and skeletal density reduction among patients with aMCI and patients with AD.

In total, 61 participants underwent PET: 31 healthy controls, 15 patients with aMCI, and 15 patients with AD. The diagnosis in one patient was modified from AD to aMCI by a neurologist; six patients with aMCI were amyloid-β-negative and reclassified as healthy controls. Eventually, 51 participants were included in analysis, including 22 amyloid-β-positive participants (11 with AD, six with aMCI, and five with normal cognition) and 29 amyloid-β-negative participants. Compared with amyloid-β-negative participants, amyloid-β-positive participants had significantly thinner macular GC-IPL. Among the amyloid-β-positive participants, cognitively impaired patients had significantly larger FAZ area, smaller fractal dimension, smaller skeleton density, larger vessel diameter index, and smaller inter-capillary area, compared with cognitively normal individuals. These differences remained significant after adjusting for age and sex.

Global amyloid burden was negatively associated with macular GC-IPL thickness (r= -0.26, 95% confidence interval [CI]= -0.49 to -0.02, P=0.03) and peripapillary RNFL thickness (r= -0.26, 95% CI= -0.52 to 0.01, P=0.06) and positively associated with FAZ area (r=0.38, 95% CI=0.11-0.66, P=0.01). Macular GC-IPL thickness, peripapillary RNFL thickness, central subfield thickness, FAZ area, fractal dimension, inter-capillary area, and non-perfusion area were also associated with amyloid and/or tau burden in specific brain regions.

Regarding correlation between retinal imaging parameters and MRI brain volume, macular RNFL thickness was positively associated with occipital lobe volume (r=0.46, 95% CI=0.15-0.76, P=0.001) and negatively associated with thalamus volume (r= -0.40, 95% CI=0.72 to -0.08, P=0.02). Non-perfusion area was positively associated with cingulate volume (r=0.76, 95% CI=0.16-1.36, P=0.01) and insula volume (r=0.70, 95% CI=0.09-1.31, P=0.03). Vessel density was negatively associated with occipital lobe volume (r= -0.40, 95% CI= -0.78 to -0.01, P=0.04) and insula volume (r= -0.55, 95% CI= -0.91 to -0.19, P=0.004). Inter-capillary area was positively associated with grey matter volume (r=0.35, 95% CI=0.01-0.70, P=0.04) and insula volume (r=0.44, 95% CI=0.11-0.77, P=0.01).

In total, 115 participants were followed up for ≥12 months and were included in longitudinal analyses; 36 of those participants had undergone PET scans. Twenty-five participants displayed a reduction of ≥3 points between the baseline and

most recent MMSE scores. Univariable analysis revealed that central subfield thickness (hazard ratio=1.612, 95% CI=1.054-2.468, P=0.0278) and mean cube thickness (hazard ratio=1.454, 95% CI=1.129-1.874), P=0.0038) were associated with a reduction of ≥3 points in MMSE score over 12 months. In multivariable analysis, these associations remained after adjusting for age and sex. However, macular GC-IPL thickness, peripapillary RNFL thickness, and OCT-angiography parameters were not associated with cognitive decline.

Discussion

Our findings revealed associations of retinal microvascular and neuronal changes with amyloid/tau burden and MRI brain volume. In particular, macular GC-IPL thickness, peripapillary RNFL thickness, and FAZ area were associated with quantitative measurements of amyloid burden in the brain. Retinal ganglion cells are neurons located in the ganglion-cell layers of the retina that receive visual information from photoreceptors and then project to the brain through the optic nerve. Therefore, amyloid and tau deposition along the axonal tracts of retinal ganglion cells may cause neuronal damage and subsequent thinning of the macular GC-IPL. Additionally, morphological and functional disruptions of cerebral capillary networks have been identified as precursors to AD-related neurodegenerative changes in animal models and post-mortem studies.[5] Changes in capillary morphology have also been identified as risk factors for small vessel diseases and neurodegeneration, which are associated with cognitive decline.[5] These findings may explain why an enlarged FAZ area, which indicates changes in capillary morphology, is associated with amyloid burden in the brain.

Our work demonstrates that retinal imaging has significant potential to identify AD-related retinal features, thereby facilitating the stratification of AD risk. Retinal imaging is non-invasive, easy to perform, and widely accessible and enables broader initial screening; patients with retinal changes can subsequently undergo more expensive brain imaging to diagnose specific subtypes of dementia. Importantly, retinal imaging enables the investigation of cerebral microvascular processes that currently cannot be discerned via MRI; these processes can be used to assess the pattern and extent of upstream brain pathology leading to cognitive decline and dementia.

Funding

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Disclosure

The results of this research have been previously published in:

1. Cheung CY, Mok V, Foster PJ, Trucco E, Chen C, Wong TY. Retinal imaging in Alzheimer's disease. J Neurol Neurosurg Psychiatry 2021;92:983-94.
2. Cheung CY, Chan VT, Mok VC, Chen C, Wong TY. Potential retinal biomarkers for dementia: what is new? Curr Opin Neurol 2019;32:82-91.
3. Chan VTT, Sun Z, Tang S, et al. Spectral-domain OCT measurements in Alzheimer's disease: a systematic review and meta-analysis. Ophthalmology 2019;126:497-510.
4. Chan VTT, Cheung CY. The role of retinal imaging in Alzheimer's disease. In: Martin CR, Preedy VR, editors. Diagnosis and Management in Dementia. Cambridge: Academic Press; 2020: 345-63.
5. Chan VTT, Wong PP, Cheung CY. Retinal vascular changes in diabetes and dementia. In: Sabanayagam C, Wong TY, editors. Diabetic Retinopathy and Cardiovascular Disease. Volume 27. Basel: S Karger; 2019: 86-99.
6. Chan VTT, Tso THK, Tang F, et al. Using retinal imaging to study dementia. J Vis Exp 2017;129:56137.

References

1. Cheung CY, Mok V, Foster PJ, Trucco E, Chen C, Wong TY. Retinal imaging in Alzheimer's disease. J Neurol Neurosurg Psychiatry 2021;92:983-94.
2. London A, Benhar I, Schwartz M. The retina as a window to the brain—from eye research to CNS disorders. Nat Rev Neurol 2013;9:44-53.
3. Jack CR Jr, Bennett DA, Blennow K, et al. NIA-AA Research Framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement 2018;14:535-62.
4. Jack CR Jr, Bennett DA, Blennow K, et al. A/T/N: an unbiased descriptive classification scheme for Alzheimer disease biomarkers. Neurology 2016;87:539-47.
5. de la Torre JC. Is Alzheimer's disease a neurodegenerative or a vascular disorder? Data, dogma, and dialectics. Lancet Neurol 2004;3:184-90.

Breastfeeding and late adolescent lipid sub-fraction: a Hong Kong birth cohort study (abridged secondary publication)

CM Schooling *, SL Au Yeung, MK Kwok, GM Leung

KEY MESSAGES

1. Apolipoprotein (Apo) B is a key lipid in cardiovascular disease.
2. In a population-representative Chinese birth cohort (n=3462), a history of exclusive breastfeeding in the first 3 months of life was associated with lower ApoB at the age of 17.6 years.
3. Waist-hip ratio may partly mediate the association between breastfeeding and ApoB.
4. Whether some effects of breastfeeding are only actuated at the completion of growth (puberty) should be investigated.

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Introduction

Associations of infant formula feeding with early life adiposity have not been confirmed in randomised trials or in observational studies from regions where breastfeeding is not associated with higher socioeconomic position.[1] The association of formula feeding with early life adiposity may be related to lipids.[2] In a randomised controlled trial of individuals born prematurely, apolipoprotein (Apo) B at a mean age of 15 years was higher in those fed pre-term formula as babies than those fed banked breast milk as babies; however, a history of formula feeding did not affect high-density lipoprotein-cholesterol (HDL-c), triglycerides, or ApoA-1.[3] Nonetheless, the trial was small and its follow-up rate was low (23%). ApoB (rather than low-density lipoprotein-cholesterol or triglycerides) is now thought to be a major lipid that contributes to ischaemic heart disease.[4]

To determine the long-term effects of breastfeeding on ApoB and hence its role in cardiovascular disease and mortality, we assessed the associations of breastfeeding with ApoB, triglycerides, and HDL-c in the Hong Kong 'Children of 1997' population-representative birth cohort in late adolescence. Considering differences in cardiovascular disease rates by sex, we also assessed whether associations varied by sex. Finally, we explored the possibility of mediation by adiposity, which could serve as a potential intervention target.

Methods

The Hong Kong 'Children of 1997' birth cohort is a population-representative cohort (n=8327) comprising 88% of births in Hong Kong during April and May 1997. Participants were recruited at all the 49 Maternal and Child Health Centres; these provide universal routine checks and vaccinations for infants and toddlers in Hong Kong. A self-administered questionnaire in Chinese was used to collect baseline information on birth and family characteristics, second-hand smoking, and infant feeding at the first visit after birth. A similar questionnaire was administered at subsequent routine visits during 1997-98 (ages 3, 9, and 18 months). Additional information was collected via record linkage to routine health checks.[1] In 2007, contact was re-established and three telephone/postal surveys were administered; a Biobank Clinical follow-up (phase 1) was conducted in 2013-16 (mean age, 17.5 years), followed by a supplementary Biobank Clinical follow-up (phase 2) in the second half of 2017 (mean age, 19.5 years). Blood samples were collected after an overnight fast and stored at -80°C. After each phase of data collection, comparisons were made with previously collected data and anomalies resolved.

Breastfeeding status during the first 3 months of life was categorised as never breastfed, mixed feeding, or exclusively breastfed. Automated nuclear magnetic resonance spectroscopy was used to obtain ApoB, HDL-c, and triglycerides measurements from serum stored at -80°C; measurements that failed quality control criteria were excluded.

Multivariable linear regression was used to assess the associations of breastfeeding with ApoB and other lipids, after adjusting for potential confounders and age at follow-up. To obtain P-for-trend values regarding the association of

breastfeeding with ApoB, the three breastfeeding categories were presented as a continuous variable (0, 1, and 2). Sex-specific estimates were compared using z-tests. Pearl's mediation formula was used to assess mediation.

Multiple imputation and inverse probability weighting were used to minimise potential biases related to missing information and any differences between respondents and non-respondents. Missing values of breastfeeding and confounders were predicted 50 times with a flexible additive regression model. Multivariable logistic regression showed that breastfeeding, gestational age, maternal age, maternal birthplace, highest parental education, the interaction of highest parental education and maternal birthplace, and possibly sex were associated with non-response to the Biobank Clinical follow-up and thus were missing ApoB values. Logistic regression was used to calculate the probability that an observation would be present. Finally, we analysed the 50 complete datasets separately, considering the probability of inclusion, then summarised the results using single estimated beta coefficients with confidence intervals adjusted for missing-data uncertainty.

Results

Of the 8327 individuals originally included in the 'Children of 1997' birth cohort, 28 had permanently withdrawn and 33 had died before the Biobank Clinical follow-up. Of the remaining 8266, 6850 were potentially contactable for the Biobank Clinical follow-up. After repeated attempts, 3460 and 158 participants attended phase 1 and phase 2 of the Biobank Clinical follow-up, respectively. Of these participants, 3462 had valid ApoB measurements (1688 girls and 1774 boys). Mean ApoB was higher in girls than in boys (0.75 vs 0.73 g/L). ApoB was strongly associated with age at follow-up in boys (0.03 g/L higher per 1 year older, 95% confidence interval=0.02-0.04, P=3.1e-12) but not in girls (0.007 g/L higher per 1 year older, 95% confidence interval= −0.001 to 0.015, P=0.13).

Participants did not differ from the original cohort with respect to socioeconomic position at birth (ie, parental income and highest parental education), parity, type of delivery, or birth weight. However, they differed in terms of maternal birthplace because some of the participants were born to migrants in Hong Kong who intended to return to China; accordingly, there were differences in breastfeeding status, maternal age, gestational age, highest parental occupation, and maternal second-hand smoking (SHS)/smoking.

During the first 3 months of life, 1811 participants were never breastfed, 1341 participants had mixed feeding, and 256 participants were exclusively breastfed; 54 participants had unknown breastfeeding status. A history of breastfeeding was not associated with birth weight, gestational age, maternal age, or age at follow-up; however, it was associated with female sex, higher parity, maternal birth outside Hong Kong, lower parental socioeconomic position, no maternal SHS/smoking, and vaginal delivery. Notably, ApoB was not associated with any other potential confounders apart from sex.

A history of exclusive breastfeeding in the first 3 months of life was associated with lower ApoB, with similar estimates by sex (Table 1). A history of breastfeeding was not associated with HDL-c or triglycerides, with similar estimates by sex (Table 2). In terms of potential mediators, a history of breastfeeding was associated with higher body mass index z-score at 3 months and a lower waist-hip ratio at 17.6 years but not with body mass index at 12 years or 17.6 years or with total fat percentage at 17.6 years. The association of breastfeeding with ApoB was slightly attenuated after adjusting for mediation by waist-hip ratio; the proportion of mediation was approximately 10%, according to causal mediation analysis.

Discussion

Consistent with the previous finding that in premature babies a history of breastfeeding was associated with lower ApoB at the age of 15 years,[3] our study showed a similar association in older adolescents (~17.6 years) but no association of breastfeeding

TABLE 1. Associations of breastfeeding in first 3 months of life with nuclear magnetic resonance spectroscopy–assessed apolipoprotein B at 17.6 years in the Hong Kong 'Children of 1997' birth cohort after adjusting for potential confounders*

Sex	Beta (95% confidence interval)	P value	P-for-trend	P value for sex difference
Overall				
Never breastfed	Reference			
Mixed feeding	-0.005 (-0.016 to 0.005)	0.33		
Exclusively breastfed	-0.027 (-0.046 to -0.007)	0.007	0.016	0.63
Boys				
Never breastfed	Reference			
Mixed feeding	-0.005 (-0.020 to 0.009)	0.48		
Exclusively breastfed	-0.021 (-0.051 to 0.008)	0.15	0.17	
Girls				
Never breastfed	Reference			
Mixed feeding	-0.007 (-0.022 to 0.009)	0.40		
Exclusively breastfed	-0.031 (-0.057 to -0.004)	0.02	0.037	

* Sex, birth weight, gestational age, maternal age, maternal birthplace, highest parental education, parental income, parental occupation, maternal second-hand smoking/smoking, age at the biobank clinical follow-up, interaction of maternal birthplace and highest parental education, and interaction of age at follow-up and sex

TABLE 2. Associations of breastfeeding in first 3 months of life with nuclear magnetic resonance spectroscopy–assessed high-density lipoprotein-cholesterol (HDL-c) and triglycerides at 17.6 years in the Hong Kong 'Children of 1997' birth cohort after adjusting for potential confounders*

Lipids	Beta (95% confidence interval)	P value	P-for-trend	P value for sex difference
HDL-c				
Never breastfed	Reference			
Mixed feeding	-0.022 (-0.046 to 0.002)	0.07		
Exclusively breastfed	-0.018 (-0.062 to 0.026)	0.43	0.11	0.56
Triglycerides				
Never breastfed	Reference			
Mixed feeding	-0.016 (-0.045 to 0.012)	0.26		
Exclusively breastfed	-0.037 (-0.090 to 0.016)	0.17	0.11	0.53

* Sex, birth weight, gestational age, maternal age, maternal birthplace, highest parental education, parental income, parental occupation, maternal second-hand smoking/smoking, age at the biobank clinical follow-up, interaction of maternal birthplace and highest parental education, and interaction of age at follow-up and sex

with HDL-c or triglycerides in individuals who had largely been born at term. The associations were similar by sex, and some mediation by current waist-hip ratio cannot be excluded.

It is unclear how breastfeeding might affect ApoB. One possible explanation is that breastfeeding contributes to nutritional programming in early life, but the effects are only actuated at the completion of growth (ie, in late adolescence). Breastfeeding largely coincides with the mini-puberty of infancy, a developmental window with effects on fertility that only become evident during or after puberty; it is unclear whether breastfeeding modulates mini-puberty or its effects in later life.

The present study had some limitations. First, similar to most long-running cohort studies, attrition and missing data occurred. We used multiple imputation and inverse probability weighting to address these issues. Second, breastfeeding status was assessed using a questionnaire, so misclassification is possible. However, misclassification is unlikely to influence ApoB many years later; thus, we expect a bias towards the absence of an effect. Third, this study was not designed to study the effects of long-term (>3 months) breastfeeding, which was uncommon in Hong Kong in 1997.

The results of this study support breastfeeding, considering its short-term benefits and potential lifelong benefits. It would be useful to explore how breastfeeding duration affects ApoB (ie, whether >6 months of breastfeeding is more beneficial than 3 months of breastfeeding); this information could have major implications for mothers' lives and maternity policies. A follow-up study examining the associations of breastfeeding with hormones would be timely and relevant, particularly because there is increasing evidence that hormones affect cardiovascular disease[5] and hormone-related cancers.

Conclusion

A history of breastfeeding was associated with lower ApoB, but not with triglycerides or HDL-c, in older adolescents; these associations were similar by sex. Breastfeeding may have important beneficial effects on the key lipid contributing to cardiovascular disease and longevity.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#17181271). The full report is available from the Health and Medical Research Fund website (https://rfs2.healthbureau.gov.hk).

Disclosure

The results of this research have been previously published in:

1. Schooling CM, Au Yeung SL, Kwok MK, Leung GM. Breastfeeding and ApoB in late adolescence: a Hong Kong birth cohort study. Eur J Pediatr 2023 Jun 8.

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References

1. Schooling CM, Hui LL, Ho LM, Lam TH, Leung GM. Cohort profile: 'children of 1997': a Hong Kong Chinese birth cohort. Int J Epidemiol 2012;41:611-20.
2. Leon DA, Ronalds G. Breast-feeding influences on later life—cardiovascular disease. Adv Exp Med Biol 2009;639:153-66.
3. Singhal A, Cole TJ, Fewtrell M, Lucas A. Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. Lancet 2004;363:1571-8.
4. Richardson TG, Sanderson E, Palmer TM, et al. Evaluating the relationship between circulating lipoprotein lipids and apolipoproteins with risk of coronary heart disease: a multivariable Mendelian randomisation analysis. PloS Med 2020;17:e1003062.
5. Luo S, Au Yeung SL, Zhao JV, Burgess S, Schooling CM. Association of genetically predicted testosterone with thromboembolism, heart failure, and myocardial infarction: mendelian randomisation study in UK Biobank. BMJ 2019;364:l476.

Maximising the cost-effectiveness of human papillomavirus testing for cervical screening in the context of routine HPV vaccination in Hong Kong: abridged secondary publication

SMK Leung *, J Wu *, KKL Chan, M Jit

KEY MESSAGES

1. Among cohorts without a human papillomavirus (HPV) vaccination programme, use of the HPV test as a standalone primary test or as a screening test after receiving cytological analysis of atypical squamous cells of undetermined significance was considered a cost-effective cervical screening strategy to reduce deaths from cervical cancer when the willingness-to-pay threshold was one gross domestic product per capita (US$46 615).
2. Reassessment of the comparative cost-effectiveness of strategies with longer routine screening intervals, a pre-defined fixed number of routine screenings per lifetime, or a later starting age is needed to identify optimal screening strategies for vaccinated cohorts, especially when newer data about the duration of vaccine protection become available.

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Introduction

The second-generation nonavalent human papillomavirus (HPV) vaccine (9vHPV) is >90% effective against seven high-risk HPV (hrHPV) types, which collectively cause >90% of cervical cancers. As HPV prevalence drops (eg, when vaccine uptake is high in a vaccination programme), the positive predictive value of cytology for cervical precancerous lesions and cancer decreases, whereas the negative predictive value of the HPV test for the same clinical outcomes increases. Consequently, incorporation of the HPV test into cytology-based screening algorithms becomes more important in the context of routine 9vHPV vaccination.

In Hong Kong, a population-based cervical screening programme was initiated in 2004, in which women aged 25 to 64 years are recommended to undergo cytology-based cervical screening every 3 years after two consecutive normal screening results.[1] Since June 2021, the updated recommendations include the use of high-risk HPV testing, either as a primary test or in combination with cytology, for women aged 30 to 64 years. Furthermore, the government recently implemented HPV vaccination for female adolescents. Beginning in the 2019-20 school year, schoolgirls in primary 5 and 6 (equivalent to age 11 to 12 years) could receive two doses of the 9vHPV vaccine at no cost. This study evaluated the cost-effectiveness of different HPV testing applications in Hong Kong.

Methods

We extended our calibrated model for HPV vaccination and cervical screening[2] to compare the cost-effectiveness of various screening strategies. Our model consisted of a deterministic age-structured compartmental dynamic model that simulates heterosexual transmission of hrHPV and a stochastic individual-based cohort model that simulates the development of cervical cancer over the lifetime of each female. We grouped hrHPV into four classes: (1) HPV-16, (2) HPV-18, (3) HPV-OV (other vaccine type that comprises the other five hrHPV targeted by the 9vHPV vaccine, namely HPV-31, 33, 45, 52, and 58), and (4) HPV-NV that comprises all non-vaccine hrHPV.

Based on the latest screening recommendations from the Cancer Expert Working Group and guidelines from the Hong Kong College of Obstetricians and Gynaecologists (HKCOG),[3,4] we considered the following screening strategies (FIG 1): A: cytology as primary screening, B: high-risk HPV DNA test (HPV test) as primary screening, and C: a combination of cytology and HPV test (co-test) as primary screening. In accordance with HKCOG guidelines, we assumed that the routine

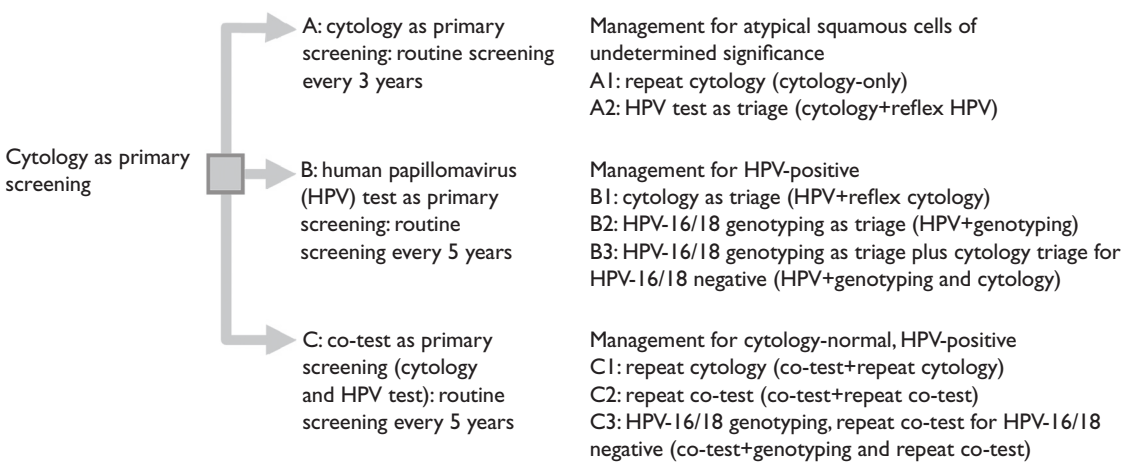

FIG 1. Cervical screening algorithm currently recommended in Hong Kong[3]

screening interval was every 3 years for cytology and every 5 years for HPV test and co-test.[3]

The findings were analysed separately for (1) cohorts who were aged ≥12 years when the routine HPV vaccination programme began in 2019 (nVP cohorts) and (2) the first 10 cohorts eligible for HPV vaccination programme during their lifetime (VP cohorts). Thus, nVP cohorts included females aged 16 to 64 years in 2022; VP cohorts included females aged 6 to 15 years in 2022. We assumed that screening uptake would be similar among vaccinated and unvaccinated individuals. We also assumed that 70% of eligible females would undergo cervical screening with full compliance.

Regarding test performance, we assumed that the mean respective sensitivities for identifying CIN1 and CIN2/3 were 0.69 and 0.76 for cytology and 0.81 and 0.93 for the HPV test. The mean specificities were 0.97 for cytology and 0.91 for the HPV test. We also assumed that colposcopy-directed biopsy was 100% accurate.

We set vaccine uptake at 85%, in accordance with statistics regarding eligible primary 5 schoolgirls who received the first dose of the 9vHPV vaccine. The class-specific vaccine efficacies of the 9vHPV vaccine were based on data from the 9vHPV vaccine trials. We considered three scenarios for the duration of vaccine protection: lifelong, 30 years, and 20 years; we tested scenarios that assumed vaccine uptake decreasing to 75%, 50%, and 25%.

The costs of screening were based on charges for private patients in public hospitals, which represent >90% of inpatient care in Hong Kong. Treatments were based on expert opinions of oncologists and gynaecologists in Hong Kong. With reference to other cost-effectiveness analysis on cervical screening, we considered both life-years (LYs) and quality-adjusted life-years (QALYs) as metrics for quantifying health outcomes. When calculating QALYs, we adopted health utility parameters from studies in other countries because there were insufficient data from Hong Kong. Both costs and health benefits were discounted by 3% per year.

We conducted probabilistic sensitivity analysis to adjust for parameter uncertainty. The analysis included 100 parameter sets related to disease epidemiology (eg, natural history of HPV transmission, vaccine efficacy, and test performance) and 100 parameter sets related to costs and health utilities. In total, 10 000 parameter combinations were studied.

The incremental cost-effectiveness ratio (ICER) was defined as the additional mean cost divided by the additional mean health outcome. The willingness to pay (WTP) threshold was set at one Hong Kong gross domestic product per capita (GDPpc; US$46 615 / HK$363 596). We used net monetary benefit (NMB) to quantify the strategies in terms of monetary value. NMB is defined as "$WTP \times E - C$", where C and E are the cost and health outcome of a particular strategy. We constructed cost-effectiveness acceptability curves and cost-effectiveness acceptability frontiers to allow simultaneous ranking of multiple strategies across a range of WTP values.

Results

For cohorts without the routine vaccination programme (nVP cohorts), the comparative cost-effectiveness of the evaluated strategies was not sensitive to vaccine uptake or duration of vaccine protection. When vaccine uptake was 85% and

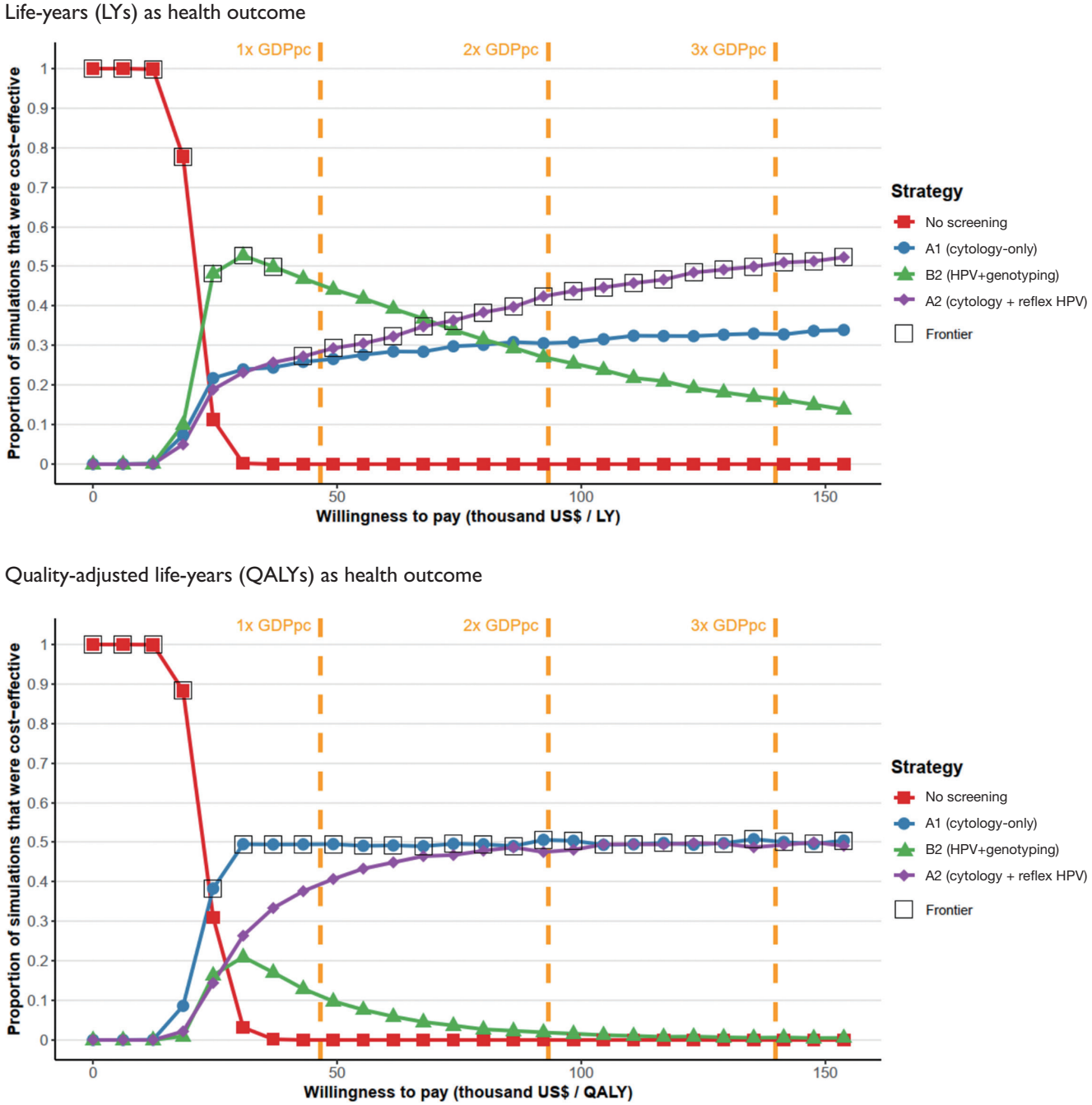

FIG 2. Cost-effectiveness acceptability curves and frontiers for cohorts without routine vaccination programme (nVP cohorts). Frontiers indicate strategies with greatest mean net monetary benefit at corresponding willingness to pay values. Plots are based on assumptions that vaccine uptake is 85% through the routine immunisation programme and that human papillomavirus (HPV) vaccines provide lifelong protection.

vaccine protection was lifelong, if LYs were used as the metric for health outcomes, B2 (HPV+genotyping) was the most cost-effective strategy, with an ICER of US$21 644 per LY gained. The next most cost-effective strategy was A2 (cytology+reflex HPV), with an ICER of US$40 137 per LY gained. The remaining strategies were either dominated or associated with ICERs >3 times GDPpc. The comparative cost-effectiveness of strategies B2 and A2 was sensitive to parameter uncertainty when the WTP threshold was near 1 GDPpc (FIG 2). However, as the WTP threshold increased, A2 (cytology+reflex HPV) was the most cost-effective strategy in more scenarios as the mean NMB increased. If QALYs were used as the metric for health outcomes, A1 (cytology-only) was the most cost-effective strategy, with an ICER

of US$23 389 per QALY gained. The next most cost-effective strategy was A2 (cytology+reflex HPV), with an ICER of US$181 297 per QALY gained. The remaining strategies were dominated. The comparative cost-effectiveness of A1 (cytology-only) and A2 (cytology+reflex HPV) was not sensitive to parameter uncertainty; thus, A1 was the most cost-effective strategy if the WTP threshold was <1 GDPpc (FIG 2). However, as the WTP threshold increased to >1.5 times GDPpc (US$70 000), A1 and A2 were the most cost-effective strategies in a similar proportion of scenarios, with A1 consistently demonstrating a higher mean NMB.

For cohorts eligible to receive routine vaccination (VP cohorts), if LYs were used as the metric for health outcomes, B2 (HPV+genotyping) was the most cost-effective strategy—regardless of vaccine uptake and duration of vaccine protection—with ICERs ranging from US$22 849 (for 25% uptake and 20 years of protection) to US$59 836 (for 85% uptake and lifelong protection) [Table]. The remaining strategies were either dominated or associated with ICERs >10 times GDPpc. If the WTP threshold was 1 GDPpc, B2 was cost-effective only if vaccine uptake was <75% and vaccine protection was <20 years (Table). For screening to be cost-effective regardless of vaccine uptake and duration of protection, the WTP threshold would need to be >1.3 times GDPpc (US$60 000). If QALYs were used as the metric for health outcomes, A1 (cytology-only) was the most cost-effective strategy regardless of vaccine uptake and duration of vaccine protection. The corresponding ICERs (compared with no screening) ranged from $24 884 (for 25% uptake and 20 years of protection) to $78 003 (for 85% uptake and lifelong protection). The remaining strategies were dominated by higher ICERs or associated with QALY loss. If the WTP threshold was 1 GDPpc, screening would be cost-effective only if vaccine uptake was ≤50% or if duration of protection was 20 years and vaccine uptake was ≤75%. For screening to be cost-effective regardless of vaccine uptake and duration of protection, the WTP threshold would need to be >1.7 times GDPpc (US$78 000). The comparative cost-effectiveness of the evaluated strategies for VP cohorts was not sensitive to parameter uncertainty.

Discussion

We compared the cost-effectiveness of different uses of HPV testing in cervical screening currently recommended by health authorities in Hong Kong.[3] Among cohorts who could not enrol in the routine 9vHPV vaccination programme (ie, nVP cohorts), when the WTP threshold is 1 GDPpc, strategy A2 (cytology+reflex HPV) and strategy A1 (cytology-only) are the optimal strategies for achieving the greatest health benefit when health outcome metrics are LYs and QALYs, respectively.

For females eligible to receive 9vHPV vaccination through the routine immunisation programme (ie, VP cohorts), the comparative cost-effectiveness of each screening strategy depends on vaccine uptake and duration of vaccine protection. If the effect of the immunisation programme is high, the marginal benefit of screening decreases and the corresponding ICER increases. The vaccine effect is highest when vaccine uptake is 85% (consistent with the latest statistics) and vaccine protection is lifelong. In this scenario, none of the evaluated screening strategies is cost-effective if the WTP threshold is 1 GDPpc. If the WTP threshold is ≥1.7 times GDPpc, the optimal screening strategy for VP cohorts is strategy B2 (HPV+genotyping) and strategy A1 (cytology-only) when health outcome metrics are LYs and QALYs, respectively.

TABLE. Incremental cost-effectiveness ratios (ICERs) of the most cost-effective screening strategies across scenarios of human papillomavirus (HPV) vaccine uptake and duration of vaccine protection for cohorts in the routine vaccination programme (VP cohorts)

Strategy	Vaccine protection	Vaccine uptake among cohorts eligible to receive routine vaccination			
		85%	75%	50%	25%
ICER per life-year gained, US$					
B2 (HPV+genotyping) vs no screening	Lifelong	59 836	49 218	32 600*	23 581*
B2 (HPV+genotyping) vs no screening	30 years	57 008	47 301	32 039*	23 491*
B2 (HPV+genotyping) vs no screening	20 years	48 509	41 511*	29 851*	22 849*
ICER per quality-adjusted life-year gained, US$					
A1 (cytology-only) vs no screening	Lifelong	78 003	61 994	38 458*	26 289*
A1 (cytology-only) vs no screening	30 years	72 943	58 506	37 177*	25 921*
A1 (cytology-only) vs no screening	20 years	59 322	49 409	33 772*	24 884*

* ICERs that are below the willingness-to-pay threshold at 1 gross domestic product per capita (US$46 615 / HK$363 596)

The guidelines-based recommendation of routine screening every 5 years may be excessive for VP cohorts when vaccine effect is high. In a study of the role of the HPV test as the primary method for cervical screening in female adolescents who were offered the 9vHPV vaccine in four high-income countries (Australia, England, New Zealand, and the United States), the most cost-effective strategy involved only two to five screenings per lifetime. Current guidelines suggest that if the HPV test is used as the primary method for routine screening, females should begin with cytology-based screening (ie, strategy A1) at age 25 years and then switch to the HPV test at age 30 years. If vaccine uptake in the routine immunisation programme is high, cytology-only screening before age 30 years may be unnecessary. If the HPV test is used as the primary method, cervical screening at age 30 or 35 years would reduce costs, potentially without significant increases in precancerous lesions and cancer cases. Further comparative analyses of the cost-effectiveness of strategies (eg, with longer routine screening intervals, a pre-defined fixed number of routine screenings per lifetime, or a later age at initial screening) are needed to identify optimal screening strategies for vaccinated cohorts, particularly as newer data about the duration of vaccine protection become available.

Funding

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Disclosure

The results of this research have been previously published in:

1. Choi HCW, Leung K, Chan KKL, Bai Y, Jit M, Wu JT. Maximizing the cost-effectiveness of cervical screening in the context of routine HPV vaccination by optimizing screening strategies with respect to vaccine uptake: a modeling analysis. BMC Med 2023;21:48.

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References

1. Cervical Screening Programme, Department of Health. Available at: https://www.cervicalscreening.gov.hk/en/index.html.
2. Choi HCW, Jit M, Leung GM, Tsui K-L, Wu JT. Simultaneously characterizing the comparative economics of routine female adolescent nonavalent human papillomavirus (HPV) vaccination and assortativity of sexual mixing in Hong Kong Chinese: a modeling analysis. BMC Med 2018;16:127.
3. The Hong Kong College of Obstetricians and Gynaecologists. Guidelines for cervical cancer prevention and screening. 2016.
4. Cancer Expert Working Group on Cancer Prevention and Screening, Centre for Health Protection. Recommendations on prevention and screening for cervical cancer. 2021. Available at: https://www.chp.gov.hk/files/pdf/cervical_cancer_professional_hp.pdf.

Climate change beliefs, perceptions of climate change–related health risk, and responses to heat-related risks among Hong Kong adults: abridged secondary publication

Q Liao *, R Fielding, WWT Lam, L Yang, L Tian, TC Lee

KEY MESSAGES

1. Psychological distance is an important contributor to low public engagement with climate change in Hong Kong.
2. A significant proportion of Hong Kong adults were sceptical about the personal and local relevance of climate change impacts, were optimistic about the ability of government, technology, and international organisations in controlling climate change, and perceived low personal responsibility for climate change mitigation.
3. Among Hong Kong adults, perceived heat-related health risk was low to moderate if heat waves occurred. Participants who were more sceptical and optimistic about climate change and disengaged regarding personal responsibility in climate change mitigation perceived lower heat-related health risk.

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Anthropogenic climate change is a major threat to the ecosystems and human society. Much attention has been paid to consequential increases in atmospheric temperature. According to the Intergovernmental Panel on Climate Change, the world's average temperature is forecasted to increase 1.5°C before 2040.[1] There is evidence that climate change is harming human health, through heat stress, extreme weather events, and infectious diseases.[2] In particular, heat stress has become more common and caused loss of numerous human lives globally.[3] People older than 65 years have limited temperature adaptive capacity and hence are more vulnerable to the harmful health impact of heat stress.[4] Hong Kong with a rapidly ageing population is particularly at risk of extreme heat compounded by the heat-island effect. It is projected that southeast Asia, including Hong Kong, will experience a sharp surge in heat-related excess mortality by the end of the 21st century under likely high anthropogenic greenhouse gas emission scenarios.[5]

However, climate change is consistently viewed as a distant, highly uncertain, and personally irrelevant threat.[6] In Hong Kong, while acknowledgement of climate change was universal, psychological distance is an important contributor to low public engagement with climate change. Of 1705 Hong Kong adults who participated in our survey, although 92% were mostly or very convinced that climate change was affecting the planet, only 58% believed that the impacts would be significant in Hong Kong in the next 5 years, and only around 40% were quite or very worried that they would be personally affected. Older adults were less worried about the impact of climate change and tended to believe that Hong Kong was not currently affected.

Climate change beliefs are multidimensional involving beliefs in its existence, anthropogenic causes, controllability, personal agency (personal responsibility in climate change mitigation), severity of impact, and personal relevance of the impact.[7] People can be classified into different groups based on the multifaced dimensions of climate change beliefs. For instance, in the United States, six groups that represented a continuum of climate change beliefs were identified: the alarmed, concerned, cautious, disengaged, doubtful, and dismissive,[8] with the latter three groups having lower engagement with climate change. In Hong Kong, our current study found that around one third of the 1705 participants had high confidence in government actions, technological solutions, and international cooperation to address climate change but were sceptical about the personal and local relevance of climate change impact (the optimistic and sceptical group). In addition, around one fourth of the participants perceived low personal responsibility or no ability to do something about climate change (the disengaged group). These two

groups also perceived lower impact of climate change on their personal health; a significant proportion of the adult population in Hong Kong are likely to have low engagement with climate-related policies and actions.

Climate beliefs are associated with perceptions of weather-related changes.[2,5] People who are more sceptical about climate change tend to downplay their subjective experience of abnormal heat.[9,10] Our study found that participants perceived low-to-moderate heat-related health risk if heat waves occurred in Hong Kong. Participants who were optimistic and sceptical about climate change and those who were more disengaged perceived lower heat-related health risk, which was associated with lower adoption of heat protection behaviours.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#16171561). The full report is available from the Health and Medical Research Fund website (https://rfs2.healthbureau.gov.hk).

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References

1. Intergovernmental Panel on Climate Change. AR6 Synthesis Report: Climate Change 2023. Available from: https://www.ipcc.ch/report/sixth-assessment-report-cycle/
2. McMichael AJ, Woodruff RE, Hales S. Climate change and human health: present and future risks. Lancet 2006;367:859-69.
3. Watts N, Amann M, Arnell N, et al. The 2020 report of the Lancet Countdown on health and climate change: responding to converging crises. Lancet 2021;397:129-70.
4. Ho HC, Lau KK, Ren C, Ng E. Characterizing prolonged heat effects on mortality in a sub-tropical high-density city, Hong Kong. Int J Biometeorol 2017;61:1935-44.
5. Gasparrini A, Guo Y, Sera F, et al. Projections of temperature-related excess mortality under climate change scenarios. Lancet Planet Health 2017;1:e360-7.
6. Spence A, Poortinga W, Pidgeon N. The psychological distance of climate change. Risk Anal 2012;32:957-72.
7. Leiserowitz A, Roser-Renouf C, Marlon J, Maibach E. Global warming's six Americas: a review and recommendations for climate change communication. Curr Opin Behav Sci 2021;42:97-103.
8. Leiserowitz A, Maibach E, Rosenthal S, Kotcher J, Lacroix K, Goldberg M. Global Warming's Six Americans, September 2021. Yale Program on Climate Change Communication. Available from https://climatecommunication.yale.edu/publications/global-warmings-six-americas-september-2021/
9. Howe PD, Leiserowitz A. Who remembers a hot summer or a cold winter? The asymmetric effect of beliefs about global warming on perceptions of local climate conditions in the U.S. Glob Environ Change 2013;23:1488-500.
10. Myers TA, Maibach EW, Roser-Renouf C, Akerlof KL, Leiserowitz A. The relationship between personal experience and belief in the reality of global warming. Nat Clim Chang 2013;3:343-7.

Effects of puerarin supplementation on cardiovascular disease risk factors: a randomised, double-blind, placebo-controlled, two-way crossover trial (abridged secondary publication)

MK Kwok, GM Leung *, L Xu, HF Tse, TH Lam, TH So, CM Schooling *

KEY MESSAGES

1. In healthy Chinese men in Hong Kong, short-term (12-week) supplementation with puerarin granules (90.2 mg daily) did not improve cardiovascular disease risk profile including lipid profile, blood pressure, testosterone, inflammation, coagulation, liver function, and renal function.
2. Fasting glucose was reduced after puerarin supplementation. Further research is needed to determine whether puerarin can help to improve glycaemic traits.

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Introduction

Pueraria (also known as 'Gegen' in Chinese and 'Kudzu' in Japanese), the root of *Pueraria lobata*, is a commonly used herb in Chinese medicine. According to the Pharmacopoeia of the People's Republic of China, dried pueraria is indicated for the treatment of fever, diarrhoea, diabetes, and hypertension, with a recommended daily dose of 10-15 g. Puerarin is the major bioactive constituent in pueraria. Over the past few decades, puerarin injections have been extensively used in China for the treatment of cerebrovascular ischaemia, cardiovascular disease (CVD), angina pectoris, cardiac infarction, and viral myocarditis. Puerarin capsules have been used in supplement form and are generally considered free of adverse effects. Previous *in vitro* and *in vivo* studies indicated that puerarin may have positive effects on the cardiovascular system.

Clinical trials have revealed conflicting results concerning the effects of puerarin on CVD risk factors in humans, although two studies showed beneficial effects on lipids and blood pressure.[1,2] Some trials investigating puerarin as an alternative to hormonal replacement therapy have shown a modest effect on cholesterol. Another trial indicated that puerarin supplementation improved insulin resistance.[3] However, a Cochrane review found that puerarin injection had a non-significant effect on survival or dependency in people with ischaemic stroke (relative risk=0.79, 95% confidence interval=0.45-1.36).[4] Another Cochrane review demonstrated that puerarin injection had a neutral effect on reducing episodes of acute angina (relative risk=0.95, 95% confidence interval=0.85-1.07).[5] Overall, the limited number of studies and small number of participants in each trial preclude definitive conclusions regarding the effect of puerarin on CVD risk, although the available evidence indicates possible benefits. This study assessed the effects of puerarin supplementation on CVD risk factors (lipid profile, blood pressure, and fasting glucose) and potential mediating pathways (including testosterone, inflammation, coagulation, and liver and renal function) in Chinese men.

Methods

This randomised, double-blind, placebo-controlled, two-way crossover trial included Chinese men aged 18 to 50 years who were willing to make return visits, were not currently taking any traditional Chinese medicine (including puerarin) supplementation, were not receiving hormone replacement therapy (currently or in the previous 12 months), were free of any congenital diseases or infectious diseases (eg, seasonal influenza), had no history of any chronic diseases including coronary heart disease (ischaemic heart disease), myocardial infarction (heart attack), stroke, diabetes, or cancer, and had a 10-year risk of ischaemic heart disease <10%. Participants were recruited by advertisements across various channels (eg, the university-wide bulk mailing system, in-class announcements, recruitment booths, and posters)

throughout Hong Kong. The trial was registered (reference: NCT03676296) and ethics approval was obtained before recruitment of participants.

Initial eligibility assessments were conducted on-site or via telephone. Participants were randomly allocated to one of two intervention sequences at a 1:1 ratio: puerarin then placebo or placebo then puerarin. Frontline staff, laboratory technicians, investigators, data analysts, and participants were masked to the intervention sequences for all participants.

Each participant took either a puerarin supplement or a placebo for 12 weeks; this was followed by a 4-week washout period, after which the participant was switched to the other intervention for 12 weeks. Fasting blood samples were collected at four timepoints: after randomisation, after 12 weeks of the first intervention, after 4 weeks of washout, and after 12 weeks of the second intervention). In total, 21 mL of fasting blood were collected at each sampling time point.

One sachet of puerarin granules contained a mixture of puerarin (90.2 mg) and excipients. The placebo was prepared with the same excipients but lacked puerarin. All granules were purchased from an approved Good Manufacturing Practice–certified manufacturer. The two types of granules were identical in weight and appearance.

The primary outcome was the lipid profile (levels of total cholesterol, low- and high-density lipoprotein cholesterol, and triglycerides). Secondary outcomes were CVD risk factors including blood pressure (systolic and diastolic) and fasting glucose, as well as potential mediating pathways such as testosterone, inflammation (high-sensitivity C-reactive protein), coagulation (prothrombin time), liver function (aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, total protein, and albumin), and renal function (urea, creatinine, sodium, and potassium).

An intention-to-treat analysis was performed, assuming no changes in baseline values for participants with missing follow-up values. Differences in outcomes between puerarin supplementation and placebo in participants were compared using paired t-tests or, if a period effect existed, crossover-based analysis. Statistical analyses were conducted using R software, version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

The trial began on 5 September 2018 and ended on 17 April 2021. Of 277 Chinese men recruited, 217 were eligible for enrolment. Participants were randomly allocated to receive puerarin then placebo (n=112) or placebo then puerarin (n=105). Baseline characteristics of the two groups were similar (Table 1). The mean participant age was 31.4 years; 77% of the participants were born in Hong Kong, while 23% were born in Mainland China or elsewhere.

Lipid profiles were similar after puerarin or placebo supplementation; the mean difference in low-density lipoprotein cholesterol was -0.02 mmol/L (Table 2). Conversely, fasting glucose was reduced after puerarin supplementation (-0.13 mmol/L). There were no significant differences in other outcomes (ie, systolic or diastolic blood pressure, testosterone, inflammation, coagulation, liver function, or renal function).

Discussion

There was little evidence that short-term (12-week) puerarin supplementation influenced the CVD risk profile in healthy Chinese men. Lipid profiles and blood pressure values were similar, regardless of puerarin or placebo supplementation. However, fasting glucose was reduced after puerarin supplementation. Because puerarin did not influence testosterone, high-sensitivity C-reactive protein, prothrombin time, liver function, or renal function, these biomarkers presumably do not underlie mediating pathways by which puerarin is linked to health outcomes.

We found no effects of puerarin supplementation on lipid profile or blood pressure. Concerning the lipid profile, two previous trials of oral puerarin supplementation (capsules, tablets, or granules) did not indicate lipid-lowering effects; however, 3 months of puerarin supplementation at a higher dose (150 mg/day in tablet form) in addition to daily metformin led to healthier lipid profiles in women with polycystic ovary syndrome (n=30). Puerarin supplementation did not lower blood pressure in one trial, but two trials showed blood pressure reduction in patients with ischaemic stroke and/or hypertension. The mixed findings thus far do not strongly support the notion that puerarin supplementation has clear lipid- or blood pressure-lowering effects.

In this trial, we found that puerarin supplementation reduced fasting glucose. Earlier trials of puerarin supplementation did not comprehensively evaluate glycaemic status. Previous trials have shown that puerarin supplementation improves insulin resistance, as indicated by a decrease in homeostatic model assessment for insulin resistance (HOMA-IR) in patients with rheumatoid arthritis[3]; conversely, it did not improve fasting glucose in women with polycystic ovary syndrome.[2] In a rodent model of diabetic injury, puerarin supplementation enhanced glycaemic status, as indicated by improvements in insulin secretion and resistance, as well as a decrease in serum glucose; these effects were potentially mediated by the reduction of oxidative stress through anti-

TABLE 1. Baseline characteristics of participants

Baseline characteristic	Overall (n=217)*	Puerarin then placebo (n=112)*	Placebo then puerarin (n=105)*	P value
Age, y	31.4±9.2	30.8±9.2	32.0±9.2	0.32
Education				0.26
Postgraduate	100 (46.5)	51 (45.9)	49 (47.1)	
Undergraduate	102 (47.4)	56 (50.5)	46 (44.2)	
Secondary or below	13 (6.0)	4 (3.6)	9 (8.7)	
Place of birth				0.46
Hong Kong	167 (77.0)	89 (79.5)	78 (74.3)	
Mainland China or elsewhere	50 (23.0)	23 (20.5)	27 (25.7)	
Smoking status				0.13
Never-smoker	185 (88.1)	97 (89.8)	88 (86.3)	
Ex-smoker	7 (3.3)	1 (0.9)	6 (5.9)	
Current smoker	18 (8.6)	10 (9.3)	8 (7.8)	
Alcohol use				0.77
Never-drinker	47 (21.7)	23 (20.5)	24 (22.9)	
Ex-drinker	55 (25.3)	27 (24.1)	28 (26.7)	
Current drinker	115 (53.0)	62 (55.4)	53 (50.5)	
Height, cm	172.6±6.8	173.1±7.1	172.1±6.6	0.31
Body mass index, kg/m^2	23.3±3.5	23.0±3.3	23.6±3.7	0.15
Systolic blood pressure, mmHg	120.1±11.5	119.3±11.1	121.1±12.0	0.25
Diastolic blood pressure, mmHg	75.9±10.1	74.7±9.9	77.2±10.2	0.06
Waist circumference, cm	85.4±10.9	84.4±11.5	86.5±10.2	0.16
Hip circumference, cm	97.3±7.7	96.8±8.0	97.9±7.4	0.27
Total body fat, %	21.8±17.4	20.9±12.9	22.8±21.2	0.43
Total muscle mass, %	53.3±7.5	53.2±8.3	53.4±6.3	0.88
Triglycerides, mmol/L	1.07±0.93	1.05±0.96	1.10±0.90	0.68
Total cholesterol, mmol/L	4.71±0.92	4.70±0.98	4.71±0.86	0.90
High-density lipoprotein cholesterol, mmol/L	1.34±0.34	1.34±0.33	1.34±0.35	0.98
Low-density lipoprotein cholesterol, mmol/L	2.90±0.81	2.91±0.85	2.89±0.77	0.89
Fasting glucose, mmol/L	5.17±0.59	5.13±0.61	5.20±0.58	0.36
Testosterone, nmol/L	20.43±7.85	20.66±7.15	20.18±8.56	0.65
C-reactive protein, mg/L	1.20±1.96	1.24±2.14	1.17±1.76	0.79
Prothrombin time, seconds	11.66±1.11	11.67±0.98	11.66±1.23	0.97
Aspartate aminotransferase, U/L	23.24±29.16	24.30±39.09	22.10±11.53	0.58
Alanine aminotransferase, U/L	34.07±23.42	31.55±18.81	36.76±27.34	0.10
Alkaline phosphatase, U/L	67.70±18.76	67.20±17.81	68.24±19.80	0.68
Gamma-glutamyl transferase, U/L	33.94±26.98	31.61±19.48	36.42±33.08	0.19
Total bilirubin, μmol/L	10.33±4.48	10.51±4.82	10.14±4.10	0.55
Total protein, g/L	75.54±4.09	75.36±4.38	75.74±3.75	0.49
Albumin, g/L	43.55±2.45	43.54±2.36	43.55±2.56	0.98
Urea, mmol/L	5.27±1.21	5.14±1.07	5.42±1.33	0.09
Creatinine, μmol/L	83.41±12.05	83.35±10.55	83.47±13.51	0.94
Sodium, mmol/L	139.01±1.61	139.04±1.60	138.97±1.63	0.74
Potassium, mmol/L	4.58±0.39	4.55±0.37	4.60±0.41	0.40

* Data are presented as mean ± standard deviation or No. (%) of participants

inflammatory processes, such as tumour necrosis factor-related pathways. Further investigations of various glycaemic traits (eg, fasting glucose, fasting insulin, glycated haemoglobin, insulin resistance, and beta-cell function) in humans are needed to clarify the effects of puerarin on glycaemic control.

Notably, puerarin supplementation had no effects on testosterone, inflammation, coagulation, renal function, or liver function in healthy Chinese men. Puerarin binds to oestrogen receptors with potentially weak antagonistic properties; it is not known to directly influence androgenic activities, although indirect modulation may occur through increased levels of serum sex hormone binding globulin.[2] Puerarin supplementation counteracted the anticoagulation effect of warfarin in rodents, but its ability to exert a similar effect in humans has not yet been examined. Previous studies in rodents have also shown that puerarin is well-tolerated by the kidneys and liver.

There were a few limitations in this trial. First, it was conducted during the COVID-19 pandemic, which delayed or disrupted recruitment and in-person clinical follow-up sessions. We recruited additional participants to allow for a high number of dropouts. Second, some outcomes may have been influenced by period effects. To adjust for these effects, we conducted crossover-based analyses of the relevant outcomes. Third, participants were highly educated; however, this presumably did not affect the internal validity of the findings because the randomisation approach minimises confounding and selection bias at the time of recruitment. Finally, the trial only included men; the findings require validation in other populations to confirm their generalisability.

TABLE 2. Treatment effects or crossover effects (if period effect is significant) of puerarin supplementation

Outcome	Mean difference (95% confidence interval)	P value
Triglycerides, mmol/L	-0.008 (-0.12 to 0.11)	0.897
Total cholesterol, mmol/L	-0.03 (-0.11 to 0.06)	0.515
High-density lipoprotein cholesterol, mmol/L	0.003 (-0.02 to 0.03)	0.833
Low-density lipoprotein cholesterol, mmol/L	-0.02 (-0.09 to 0.06)	0.648
Systolic blood pressure, mmHg	-0.35 (-1.91 to 1.20)	0.652
Diastolic blood pressure, mmHg	-0.08 (-1.53 to 1.37)	0.912
Fasting glucose, mmol/L	-0.13 (-0.25 to -0.008)	0.036
Testosterone, nmol/L	0.52 (-0.37 to 1.42)	0.250
C-reactive protein, mg/L	0.33 (-0.72 to 1.37)	0.541
Prothrombin time, seconds	0.04 (-0.13 to 0.21)	0.604
Aspartate aminotransferase, U/L	-1.99 (-5.73 to 1.74)	0.295
Alanine aminotransferase, U/L	-0.64 (-3.35 to 2.08)	0.644
Alkaline phosphatase, U/L	-0.35 (-1.82 to 1.12)	0.639
Gamma-glutamyl transferase, U/L	1.25 (-2.17 to 4.66)	0.473
Total bilirubin, μmol/L	-0.09 (-0.92 to 0.75)	0.840
Total protein, g/L	-0.62 (-1.26 to 0.03)	0.060
Albumin, g/L	-0.22 (-0.63 to 0.20)	0.303
Urea, mmol/L	-0.01 (-0.22 to 0.19)	0.912
Creatinine, nmol/L	0.14 (-1.15 to 1.43)	0.832
Sodium, mmol/L	-0.27 (-0.63 to 0.09)	0.140
Potassium, mmol/L	-0.001 (-0.07 to 0.07)	0.980
Height, cm	6.87 (-2.55 to 16.28)	0.152
Body mass index, kg/m^2	0.76 (-0.63 to 2.16)	0.283
Waist circumference, cm	-0.26 (-1.13 to 0.61)	0.556
Hip circumference, cm	0.07 (-1.06 to 1.19)	0.903
Total muscle mass, %	-2.77 (-7.57 to 2.02)	0.256
Total body fat, %	-0.05 (-2.32 to 2.22)	0.965

Conclusion

In healthy Chinese men in Hong Kong, short-term (12-week) puerarin supplementation (90.2 mg daily) did not improve CVD risk profile including the lipid profile, blood pressure, testosterone, inflammation, coagulation, liver function, and renal function. Further research is needed to determine whether puerarin can help to improve glycaemic traits such as fasting glucose.

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Disclosure

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1. Kwok MK, Leung GM, Xu L, Tse HF, Lam TH, Schooling CM. Effect of puerarin supplementation on cardiovascular disease risk factors: a randomized, double-blind, placebo-controlled, 2-way crossover trial. Biomed Pharmacother 2022;153:113472.

References

1. Verma SK, Jain V, Singh DP. Effect of *Pueraria tuberosa* DC. (Indian Kudzu) on blood pressure, fibrinolysis and oxidative stress in patients with stage 1 hypertension. Pak J Biol Sci 2012;15:742-7.
2. Li W, Hu H, Zou G, Ma Z, Liu J, Li F. Therapeutic effects of puerarin on polycystic ovary syndrome: a randomized trial in Chinese women. Medicine (Baltimore) 2021;100:e26049.
3. Yang M, Luo Y, Liu T, et al. The effect of puerarin on carotid intima-media thickness in patients with active rheumatoid arthritis: a randomized controlled trial. Clin Ther 2018;40:1752-64.e1.
4. Liu B, Tan Y, Wang D, Liu M. Puerarin for ischaemic stroke. Cochrane Database Syst Rev 2016;2:CD004955.
5. Wang Q, Wu T, Chen X, et al. Puerarin injection for unstable angina pectoris. Cochrane Database Syst Rev 2006;3:CD004196.

Cardiac magnetic resonance assessment of heart failure with preserved ejection fraction: abridged secondary publication

MY Ng *, V Vardhanabhuti, KH Yiu, SH Hai

KEY MESSAGES

1. Left atrial (LA) reservoir strain was the best cardiac magnetic resonance (CMR) strain parameter for diagnosing heart failure with preserved ejection fraction (HFpEF) in clinically suspected patients, with the area under the curve (AUC) of 0.804.
2. Two other CMR parameters with high diagnostic accuracy were LA area indexed and LA volume indexed, with AUCs of 0.815 and 0.776, respectively.
3. Tagging, CMR-feature tracking in the left ventricle, and CMR-feature tracking in the right ventricle parameters may be less useful than expected in the diagnosis of HFpEF.

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Introduction

Heart failure with preserved ejection fraction (HFpEF) is a persistent diagnostic challenge.[1] Cardiac magnetic resonance (CMR) atrial measurements, feature tracking (FT), and tagging are proposed parameters for diagnosis of HFpEF; they may complement echocardiography, particularly when echocardiography findings are inconclusive.[2] To our knowledge, there are no data supporting the use of atrial measurements, CMR-FT, or tagging. We conducted a prospective case-control study to assess the diagnostic accuracy of CMR atrial volume/area, CMR-FT, and tagging in the diagnosis of HFpEF among clinically suspected patients.

Methods

Patients with suspected HFpEF were prospectively recruited from four centres. Diagnoses of HFpEF were made on the basis of echocardiography, CMR, and N-terminal pro-B-type natriuretic peptide (NT-proBNP) measurements within 24 hours. Patients without a diagnosis of HFpEF were confirmed by catheter pressure measurements or stress echocardiography. Areas under the curve (AUCs) were determined by comparing patients with and without HFpEF.

Results

In total, 142 patients were initially recruited. There were 53 patients with HFpEF (median [interquartile range] age, 78 (74-82) years) and 38 patients without HFpEF (median [interquartile range] age, 70 (64-76) years) after application of exclusion criteria as well as stress echocardiography and invasive catheter pressure measurements. Parameters of CMR left atrial (LA) reservoir strain (ResS), LA area indexed (LAAi), and LA volume indexed (LAVi) had the highest diagnostic accuracy (AUCs: 0.804, 0.815, and 0.776, respectively) [Table]. Parameters of LA ResS, LAAi, and LAVi had significantly better diagnostic accuracy than the CMR-FT left ventricle (LV)/right ventricle (RV) parameters and tagging ($p<0.01$). The CMR-FT LV and RV strain parameters showed poor diagnostic accuracy, with circumferential strain having the highest AUC (0.603) to distinguish patients with HFpEF from patients without HFpEF (Fig). Tagging circumferential strain and radial strain also showed poor diagnostic accuracy (AUCs=0.644 and 0.541, respectively).

Conclusion

Among patients with clinically suspected HFpEF, CMR LA ResS, LAAi, and LAVi had the highest diagnostic accuracy to distinguish patients with HFpEF from patients without HFpEF, whereas the CMR-FT LV/RV parameters and tagging had low diagnostic accuracy.

TABLE. Accuracies of cardiac magnetic resonance and echocardiography parameters to distinguish patients with heart failure with preserved ejection fraction (HFpEF) [n=53] from patients without HFpEF (n=38)

Parameter	Area under the curve (confidence interval)	Sensitivity, %	Specificity, %	Diagnostic accuracy, %	Cut-off
Feature tracking in left ventricle (LV)					
Radial strain, %	0.602 (0.475-0.713)	63.2	66.0	64.8	30.0
Radial early diastolic strain rate, 1/s	0.581 (0.455-0.695)	55.3	66.0	61.5	1.33
Circumferential strain, %	0.603 (0.477-0.714)	68.4	60.4	63.7	17.2
Circumferential early diastolic strain rate, 1/s	0.517 (0.401-0.645)	2.6	100	59.3	1.43
Longitudinal strain, %	0.522 (0.412-0.652)	0	100	58.2	5.5
Longitudinal early diastolic strain rate, 1/s	0.568 (0.446-0.692)	47.4	73.6	62.6	0.49
Feature tracking in right ventricle (RV)					
RV radial strain, %	0.530 (0.409-0.651)	100	0	58.2	8.8
RV longitudinal strain, %	0.501 (0.387-0.629)	98.1	2.6	58.2	8.4
Feature tracking in left atrium (LA)					
LA reservoir, %	0.804 (0.714-0.893)	81.1	68.4	75.8	25.9
LA booster, %	0.746 (0.649-0.847)	64.2	76.3	69.2	12.6
LA conduit, %	0.731 (0.619-0.831)	75.5	63.2	70.3	12.8
Atrial size					
LA volume indexed, ml/m^2	0.776 (0.681-0.871)	82.7	60.5	73.3	44.6
LA area indexed on 4-chamber, cm/m^2	0.815 (0.730-0.901)	66.0	86.8	74.7	15.3
RA area indexed on 4-chamber, cm/m^2	0.700 (0.595-0.812)	83.0	57.9	72.5	11.3
Tagging strain parameters					
Circumferential strain, %	0.644 (0.527-0.761)	75.5	55.3	65.9	15.9
Radial strain, %	0.541 (0.420-0.662)	69.8	42.1	58.2	24.6
Cardiac magnetic resonance LV myocardial mass					
LV myocardial mass indexed, g/m^2	0.523 (0.415 -0.656)	0	100	58.2	87.2
Echocardiography parameters					
Septal wall e', m/s	0.555 (0.426-0.663)	100	0	58.2	0.14
Lateral wall e', m/s	0.579 (0.470-0.706)	100	2.6	59.3	0.05
Mean E/e' ratio	0.584 (0.451-0.692)	84.9	28.9	61.5	9.3
Tricuspid regurgitation peak velocity, ms	0.768 (0.661-0.861)	79.2	60.5	71.4	2.3
LV mass indexed, g/m^2	0.621 (0.510-0.745)	96.2	21.0	64.8	74.6
LA volume indexed, ml/m^2	0.766 (0.666-0.860)	56.6	89.5	70.3	46.0

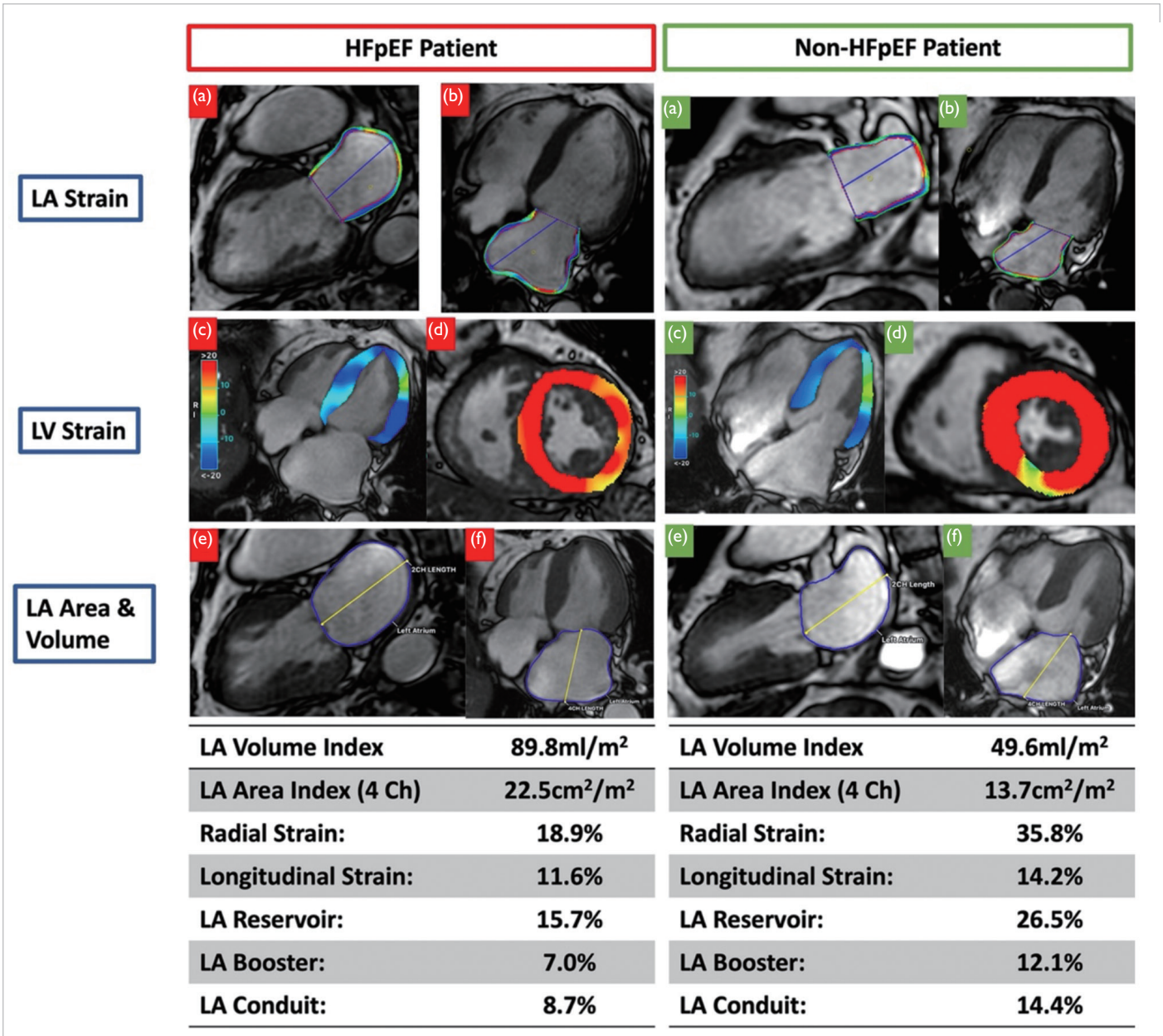

FIG. A patient with heart failure with preserved ejection fraction (HFpEF) has lower left atrial (LA) strain (reservoir, conduit, and booster) and larger LA volume/area, compared with a patient without HFpEF: (a and b) contours of the LA in 2- and 4-chamber cines for LA strain, (c and d) radial and longitudinal strain assessment in the short axis and 4-chamber cines using cardiac magnetic resonance feature tracking, and (e and f) contours of the LA and right atrial in 2- and 4-chamber cines in end-systole for volume/area.

Funding

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Disclosure

The results of this research have been previously published in:

1. Ng MY, Kwan CT, Yap PM, et al. Diagnostic accuracy of cardiovascular magnetic resonance strain analysis and atrial size to identify heart failure with preserved ejection fraction. Eur Heart J Open 2023;3:oead021.

References

1. Pieske B, Tschope C, de Boer RA, et al. How to diagnose heart failure with preserved ejection fraction: the HFA-

PEFF diagnostic algorithm: a consensus recommendation from the Heart Failure Association (HFA) of the European Society of Cardiology (ESC). Eur Heart J 2019;40:3297-317.

2. Chamsi-Pasha MA, Zhan Y, Debs D, Shah DJ. CMR in the evaluation of diastolic dysfunction and phenotyping of HFpEF: current role and future perspectives. JACC Cardiovasc Imaging 2020;13:283-96.

Nurse-led sexual rehabilitation to rebuild sexuality and intimacy after treatment for gynaecological cancer: a randomised controlled trial (abridged secondary publication)

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KEY MESSAGES

1. A nurse-led sexual rehabilitation programme is effective in improving sexual function in terms of reducing vaginal problems during sexual intercourse and enhancing partners' sexual interest, compared with traditional care, among Hong Kong Chinese women treated for gynaecological cancer.
2. Qualitative comments from participants indicated that the interventions provided informational and psychological support, which facilitated rebuilding sexuality and intimacy.
3. Sexual rehabilitation interventions delivered by trained nurses should be included in routine clinical practice for patients with gynaecological cancer in Hong Kong.

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Introduction

Gynaecological cancer (GC) is the second most common female cancer in Hong Kong. The incidences of uterine and ovarian cancers are expected to increase. Because the GC survival rate increases with treatment improvements, quality of life (intimacy and sexuality) is essential for women undergoing treatment for GC.[1]

Sexual dysfunction, a common problem among women undergoing treatment for GC, may manifest as vaginal dryness, dyspareunia, shortened vagina, difficulty in reaching orgasm, and diminished sexual desire.[1] Affected women refrain from sexual activity because of their fear of painful sexual intercourse and uncertainty about their illness; this can lead to distress and disruptions to intimacy and relationship quality.[2] Therefore, rebuilding sexuality is pivotal for these women to maintain intimate relationships with their partners.

Sexual rehabilitation is characterised by comprehensive assessment and appropriate interventions from a holistic perspective.[3] In Hong Kong, women undergoing treatment for GC do not receive support in areas of sexuality and intimacy. We implemented a nurse-led sexual rehabilitation programme to help Hong Kong Chinese women with GC and their partners to resume a satisfying intimate relationship or adapt to permanent sexual dysfunction. We hypothesised that the intervention would improve sexual function and marital relationships while reducing the level of sexual distress. We evaluated the effects of the programme on sexual function, sexual distress, and marital relationships.

Methods

Participants were recruited from gynaecological oncology clinics or wards at three regional hospitals in Hong Kong between July 2019 and July 2021. Inclusion criteria were women aged >18 years with non-terminal primary GC (uterine, ovarian, or cervical cancer) diagnosed in the preceding 3 months who had a regular sexual partner and could speak Cantonese and read Chinese. Women with known pre-existing psychotic illness were excluded.

Women with GC were randomly assigned to the intervention group or control group. Women in the intervention group completed a nurse-led sexual rehabilitation programme, as described in a previous study.[3] Briefly, the programme was delivered individually, with or without partner involvement, by a trained research nurse at four sessions: before start of treatment and 1 month, 2 months, and 6 months after treatment completion. Each session lasted 45 to 60 minutes. The programme's key components were information provision, cognitive behavioural therapy, and counselling using motivational interviewing techniques. Participants in the control group received four sessions of attention from the research nurse. The nurse delivered general advice to participants over the phone during follow-ups but did not provide any intervention.

Participants were asked to complete a set of questionnaires that included the Chinese versions of the Sexual Function-Vaginal Changes Questionnaire (SVQ), Female Sexual Distress Scale-Revised (FSDS-R), and ENRICH Marital Satisfaction Scale (EMS), as well as the sociodemographic survey at baseline (T0). Follow-up telephone surveys were conducted 1 month after completion of cancer treatment (T1), immediately after completion of the programme (T2), and 12 months after treatment (T3). Additionally, intervention participants completed semi-structured interviews with another research nurse immediately after the last session of the programme to record their experiences and feelings regarding the programme.

The homogeneity of the intervention and control groups was tested using the independent *t*-test, Chi squared test, or Fisher's exact test, as appropriate. Generalised estimating equation models were used to compare changes in sexual function, sexual distress, and marital satisfaction from T0 to T1, T2, and T3 between the two study arms with adjustment for potential confounders (age, number of children, type of GC, and stage of cancer). All statistical tests were two-sided, and the significance level was set at P<0.05. Qualitative data were assessed via content analysis by two researchers who were not involved in data collection. The recorded tapes were transcribed verbatim, and the transcripts were analysed to identify themes and categories, which were then translated into English. The quantitative and qualitative findings were compared and contrasted.

TABLE 1. Baseline characteristics and outcomes of participants (n=150)

Characteristic	Control (n=72)*	Intervention (n=78)*	P value
Age, y	52.5±7.1	45.7±7.7	<0.001
Educational level			0.661
Secondary or below	61 (84.7)	64 (82.1)	
Tertiary or above	11 (15.3)	14 (17.9)	
Monthly household income, HK$			0.662
<20 000	41 (56.9)	38 (49.4)	
20 000-29 999	17 (23.6)	20 (26.0)	
≥30 000	14 (19.4)	19 (24.7)	
No. of children			0.018
0	13 (18.1)	25 (32.1)	
1	21 (29.2)	29 (37.2)	
≥2	38 (52.8)	24 (30.8)	
Type of gynaecological cancer			0.012
Corpus uteri	44 (61.1)	29 (37.2)	
Cervical	22 (30.6)	36 (46.2)	
Ovarian and other	6 (8.3)	13 (16.7)	
Stage of cancer			0.038
I	40 (55.6)	50 (64.1)	
II	17 (23.6)	13 (16.7)	
III	13 (18.1)	6 (7.7)	
Unknown	2 (2.8)	9 (11.5)	
Type of treatment			0.735
Surgery only	36 (50.0)	43 (55.1)	
Chemotherapy only	15 (20.8)	16 (20.5)	
Surgery + chemotherapy	9 (12.5)	4 (5.1)	
Surgery + radiation	6 (8.3)	7 (9.0)	
Surgery + chemotherapy	5 (6.9)	7 (9.0)	
Unknown	1 (1.4)	1 (1.3)	
Sexual Function-Vaginal Changes Questionnaire score			
Intimacy	3.87±2.10	5.01±2.28	0.002
Global sexual satisfaction	8.25±1.90	8.32±1.69	0.810
Sexual interest	1.68±0.96	2.10±1.09	0.013
Vaginal changes	13.86±1.68	12.53±2.23	0.084
Sexual function	10.71±1.11	10.87±1.13	0.180
Female Sexual Distress Scale-Revised score	3.44±5.42	5.24±7.16	0.770
ENRICH Marital Satisfaction Scale score	33.71±5.87	35.58±5.14	0.046

* Data are presented as mean ± standard deviation or No. (%) of participants

Results

In total, 150 participants (mean age, 49.0 years) were randomly allocated to the intervention group (n=78) or control group (n=72). The baseline characteristics of the two groups were comparable, except for age, number of children, type of GC, and stage of cancer (Table 1). The two groups were comparable in terms of SVQ global sexual satisfaction, vaginal changes, sexual function subscale scores, and the FSDS-R score. Compared with the control group, the intervention group had significantly higher SVQ intimacy and sexual interest subscale scores, as well as a higher EMS score (Table 1).

Generalised estimating equation analyses revealed a significant group × time interaction in the SVQ vaginal changes subscale score at T3 (B=2.44, 95% confidence interval=0.09-4.78, P=0.041, Table 2), indicating that the intervention group experienced significantly fewer vaginal problems and related concerns during sexual intercourse, compared with the control group. No significant group × time interactions in the subscale scores of intimacy, global sexual satisfaction, sexual interest, and sexual function were observed between the two groups. Notably, women in the intervention group were more likely to perceive greater sexual interest from their partners at T3 (P=0.001), compared with women in the control group. There were no significant group × time interactions in the FSDS-R score for sexual distress or EMS score for marital satisfaction at T1, T2, and T3.

TABLE 2. Generalised estimating equation analyses of sexual function, sexual distress, and marital satisfaction across study time points between intervention and control groups

Outcome	Crude model		Adjusted model	
	B (95% confidence interval)	P value	B (95% confidence interval)	P value
Sexual Function-Vaginal Changes Questionnaire				
Intimacy				
Group	1.14 (0.44 to 1.84)	0.001	0.65 (-0.15 to 1.45)	0.111
T1	0.31 (-0.08 to 0.70)	0.120	0.29 (-0.10 to 0.67)	0.149
T2	0.51 (0.10 to 0.92)	0.015	0.49 (0.08 to 0.90)	0.020
T3	0.62 (0.19 to 1.05)	0.005	0.60 (0.17 to 1.03)	0.007
Group × T1	0.55 (-0.11 to 1.21)	0.103	0.56 (-0.10 to 1.22)	0.098
Group × T2	0.31 (-0.35 to 0.97)	0.353	0.32 (-0.34 to 0.98)	0.342
Group × T3	0.41 (-0.26 to 1.07)	0.234	0.41 (-0.26 to 1.07)	0.235
Global sexual satisfaction				
Group	0.07 (-0.50 to 0.64)	0.809	0.32 (-0.30 to 0.95)	0.307
T1	0.51 (0.07 to 0.95)	0.023	0.51 (0.07 to 0.94)	0.024
T2	0.53 (0.10 to 0.95)	0.015	0.52 (0.10 to 0.95)	0.016
T3	0.44 (-0.03 to 0.91)	0.067	0.43 (-0.04 to 0.90)	0.073
Group × T1	-0.07 (-0.70 to 0.56)	0.824	-0.07 (-0.69 to 0.56)	0.840
Group × T2	-0.63 (-1.30 to 0.040	0.067	-0.62 (-1.29 to 0.05)	0.070
Group × T3	-0.46 (-1.16 to 0.24)	0.195	-0.45 (-1.15 to 0.25)	0.207
Sexual interest				
Group	0.42 (0.10 to 0.75)	0.011	0.16 (-0.14 to 0.46)	0.296
T1	0.06 (-0.17 to 0.29)	0.611	0.04 (-0.19 to 0.27)	0.731
T2	0.12 (-0.10 to 0.34)	0.286	0.10 (-0.12 to 0.32)	0.367
T3	0.09 (-0.14 to 0.32)	0.431	0.07 (-0.16 to 0.29)	0.549
Group × T1	0.01 (-0.38 to 0.40)	0.966	0.02 (-0.37 to 0.41)	0.927
Group × T2	0.04 (-0.30 to 0.37)	0.837	0.04 (-0.29 to 0.38)	0.805
Group × T3	0.07 (-0.29 to 0.42)	0.712	0.07 (-0.28 to 0.42)	0.688
Vaginal changes				
Group	-1.64 (-3.51 to 0.23)	0.085	-2.50 (-4.63 to -0.37)	0.021
T1	1.68 (-0.46 to 3.82)	0.123	1.52 (-0.50 to 3.53)	0.140
T2	-1.02 (-3.12 to 1.08)	0.341	-1.29 (-3.43 to 0.86)	0.238
T3	-1.59 (-3.48 to 0.30)	0.099	-1.83 (-3.77 to 0.12)	0.065
Group × T1	-0.33 (-3.20 to 2.53)	0.820	0.08 (-2.72 to 2.87)	0.957
Group × T2	0.84 (-1.64 to 3.32)	0.505	1.30 (-1.21 to 3.80)	0.310
Group × T3	2.08 (-0.19 to 4.36)	0.073	2.44 (0.09 to 4.78)	0.041
Sexual function				
Group	-0.10 (-1.37 to 1.17)	0.877	0.20 (-1.24 to 1.64)	0.787
T1	-1.11 (-3.19 to 0.97)	0.295	-1.10 (-3.17 to 0.97)	0.299
T2	-1.23 (-2.76 to 0.29)	0.114	-1.22 (-2.77 to 0.33)	0.122
T3	-1.13 (-2.57 to 0.31)	0.125	-1.13 (-2.59 to 0.35)	0.134
Group × T1	-0.34 (-2.73 to 2.06)	0.782	-0.37 (-2.73 to 2.00)	0.761
Group × T2	-0.19 (-1.85 to 1.47)	0.818	-0.18 (-1.85 to 1.49)	0.831
Group × T3	-0.09 (-1.61 to 1.43)	0.905	-0.05 (-1.58 to 1.48)	0.948
Female Sexual Distress Scale-Revised				
Group	0.35 (-0.09 to 0.79)	0.123	0.19 (-0.32 to 0.69)	0.464
T1	0.09 (-0.25 to 0.43)	0.616	0.09 (-0.25 to 0.43)	0.616
T2	0.35 (0.01 to 0.68)	0.042	0.35 (0.01 to 0.68)	0.042
T3	0.46 (0.06 to 0.86)	0.023	0.46 (0.06 to 0.86)	0.023
Group × T1	-0.02 (-0.49 to 0.45)	0.930	-0.03 (-0.50 to 0.44)	0.913
Group × T2	0.02 (-0.51 to 0.56)	0.932	0.02 (-0.52 to 0.55)	0.956
Group × T3	0.19 (-0.39 to 0.78)	0.521	0.18 (-0.41 to 0.76)	0.550
ENRICH Marital Satisfaction Scale				
Group	1.85 (0.04 to 3.66)	0.045	2.21 (0.06 to 4.36)	0.044
T1	0.24 (-0.74 to 1.220	0.629	0.21 (-0.77 to 1.20)	0.670
T2	0.96 (0.00 to 1.92)	0.051	0.93 (-0.04 to 1.89)	0.059
T3	0.51 (-0.40 to 1.42)	0.273	0.48 (-0.44 to 1.39)	0.309
Group × T1	0.22 (-1.06 to 1.49)	0.738	0.22 (-1.05 to 1.50)	0.731
Group × T2	-0.85 (-2.29 to 0.60)	0.251	-0.84 (-2.28 to 0.61)	0.257
Group × T3	-0.62 (-2.20 to 0.95)	0.438	-0.60 (-2.18 to 0.97)	0.453

Of the 66 women who completed the intervention, 42 attended the semi-structured interviews. The mean interview duration was approximately 30 minutes. Content analysis revealed two themes: effective features of the programme and positive experiences in rebuilding sexuality and intimacy (Table 3). Most participants considered the intervention to be useful; they greatly appreciated the informational and psychological support provided by the nurse intervener. Most participants considered that the programme format and delivery were appropriate. Some participants shared positive experiences in improving sexual function, sexual distress, and intimate relationships.

Discussion

Women in the intervention group reported significantly fewer vaginal problems (eg, vaginal dryness and painful sensation during sexual intercourse) and related concerns at 12 months post-treatment, compared with women in the control group. Similarly, a study showed that a nurse-led sexual rehabilitation intervention had a positive effect on overall sexual function in Chinese women who received treatment for cervical cancer.[4] Most participants resumed sexual activity within 1 to >3 years of treatment. These findings suggest that the beneficial effects of sexual rehabilitation

TABLE 3. A summary of themes identified from qualitative content analysis

Theme and code	Sample quote
Effective features of the programme	
Informational support	
Information needs	*I was lost. I didn't know what I could or could not do, what I could or could not eat. I learned more by talking to the nurse intervener. Now I know what is good for me, for example, exercise. And I know what I can't eat. I think this programme is very good and important. I was lucky to be able to ask her [the nurse intervener]. I feel relieved now.*
Credible and personalised information	*During follow-up with the nurse [intervener], she can explain things to me because she has taken care of many patients. She can help me in many ways. There is a lot of information online, but some may not be credible. In this programme, I can ask a professional directly.*
Psychological support	
Uncertainty	*There are many uncertainties and concerns that can cause patients to worry. This programme can support them. It's very helpful.*
Someone to share feelings with	*I am suffering from this disease. I don't want my friends and family to worry about me. I could talk about this with someone [the nurse intervener]. It's like counselling and making a new friend. I feel more at ease.*
Relief from negative emotions	*Whenever I met the nurse [intervener], it made me feel good and relaxed. I realised that it's not really a big deal. I feel as if someone supports me.*
Appropriate format and delivery	
Format and schedule	*If possible, I wish I could share more with the nurse [intervener] in the future. Sometimes I want to share my true feelings, but I may not be able to share them with others. They may not understand, but the nurse [intervener] does.*
Importance of nurse intervener	*When it comes to sexual life, you simply don't want to share with someone you know. But if there is a health professional, I am more at ease. It would be embarrassing to share it with a male attending doctor... but that's not the case for female nurses. She [the nurse intervener] understands my situation and struggle from a woman's perspective.*
Positive experiences in rebuilding sexuality and intimacy	
Sexual function	
Sexual difficulties	*For example, vaginal dryness and other sexual difficulties that the nurse [intervener] mentioned. When I had sexual intercourse, I encountered these [difficulties].*
Coping strategies	*Yes, it is a bit dry. Then I tried the gel that the nurse intervener recommended. It works.*
Sexual distress	
Fear of resuming sexual activity	*Some women, like me, were hesitant to resume sexual activity due to fear of pain or fear of infection.*
Clearing up misconceptions	*The nurse intervener explained that the pain was caused by [vaginal] contraction. She also told me how to solve this problem. I know there is something that can help relieve the pain. So I don't think it's a big deal anymore.*
Intimate relationships	
Sexual expression	*Sometimes, we hug and kiss each other. We say "I love you" to each other more. Things like that. We are happy [with our relationship] even without physical intimacy.*
Communication	*After hearing from the nurse intervener, I learned how to communicate better with my partner. Before this programme, we didn't know how to communicate well.*

interventions may require a long interval to become apparent. Additionally, partners play an important role in the abilities of patients with GC to adjust to sexual changes. Compared with control participants, intervention participants perceived greater sexual interest from their partners at 12 months post-treatment; however, only 20 (30.3%) of 66 intervention participants' partners attended the sessions. Future research should include participants' partners and a longer follow-up.

This study did not demonstrate a significant intervention effect on sexual distress. However, sexual distress was extremely low in both groups of participants at all time points, indicating that the participants did not worry about sexual relationships or function. Additionally, nearly half of participants were sexually inactive after treatment. This low level of sexual distress and high prevalence of sexual inactivity might be attributed to Chinese culture, which emphasises abstinence from sexual activity while recovering from illness, and the Confucian perspective that sexuality primarily serves a reproductive function.[5]

This study did not show a significant intervention effect on marital satisfaction. At baseline, both groups of participants regarded their marital relationships as somewhat satisfactory to mostly satisfactory. The inclusion criterion of having a regular sexual partner might have introduced recruitment bias, such that only women with satisfying sexual relationships were recruited.

The qualitative findings complemented the quantitative findings. First, the significant improvement in vaginal problems was consistent with the qualitative finding that participants reported relief from vaginal dryness when using the vaginal lubricant recommended by the nurse intervener. Second, although quantitative analysis did not show significant improvements in sexual distress and marital relationships, qualitative analysis revealed that some women had lower sexual distress and better intimate relationships with their partners because of support they received from the nurse intervener. Third, the qualitative findings suggested that in addition to sexual concerns, the intervention can help to address women's informational and psychological needs.

Limitations of this study included over-representation of patients with early-stage GC, the small number of partners who attended intervention sessions with the participants, the lack of qualitative data regarding partners' opinions, the small proportion of participants who reported resuming sexual activity within 6 months post-treatment, and the unknown cost-effectiveness of the programme.

Conclusion

Our findings suggest that sexual rehabilitation interventions delivered by trained nurses should be included in routine clinical practice for patients with gynaecological cancer in Hong Kong.

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Disclosure

The results of this research have been previously published in:

1. Chow KM, Porter-Steele J, Siu KY, Choi KC, Chan CWH. A nurse-led sexual rehabilitation programme for rebuilding sexuality and intimacy after treatment for gynaecological cancer: study protocol for a randomized controlled trial. J Adv Nurs 2022;78:1503-12.

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References

1. Bal MD, Yilmaz SD, Beji NK. Sexual health in patients with gynecological cancer: a qualitative study. Sex Disabil 2013;31:83-92.
2. Lee JT, Lin HH, Tsai JL, Chen CP, Huang KG, Lien AS. Transformation of sexual expression in Taiwanese women after treatment for gynecological cancer. Cancer Nurs 2015;38:475-83.
3. Chow KM, Porter-Steele J, Siu KY, Choi KC, Chan CWH. A nurse-led sexual rehabilitation programme for rebuilding sexuality and intimacy after treatment for gynaecological cancer: study protocol for a randomized controlled trial. J Adv Nurs 2022;78:1503-12.
4. Shi Y, Cai J, Wu Z, et al. Effects of a nurse-led positive psychology intervention on sexual function, depression and subjective well-being in postoperative patients with early-stage cervical cancer: a randomized controlled trial. Int J Nurs Stud 2020;111:103768.
5. Khoo SB. Impact of cancer on psychosexuality: cultural perspectives of Asian women. Int J Nurs Pract 2009;15:481-8.

Extracellular CD147 as a diagnostic marker for defective acrosome reaction in asthenozoospermia and idiopathic infertility: abridged secondary publication

E Fok*

KEY MESSAGES

1. Sperm from patients with asthenozoospermia exhibits reduced motility and decreased induction of the acrosome reaction (AR), which may be associated with CD147 deficiency.
2. CD147 plays an important role in fertilisation by regulating sperm motility and the AR.
3. Recombinant CD147 treatment could improve sperm function and fertilisation outcomes in sperm from patients with asthenozoospermia.
4. The soluble CD147 levels in seminal plasma and follicular fluid can serve as predictive markers for fertilisation rates and in vitro fertilisation outcomes.

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Introduction

Successful fertilisation requires the maintenance of sperm motility and induction of the acrosome reaction (AR), a process of enzyme release from the sperm head that allows penetration of the egg. Asthenozoospermia is a condition in which sperm display insufficient motility. Such sperm also exhibits a significantly lower rate of AR induction. However, the molecular mechanisms underlying defects in asthenozoospermia that lead to infertility remain unclear.

CD147 is a member of the immunoglobulin superfamily that is commonly detected in the reproductive tract and other glandular epithelial cells of various organs. CD147 is also highly expressed in cancers; it has been associated with tumour progression and invasion. CD147 is expressed in both membrane-bound and soluble forms; these two forms interact and form dimers that promote tumour invasion.[1] CD147 has essential roles in reproduction. CD147 knockout mice display both male and female infertility.[2] Loss of CD147 in oocytes and surrounding cumulus cells leads to a significant decrease in the rate of fertilisation with wild-type sperm during in vitro fertilisation (IVF),[3] suggesting an essential role for cumulus cell-derived CD147 in fertilisation. CD147 is also expressed in sperm. However, the functions of CD147 in sperm and during fertilisation remain elusive.

This study aimed to characterise the expression patterns of CD147 in sperm, seminal plasma, and follicular fluid (FF), as well as the associations of CD147 with sperm parameters and pregnancy outcomes, and to evaluate the effects of CD147 on the AR and the fertilisation potential of sperm from patients with infertility.

Methods

Semen and FF samples were collected from patients with asthenozoospermia and couples with unexplained infertility. To evaluate the expression of CD147 in extracellular compartments, extracellular vesicles (ie, membranous vesicles secreted by cells into the extracellular space) were isolated. All samples were used for expression profiling to evaluate the level of CD147 protein on extracellular vesicles, on sperm, and in FF. Selected samples were used as in vitro models to study the effects of extracellular/soluble CD147 on sperm function, along with the underlying molecular mechanisms.

The levels of CD147 on sperm, in seminal plasma, and in FF were assessed by enzyme-linked immunosorbent assays (ELISAs), immunofluorescence staining, and semi-quantitative Western blotting. The effects of CD147 on sperm function were examined by treating sperm samples with CD147-neutralising antibody or soluble CD147 (recombinant CD147 [rCD147]). Sperm motility and hyperactivated motility were measured by computer-assisted sperm analysis (CASA); AR was evaluated by fluorescein-*Pisum sativum* agglutinin (FITC-PSA) assays. Fertilisation capacity was measured by hyaluronan-binding and sperm penetration assays. Detailed experimental protocols are available in previous publications.[4,5]

Results

Under immunofluorescence staining, CD147

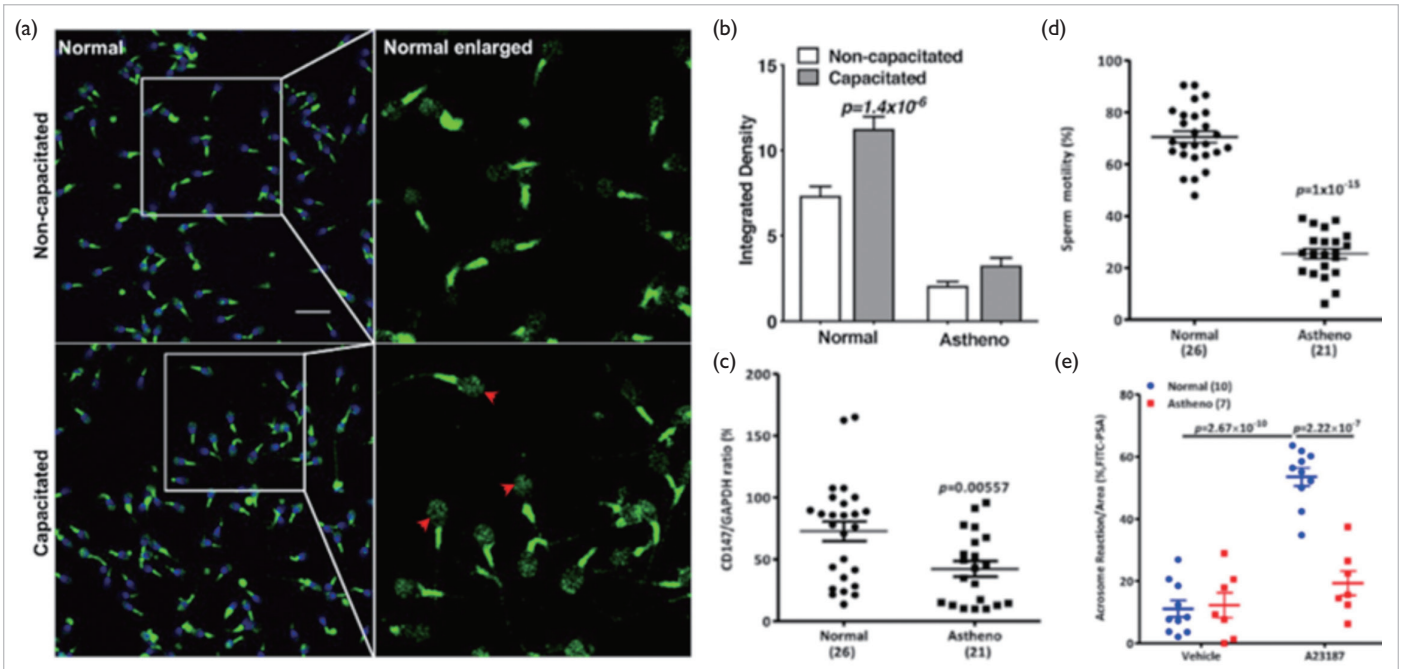

FIG 1. CD147 deficiency and acrosome reaction impairment in sperm from patients with asthenozoospermia. (a) Representative confocal images showing the expression and localisation of CD147 (green) in the head and midpiece before capacitation in normal sperm. The intensity of CD147 on the sperm head is increased after capacitation (red arrowheads). (b) Quantification of fluorescent signals showing a significant increase in the integrated density of CD147 in the normal sperm head region after capacitation. (c) Quantification of Western blots showing decreased CD147 expression in patients with asthenozoospermia, compared with healthy men. (d) Computer-assisted sperm analysis of motility in sperm from healthy men and patients with asthenozoospermia. (e) Quantification of ionophore (A23187)-induced initiation of the acrosome reaction, as measured by fluorescein-*Pisum sativum* agglutinin staining. Initiation of the acrosome reaction is significantly reduced in sperm from patients with asthenozoospermia than in sperm from healthy men.

protein levels were lower in asthenozoospermic sperm samples than in normal sperm samples (Fig 1). A stronger CD147 signal was observed in the head region upon capacitation, a process induced by the female reproductive tract essential for sperm to fertilise. The reduced expression of CD147 in patients with asthenozoospermia was confirmed by Western blotting. In addition, ionophore-induced AR was also reduced in asthenozoospermic sperm. These results suggest that sperm from patients with asthenozoospermia exhibits reduced motility and decreased induction of the AR, which may be associated with CD147 deficiency.

CD147 was expressed in membrane-bound and soluble forms; thus, the female reproductive tract was assumed to secrete soluble CD147 and regulate sperm function. Indeed, ELISAs showed that soluble CD147 was present in human FF (Fig 2). CD147 was identified in a subpopulation of extracellular vesicles isolated from FF.

To investigate the role of CD147 in sperm function, sperm-bound CD147 was blocked by a neutralising antibody. The effect of a rCD147 protein that resembled soluble CD147 was tested. Although rCD147 treatment did not increase sperm motility, anti-CD147 antibody treatment significantly reduced sperm motility (Fig 2), suggesting that sperm-bound CD147 contributes to the maintenance of sperm motility. Hyperactivated motility and the AR, both associated with capacitation, were assessed by CASA and *Pisum sativum* agglutinin (PSA) staining, respectively. Although progesterone, a physiological inducer of hyperactivation and the AR, induced a twofold increase in the percentage of sperm with hyperactivated motility, rCD147 treatment did not affect this process. These findings suggested that soluble CD147 is not essential for sperm hyperactivation. Notably, rCD147 treatment induced a threefold increase in AR, comparable with the increase triggered by progesterone, indicating that rCD147 is a potent AR inducer. Effects of CD147 on fertilisation outcomes were examined using hyaluronan-binding and human sperm-hamster oocyte penetration assays. Anti-CD147 antibody treatment significantly decreased the percentage of sperm bound to hyaluronan and the number of oocyte-penetrating sperm. rCD147 treatment significantly increased the number of oocyte-penetrating sperm but showed only a modest increase in the percentage of sperm bound to

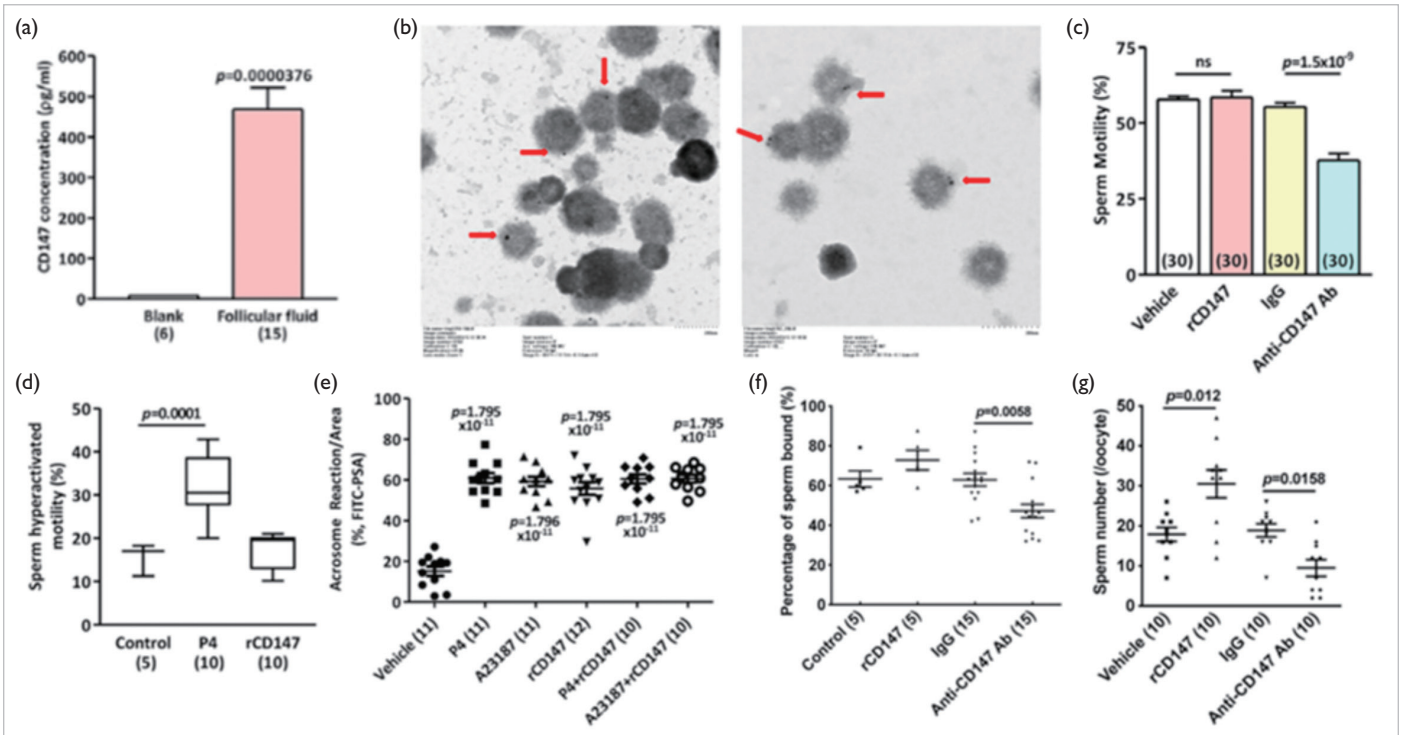

FIG 2. Soluble CD147 promotes sperm motility and induces the acrosome reaction. (a) Enzyme-linked immunosorbent assay results showing the presence of soluble CD147 in human follicular fluid. (b) Representative electronic microscopy images showing the presence of CD147-labelled gold particles (red arrow) on extracellular vesicles isolated from follicular fluid. (c) Computer-assisted sperm analysis (CASA) results showing the effect of recombinant CD147 (rCD147) or CD147-neutralising antibody (anti-CD147 Ab) treatment on sperm motility. (d) CASA results showing the effect of rCD147 and progesterone on hyperactivated motility in capacitated sperm. (e) Fluorescein-*Pisum sativum* agglutinin staining results showing the effect of rCD147, progesterone, the ionophore A23187, or the indicated combined treatments on the acrosome reaction in sperm. (f) Hyaluronan-binding assay (g) and human sperm-hamster oocyte penetration assay results showing the effect of rCD147 or anti-CD147 Ab treatment on hyaluronan-binding and oocyte-penetrating abilities in sperm.

hyaluronan. These results suggest that CD147 plays an important role in fertilisation by regulating sperm motility and the AR.

Sperm function was tested after augmenting the dimerisation of CD147 through increasing the amount of soluble CD147. Results showed that rCD147 significantly enhanced motility in sperm and that this effect could be abolished by treatment with the CD147-neutralising antibody (Fig 3). Despite a decrease in the level of AR induction, rCD147 treatment was able to trigger a 3.7-fold increase in AR induction, which could be inhibited by anti-CD147 antibody treatment. Importantly, rCD147 treatment significantly increased the percentage of bound sperm in samples from patients with asthenozoospermia. These results demonstrated that rCD147 treatment could improve sperm function and fertilisation outcomes in sperm from patients with asthenozoospermia.

The level of CD147 could serve as a predictive marker for fertilisation outcomes. Couples with unexplained (idiopathic) infertility who were undergoing IVF treatment were recruited. The soluble CD147 level in FF from women with idiopathic infertility significantly decreased, compared with women whose partners were diagnosed with male infertility (Fig 3), suggesting that soluble CD147 in FF can be a diagnostic marker for AR defects in couples with idiopathic infertility. Furthermore, the level of CD147 in seminal plasma was positively correlated with the fertilisation rate. The level of CD147 in seminal plasma was significantly lower in sperm from men whose partners did not become pregnant after IVF treatment. This suggested an association between seminal CD147 levels and IVF outcomes. Thus, the soluble CD147 levels in seminal plasma and FF can serve as predictive markers for fertilisation rates and IVF outcomes.

Discussion

Sperm-bound CD147 is involved in sperm motility, the AR, and sperm-egg interactions. Deficiency in sperm-bound CD147 may result in poor motility and AR in patients with asthenozoospermia; therefore, CD147 is a potential diagnostic marker for male infertility.

The ability of rCD147 to trigger AR induction

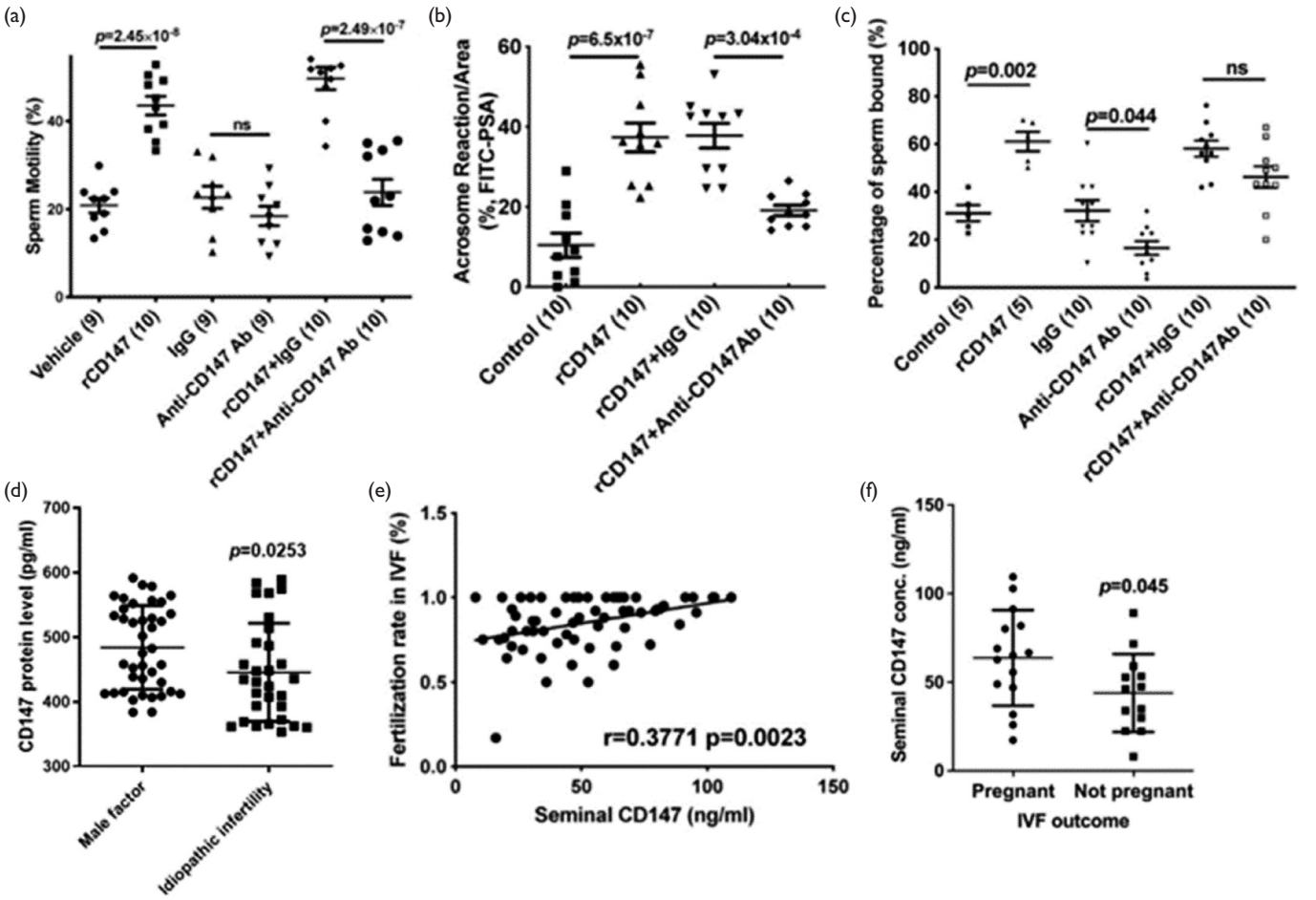

FIG 3. Potential application of soluble CD147 level in assisted reproduction. (a) Computer-assisted sperm analysis results showing the effect of CD147-neutralising antibody (anti-CD147 Ab) and/or recombinant CD147 (rCD147) treatment on motility in sperm from patients with asthenozoospermia. (b) Fluorescein-*Pisum sativum* agglutinin (FITC-PSA) staining results showing the effect of rCD147 on the acrosome reaction in sperm from patients with asthenozoospermia, in the presence or absence of anti-CD147 Ab. (c) Hyaluronan-binding assay results showing the effect of anti-CD147 Ab and/or rCD147 on hyaluronan-binding ability in sperm from patients with asthenozoospermia. (d) Enzyme-linked immunosorbent assay (ELISA) results showing reduced level of CD147 in follicular fluid from women with idiopathic infertility. Female partners with normal profiles and their male partners diagnosed with male infertility were recruited as controls. (e) Correlation analysis of seminal plasma CD147 level with the fertilisation rate after in vitro fertilisation (IVF). (f) ELISA results showing the level of CD147 in seminal plasma from men with infertility, according to IVF outcome.

and augment CD147 signalling, thereby improving sperm motility and fertilising capacity, has high translational value. Successful IVF and intrauterine insemination outcomes rely on sperm motility. The use of rCD147, which mimics the physiological source of soluble CD147 in semen and the female reproductive tract, may improve the success rate by restoring sperm motility and enhancing fertilising capacity.

Intracytoplasmic sperm injection (ICSI) is usually conducted when patients lack motile or morphologically normal sperm. Assessment of AR capability has been proposed to guide the selection of IVF or ICSI. The level of soluble CD147 in seminal plasma was correlated with the rates of fertilisation and pregnancy, suggesting that CD147 could be an indicator to guide the assisted reproductive technology regimens. Additionally, introduction of large amounts of acrosomal enzymes into the oocyte cytoplasm disrupts the cytoskeleton and damages oocytes and decreases the success rate of ICSI. The removal of acrosomes before ICSI improves oocyte survival and embryonic development. The AR can be triggered by rCD147 treatment, which efficiently removes acrosomal enzymes and increases the success rate of ICSI.

Conclusion

CD147 has a key role in sperm function. CD147 deficiency results in poor sperm motility and AR, contributing to infertility outcomes associated with asthenozoospermia. The levels of soluble CD147 in FF and seminal plasma may serve as indicators

to guide the personalised assisted reproductive technology regimens.

Funding

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Disclosure

The results of this research have been previously published in:

1. Chen H, Shi X, Li X, et al. CD147 deficiency is associated with impaired sperm motility/acrosome reaction and offers a therapeutic target for asthenozoospermia. Mol Ther Nucleic Acids 2021;26:1374-86.

References

1. Wu J, Hao ZW, Zhao YX, et al. Full-length soluble CD147 promotes MMP-2 expression and is a potential serological marker in detection of hepatocellular carcinoma. J Transl Med 2014;12:190.
2. Bi J, Li Y, Sun F, et al. Basigin null mutant male mice are sterile and exhibit impaired interactions between germ cells and Sertoli cells. Dev Biol 2013;380:145-56.
3. Kuno N, Kadomatsu K, Fan QW, et al. Female sterility in mice lacking the basigin gene, which encodes a transmembrane glycoprotein belonging to the immunoglobulin superfamily. FEBS Lett 1998;425:191-4.
4. Choy KHK, Chan SY, Lam W, et al. The repertoire of testicular extracellular vesicle cargoes and their involvement in inter-compartmental communication associated with spermatogenesis. BMC Biol 2022;20:78.
5. Chen H, Shi X, Li X, et al. CD147 deficiency is associated with impaired sperm motility/acrosome reaction and offers a therapeutic target for asthenozoospermia. Mol Ther Nucleic Acids 2021;26:1374-86.

Perioperative hypothermia and myocardial injury after non-cardiac surgery: abridged secondary publication

MTV Chan *, CKM Lam, BCP Cheng, T Gin, CW Cheung

KEY MESSAGES

1. In patients who were randomly assigned to either aggressive warming with targeted intraoperative temperatures of 37.0°C or routine thermal care with temperatures around 35.5°C, there were similar incidences of myocardial injury (9.9% vs 9.6%), surgical site infection (7.2% vs 6.3%), and need for transfusion (10% vs 9.5%).
2. Over a range of 1.5°C—from very mild hypothermia to full normothermia—there was no evidence of any substantial effect on patient outcomes. The maintenance of core temperature of ≥35.5°C in surgical patients appears to be sufficient to avoid major hypothermia-related complications.

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Background

Myocardial injury is among the most common causes of death within 1 month of non-cardiac surgery. Hypothermia increases sympathetic activation, promotes tachycardia, and causes hypertension; all of these can lead to myocardial injury. Mild hypothermia (35.5°C) increases morbid myocardial outcomes. Moderate perioperative hypothermia (34.5°C) increases the risk of surgical site infections and increases transfusion requirements. Consequently, international guidelines recommend maintaining normothermia in surgical patients, and active intraoperative warming has become routine. We tested the primary hypothesis that aggressive intraoperative warming reduces the incidence of major cardiovascular complications in patients undergoing major non-cardiac surgery.

Methods

In a multicentre, parallel group, superiority trial (perioperative hypothermia and myocardial injury after non-cardiac surgery trial [PROTECT]), adult patients at risk for postoperative cardiovascular complications who underwent major non-cardiac surgery at 12 sites in China and at the Cleveland Clinic in the United States were randomly assigned to receive either 'aggressive warming' to achieve a target intraoperative core temperature of 37°C or 'routine thermal management' with rescue intraoperative forced-air warming to prevent core temperature from decreasing below 35.5°C. Patient characteristics and perioperative details were recorded. In addition, venous blood samples for plasma cardiac troponin assays were collected before surgery and on each of the first 3 days after surgery.

The primary outcome was a composite of myocardial injury (elevated troponin because of ischaemia), non-fatal cardiac arrest, and all-cause mortality within 30 days of surgery. Secondary 30-day outcomes were deep or organ-space surgical site infection (ie, serious wound infection), intraoperative transfusion requirement, duration of hospitalisation, and readmission.

Results

In total, 5013 patients (mean age, 67 years, 33% women) were randomly assigned to receive 'aggressive warming' (n=2507) or 'routine thermal management' (n=2506) during surgery (52% of surgeries were laparoscopic intra-abdominal procedures). The final intraoperative temperatures in the respective groups were 37.1±0.3°C and 35.6±0.3°C. The two groups

were similar in terms of the incidences of major cardiovascular complications (9.9% vs 9.6%, relative risk [RR]=1.04, 95% confidence interval [CI]=0.87-1.24, P=0.69), serious wound infection (7.2% vs 6.3%, RR=1.13, 95% CI=0.87-1.47, P=0.25), transfusion requirement (10.2% vs 9.5%, RR=1.07, 95% CI=0.87-1.33, P=0.41), duration of hospitalisation (hazard ratio=0.98, 95% CI=0.91-1.05, P=0.46), and readmission (6.5% vs 5.5%, RR=1.19, 95% CI=0.89-1.57, P=0.13).

Discussion

PROTECT is more than tenfold larger than prior thermoregulatory trials, most of which were conducted >20 years ago. Despite a difference of 1.5°C (ranging from very mild hypothermia to full normothermia), there was no significant or clinically meaningful difference in the primary composite outcome of myocardial injury, non-fatal cardiac arrest, and all-cause mortality within 30 days after surgery. The incidence of myocardial infarction was also similar. Furthermore, the 95% CIs around the primary outcome were relatively small, indicating that a type II error was relatively unlikely.

Our findings differ from the those of prior trials in which mild hypothermia did not influence postoperative cardiovascular events.[1] However, the previous trials had few events and did not use the sensitive cardiac troponin marker to detect myocardial injury; therefore, they were prone to bias.[2] We also found no difference in the incidences of serious wound infection between groups. This finding also distinctly differed from that of previous observational analyses[3,4] and a randomised trial.[5] Importantly, the extent of hypothermia was more severe in previous studies. Furthermore, the previous studies had a small sample size and wide confidence intervals; its finding of a threefold reduction in the incidence of infection seems biologically implausible. The results of the present study indicate that mild hypothermia of ≥35.5°C did not result in serious wound infections, although more severe hypothermia might have different effects.

There were some limitations in this study. First, the preoperative and intraoperative teams could not be blinded to thermal management. Similarly, patients knew that they were pre-warmed, although they were not informed about possible consequences. However, the primary outcome (a composite of myocardial injury, non-fatal cardiac arrest, and all-cause mortality) was objective and unlikely to be influenced by patient perception of warming. Secondary outcomes were also objective, and they were unlikely to be influenced by the lack of blinding. Second, we did not evaluate some thermoregulatory responses such as postoperative thermal comfort or shivering. However, both complications are minor and transient. Similar to other trials, our conclusions are directly applicable to the enrolled patients and can reasonably extrapolated to similar patients. Results may differ in obese patients, patients undergoing emergency surgery, and patients with greater risk of cardiovascular complications.

Conclusions

In patients randomly assigned to receive either 'aggressive warming' or 'routine thermal management' during surgery, there were similar incidences of composite major cardiovascular outcomes within 30 days after surgery. The incidences of serious wound infection and transfusion were also similar, as were the duration of hospitalisation and incidence of hospital readmission. The maintenance of core temperature of ≥35.5°C in surgical patients appears to be sufficient to avoid major perioperative adverse outcomes.

Funding

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Disclosure

The results of this research have been previously published in:

1. Sessler DI, Pei L, Li K, et al. Aggressive intraoperative warming versus routine thermal management during non-cardiac surgery (PROTECT): a multicentre, parallel group, superiority trial. Lancet 2022;399:1799-808.

References

1. Frank SM, Fleisher LA, Breslow MJ, et al. Perioperative maintenance of normothermia reduces the incidence of morbid cardiac events. A randomized clinical trial. JAMA 1997;277:1127-34.
2. Devereaux PJ, Chan MT, Eisenach J, Schricker T, Sessler DI. The need for large clinical studies in perioperative medicine. Anesthesiology 2012;116:1169-75.
3. Scott AV, Stonemetz JL, Wasey JO, et al. Compliance with surgical care improvement project for body temperature management (SCIP Inf-10) is associated with improved

clinical outcomes. Anesthesiology 2015;123:116-25.
4. Seamon MJ, Wobb J, Gaughan JP, Kulp H, Kamel I, Dempsey DT. The effects of intraoperative hypothermia on surgical site infection: an analysis of 524 trauma laparotomies. Ann Surg 2012;255:789-95.
5. Kurz A, Sessler DI, Lenhardt R. Perioperative normothermia to reduce the incidence of surgical-wound infection and shorten hospitalization. Study of Wound Infection and Temperature Group. N Engl J Med 1996;334:1209-15.

A new class of antimicrobial therapeutics targeting the envelope stress response of Gram-negative bacteria: abridged secondary publication

SW Tang, SH Kwok, X Li, KH Tang, JA Kubi, AS Brah, K Yeung, M Dong, YW Lam *

KEY MESSAGES

1. BING is a novel amphipathic peptide with broad-spectrum antibacterial activity. Genome-wide transcriptomic analysis suggests that BING represents a new class of antibiotics.
2. BING suppresses the expression of genes involved in flagellar biosynthesis and chemotaxis, thereby inhibiting motility, in *Escherichia coli* and *Salmonella enterica* serovar Typhimurium.
3. BING has synergistic effects with ampicillin, amoxicillin, and novobiocin in *E. coli* and *Pseudomonas aeruginosa*; it can suppress ampicillin resistance in *P. aeruginosa*.
4. Amidation and D-amino acid substitution can stabilise BING against serum degradation and heat inactivation.
5. BING and its derivatives are non-haemolytic and exhibit low in vivo toxicity.

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Introduction

BING is a novel antimicrobial peptide (AMP) discovered by proteomic characterisation of plasma peptides in medaka fish.[1] This 13-mer amphipathic peptide displays broad-spectrum toxicity against pathogenic bacteria—including drug-resistant strains—at concentrations that are relatively non-toxic to mammalian cell lines. BING suppresses the expression of *cpxR*,[1] an upstream regulator of envelope stress responses that plays important roles in antimicrobial resistance (eg, by regulating drug efflux genes). In this study, we investigated the effects of BING-mediated *cpxR* downregulation by performing genome-wide transcriptomic analysis of BING-treated bacteria. We tested the combined toxicities of BING and other classes of antibiotics, mixed at various ratios, against pathogenic bacteria; we also investigated the effects of sublethal doses of BING on the generation of antibiotic resistance in serial passage experiments. Furthermore, we performed lead optimisation by introducing C-terminal amidation and D-amino acid substitution, then analysing how these modifications influenced antibacterial activity and stability at high temperature and in animal serum. Finally, to facilitate future clinical applications of BING, we conducted haemolytic assays and in vivo toxicity tests.

Methods

Detailed procedures for bacterial culture, determination of minimum inhibitory concentration (MIC), checkerboard experiments, and assessment of antibiotic resistance are described in our previous study.[1] RNA samples from BING-treated cultures and untreated control cultures (n=3 for each treatment) were isolated and reverse-transcribed as described. cDNA samples were analysed using an Illumina PE150 sequencer. To measure motility, we inoculated *Escherichia coli* and *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) on 0.3% soft swimming nutrient broth agar, then recorded colony diameters after incubation for 24 hours. Bacterial swimming behaviour was recorded at a rate of 10 frames per second. For assessments of haemolytic activity, human or mouse red blood cells were incubated with AMPs and antibiotics for 1 hour at 37°C, then centrifuged. The optical density of each supernatant was measured at 540 nm. For analyses of acute toxicity, a standard up-and-down procedure (Organisation for Economic Co-operation and Development test guideline 425) was performed on adult male C57BL/6 mice, using a starting dose of 62.5 mg CD-BING per 1 kg body weight.

Results

BING downregulates flagellar biosynthesis and affects bacterial motility

We conducted genome-wide RNA-Seq analysis of *E. coli* that had been treated with BING at the MIC for 60 minutes. We selected this treatment

time to observe initial responses to BING without interference from secondary effects (eg, cell stress or death). We found that BING induced differential expression (fold change >2, P<0.05) of 402 genes. Compared with the gene expression profiles of *E. coli* that had been separately treated with 37 antibiotics, representing the six classes of antibiotics currently in use,[2] transcriptomic changes induced by BING were highly unique (unpublished data). These findings suggest that the antibacterial mechanism of BING may be distinct from the mechanisms exhibited by other cationic AMPs. Consistent with this speculation, we did not detect any disruptive effects of BING on membrane integrity (Fig 1).

Additionally, BING caused widespread downregulation of flagellar and chemotactic genes. Reverse transcription polymerase chain reaction analysis confirmed that levels of *fliA* and *motA* were dramatically reduced in *E. coli* and *S.* Typhimurium after BING treatment; ampicillin treatment did not affect these genes (data not shown). We tested the effects of BING on motility in *E. coli* and *S.* Typhimurium by measuring surface colonisation rates and swimming trajectories; even at a sublethal dose (0.5× MIC), BING reduced bacterial motility to quasi-Brownian motion. Ampicillin did not significantly affect bacterial motility (unpublished data).

Structure of BING

Next, we investigated the structure of BING by circular dichroism spectroscopy (Fig 1). Because BING presumably exerts its effects through membrane interactions, we performed circular dichroism spectroscopy in hydrophobic environments. In 50% trifluoroethanol, BING displayed prominent changes in mean residue ellipticity at 191 and 215 nm. Deconvolution analysis revealed substantial β-sheet composition. Similar phenomena were observed in a membrane-mimetic condition containing sodium dodecyl sulphate. Structural modelling by PEP-FOLD42 indicated that a hydrophilic patch was formed by Arg-2, Arg-6, and Lys-12, whereas a hydrophobic patch was formed by Ile-3, Ala-9, Leu-10, and Ile-13 on the opposite surface; Glu-8 was located at the 'hinge' (Fig 1).

BING suppresses drug efflux expression and acts synergistically with antibiotics

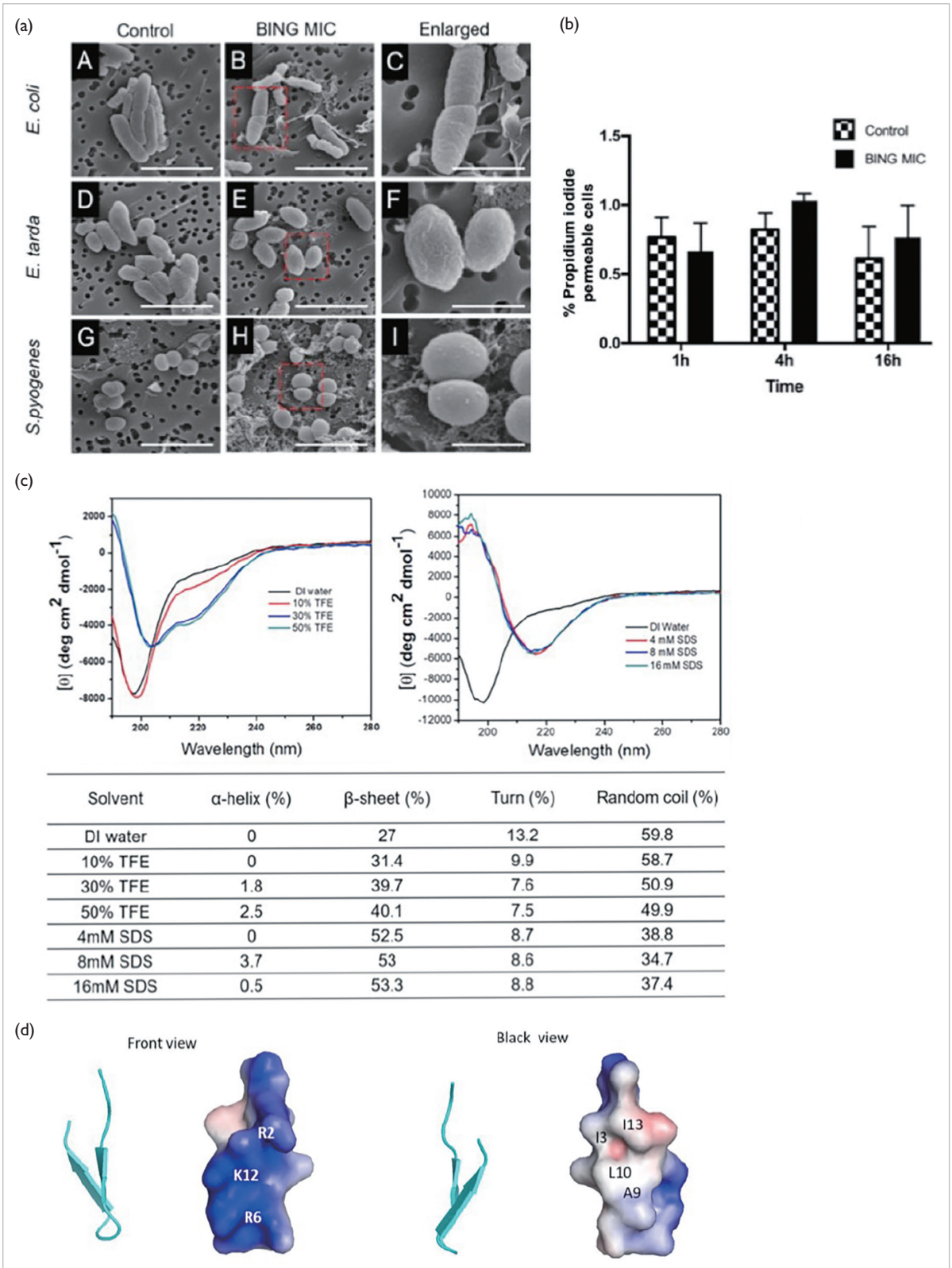
BING downregulates the RNA level of *cpxR*, a gene known to regulate bacterial drug efflux, in *P. aeruginosa* (Fig 2) and other Gram-negative bacteria (data not shown). We tested whether BING-induced downregulation of *cpxR* could alter the expression of efflux pump components. In *P. aeruginosa*, the expression levels of the transporter genes *mexB*, *mexY*, and *oprM* were significantly reduced after exposure to BING. In contrast, ampicillin, which upregulates *cpxR* (data not shown), enhanced the expression of these genes. Next, we investigated whether BING could sensitise bacteria to antibiotic toxicity, using two-dimensional checkerboard experiments to determine the combined effects of BING and antibiotics (Fig 2). The presence of BING significantly enhanced the effects of ampicillin (fractional inhibitory concentration index [FICI]=0.4), amoxicillin (FICI=0.39), and novobiocin (FICI=0.16). A synergistic effect of BING and ampicillin was also observed in *P. aeruginosa* that had previously been selected for ampicillin resistance (FICI=0.42), suggesting that BING can restore antibiotic sensitivity in drug-resistant bacteria.

Subsequently, we examined whether BING could attenuate the development of antimicrobial resistance. *E. coli* were passaged daily with increasing titres of kanamycin or ampicillin for 7 days, resulting in an increase in the kanamycin MIC by ≥8-fold and an increase in the ampicillin MIC by ≥4-fold (Fig 2). In the presence of a sublethal dose of BING (3.9 μg/mL) for 7 days, increases in the MICs of both antibiotics were limited, suggesting that exposure to BING can delay the development of antimicrobial resistance. The deletion of *cpxR* also led to a similar delayed onset of ampicillin resistance in *E. coli* even in the absence of BING, consistent with the notion that the effects of BING on antibiotic resistance are mediated by downregulation of *cpxR*. Similar results were observed in the development of resistance to ampicillin, meropenem, and imipenem in *P. aeruginosa*. Importantly, meropenem and imipenem belong to the carbapenem class of antibiotics; they are among the drugs of last resort.

In vivo toxicity of BING

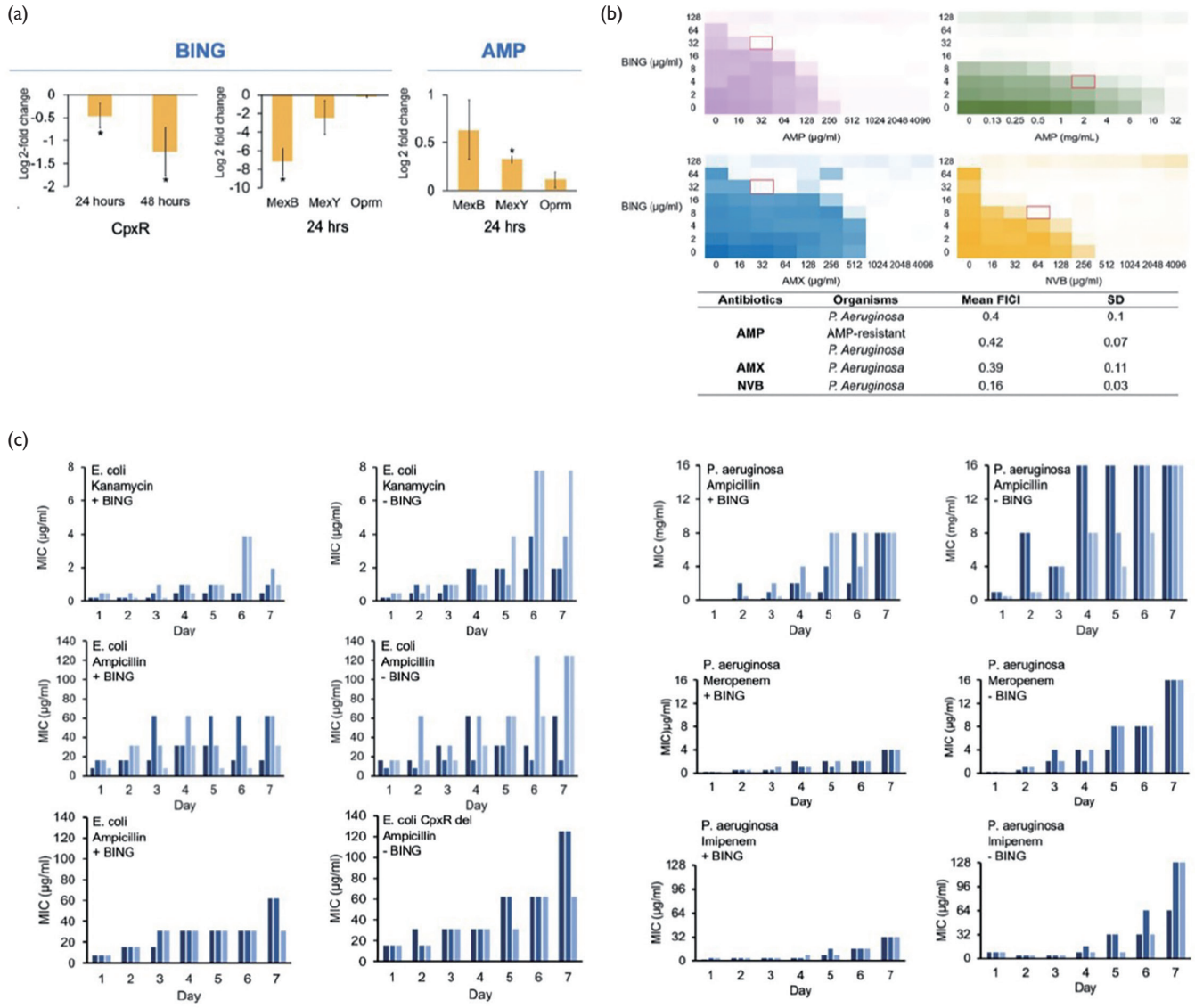
C-terminus amidation (C-BING), complete D-amino acid substitution (D-BING), and double modification (CD-BING) of BING increased its stability without compromising its antibacterial activity (Fig 3). We tested the potential haemolytic effects of BING and CD-BING. Neither peptide caused measurable haemolysis even at 200 mg/L, which is an order of magnitude greater than the MIC of each peptide for most pathogenic bacteria. Melittin, another AMP, induced significant haemolysis.

We then performed acute toxicity testing of CD-BING by intraperitoneal injection into adult mice. To reduce the use of experimental animals, we did not perform the test on BING; thus far, our data indicate that CD-BING is a more promising candidate for clinical translation. We used a standard up-and-down procedure (Organisation for Economic Co-operation and Development test guideline 425), which further minimised the number of animals required. At a dose of <200 mg/kg body weight, CD-BING did not cause any observable adverse effect for up to 5 days (data not shown). At 650 mg/kg



Solvent	α-helix (%)	β-sheet (%)	Turn (%)	Random coil (%)
DI water	0	27	13.2	59.8
10% TFE	0	31.4	9.9	58.7
30% TFE	1.8	39.7	7.6	50.9
50% TFE	2.5	40.1	7.5	49.9
4mM SDS	0	52.5	8.7	38.8
8mM SDS	3.7	53	8.6	34.7
16mM SDS	0.5	53.3	8.8	37.4

FIG 1. (a) Scanning electron micrographs of *Escherichia coli*, *Edwardsiella tarda*, and *Streptococcus pyogenes* incubated with 7.8, 10, and 50 μg/mL of BING or culture medium for 1 hour. (b) Percentages of unfixed bacterial cells labelled with propidium iodide after treatment with or without BING (at minimum inhibitory concentration of 4 ug/mL) for 1, 4, and 16 hours. (c) Secondary structure analysis of BING peptide by far-ultraviolet circular dichroism spectroscopy. The circular dichroism spectra of BING peptide were measured in aqueous solution with increasing concentrations of trifluoroethanol (TFE) and sodium dodecyl sulphate (SDS). The relative contents of secondary structure elements are presented as mean residue ellipticity (Θ). (d) Ribbon diagram of the BING peptide three-dimensional model and electrostatic potential mapped onto the accessible surface area of the model structure. Positive electrostatic surfaces are shown in blue and negative electrostatic surfaces are shown in red; all surfaces are drawn at ±3 kT/e.



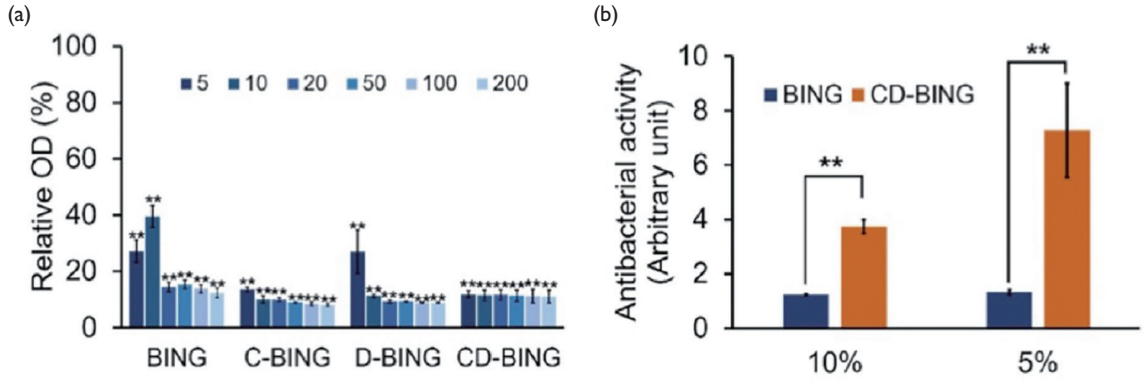
Antibiotics	Organisms	Mean FICI	SD
AMP	*P. Aeruginosa*	0.4	0.1
	AMP-resistant *P. Aeruginosa*	0.42	0.07
AMX	*P. Aeruginosa*	0.39	0.11
NVB	*P. Aeruginosa*	0.16	0.03

FIG 2. (a) *cpxR* gene expression in *Pseudomonas aeruginosa* treated with BING at 25 μg/mL for 24 hours and 48 hours, respectively. Expression levels of *mexB*, *mexY*, and *oprM* in *P. aeruginosa* after treatment with BING (25 μg/mL) and ampicillin (25 μg/mL) for 48 hours, respectively. (b) Combined effects of BING and antibiotics: checkerboard assays on the growth of *P. aeruginosa* or ampicillin-resistant *P. aeruginosa* treated with BING, ampicillin (AMP), amoxicillin (AMX), or novobiocin (NVB). Fractional inhibitory concentration indices (FICIs) of all checkerboards are shown. (c) Effect of BING on the development of antibiotic resistance in *Escherichia coli* and *P. aeruginosa*, which were cultured in increasing concentrations of antibiotics; cells that survived the highest antibiotic concentration were passaged daily for 7 days. Minimum inhibitory concentrations (MICs) of the selected cells on each day are shown.

body weight, CD-BING caused significant weight loss; lethality was observed at 2000 mg/kg body weight. Calculations using the Uniform Appraisal Dataset guideline indicated that the median lethal dose of CD-BING was 1200 mg/kg body weight (unpublished data).

Discussion

Antibiotic-resistant bacteria are generally sensitive to AMPs,[3] which makes AMPs promising candidates for combined antibiotic therapy. Similar to most known AMPs, BING is cationic with ~50% hydrophobic residues. This charge distribution presumably AMPs to disrupt bacterial membranes. However, our data suggest that BING inhibits bacterial growth through a different mechanism. First, BING did not cause detectable permeabilisation of bacterial membranes. Second, amino acid substitution experiments showed that the antibacterial activity of BING could be lost despite maintenance of its charge and hydrophobicity. The amide side chain at position



(c)

T(°C) \ t (hours)	Antimicrobial activity MIC (mg/L) BING 0.5	BING 3	BING 24	CD-BING 0.5	CD-BING 3	CD-BING 24
25	5	5	5	2.5	2.5	2.5
37	5	5	5	2.5	2.5	2.5
90	5	5	5	2.5	2.5	2.5

FIG 3. CD-BING is a stabilised form of BING with relatively low in vivo toxicity. (a) Relative viability (optical density [OD]) of *Escherichia coli* treated with BING, C-amidated BING (C-BING), D-isomer substituted BING (D-BING), and both C-amidated and D-isomer substituted BING (CD-BING) for 16 hours. (b) Minimum inhibitory concentrations (MICs) of BING and CD-BING against *E. coli* in the presence of 5% and 10% fetal bovine serum. (c) MICs of BING and CD-BING pre-incubated at different temperatures for 0.5, 3, and 24 hours (n=3).

8 is required for the antibacterial effect of BING. This degree of sequence dependence suggests that BING exerts its antibacterial effect through interactions with intracellular molecules in a specific ligand-targeting manner.

Our RNA-Seq data indicated that BING causes significant suppression of flagellar and chemotactic gene expression, resulting in bacterial immobilisation. This is an interesting observation because motility and chemotaxis are associated with antibiotic evasion and pathogenicity.

We showed that C-terminus amidation and D-amino acid substitution, modifications known to increase peptide stability in vivo, could increase BING potency and its stability in animal serum. Importantly, BING and CD-BING were extremely thermostable, maintaining their MIC even after 24 hours at 90°C. The stability of BING may be related to its existence in fish blood where it is exposed to serum protease activities and changing environmental conditions. Our data from haemolysis assays and in vivo toxicity tests showed that CD-BING has relatively low toxicity, consistent with our cytotoxicity findings.[1] To our knowledge, CD-BING has much lower acute toxicity in mice, compared with any other AMP for which data are available. Overall, our findings indicate that CD-BING has potential for clinical translation. Future investigations will explore the use of CD-BING as a novel antibacterial agent in animal models.

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This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#18170572). The full report is available from the Health and Medical Research Fund website (https://rfs2.healthbureau.gov.hk).

Disclosure

The results of this research have been previously published in:

1. Dong M, Kwok SH, Humble JL, et al. BING, a novel antimicrobial peptide isolated from Japanese medaka plasma, targets bacterial envelope stress response by suppressing cpxR expression. Sci Rep 2021;11:12219.

References

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2. O'Rourke A, Beyhan S, Choi Y, et al. Mechanism-of-action classification of antibiotics by global transcriptome profiling. Antimicrob Agents Chemother 2020;64:e01207-19.
3. Lázár V, Martins A, Spohn R, et al. Antibiotic-resistant bacteria show widespread collateral sensitivity to antimicrobial peptides. Nat Microbiol 2018;3:718-31.

Development of an antigen capture assay for melioidosis caused by *Burkholderia pseudomallei*: abridged secondary publication

JLL Teng *, PCY Woo, E Chan

KEY MESSAGES

1. Melioidosis is a potentially fatal disease caused by *Burkholderia pseudomallei*. There is an urgent need for a diagnostic method that can rapidly detect *B. pseudomallei* infection.
2. We generated and purified multiple monoclonal antibodies with high reactivity to *B. pseudomallei*.
3. We developed a simple and reliable rapid antigen capture assay with high sensitivity (85%) and specificity (100%) for the detection of *B. pseudomallei*. The assay is user-friendly and rapid, allowing inexpensive laboratory diagnosis of melioidosis. It has potential for development into lateral flow immunoassays that allow rapid on-site diagnosis of melioidosis in humans and animals.

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Introduction

Melioidosis, a potentially fatal disease caused by *Burkholderia pseudomallei*, is an emerging health concern in various regions, particularly Southern Asia and northern Australia. *B. pseudomallei* also causes melioidosis in various animals within endemic areas.[1] In Hong Kong, melioidosis is endemic in humans, captive marine mammals (eg bottlenose dolphins, California sea lions, and pilot whales), and captive birds such as zebra doves.[2] Accurate and timely diagnosis of melioidosis is essential for effective treatment; however, conventional culture-based identification is time-consuming and often yields false-negative results, whereas molecular techniques such as polymerase chain reaction require specialised equipment and expertise. Thus, serological tests for detecting melioidosis are needed.

Methods and results

We generated and evaluated three monoclonal antibodies (mAbs) specifically targeting the lipopolysaccharide of *B. pseudomallei*. The mAb with the highest reactivity, designated B5 (subclass IgG2b with λ light chains), was used to develop the antigen capture assay. This sandwich enzyme-linked immunosorbent assay–based method used B5 as the capture antibody and an in-house polyclonal anti–*B. pseudomallei* antibody as the detection antibody. Evaluation of the antigen capture assay showed promising results. The diagnostic sensitivity and specificity of the developed antigen capture assay was assessed using 20 melioidosis-positive and 25 melioidosis-negative urine samples. When tested with clinical samples, the assay exhibited high sensitivity (17/20, 85%) and specificity (25/25, 100%) for the detection of melioidosis.

Discussion

Our assay offers several advantages over existing diagnostic methods. It is simple and reliable and can be used in a standard diagnostic laboratory without requiring expensive equipment or reagents. The rapid turnaround time can facilitate timely diagnosis. Furthermore, the antigen capture assay has the potential for adaptation into specific lateral flow immunoassays such as point-of-care test strips and cassettes, thereby enabling rapid on-site diagnosis of melioidosis in both humans and animals. Although the mAb B5 exhibited some cross-reactivity with bacterial lysates of closely related *Burkholderia* species (*B. mallei* and *B. thailandensis*), these species have limited clinical significance in human infections. *B. thailandensis* is considered rare and non-pathogenic in humans, whereas *B. mallei* primarily infects equids (eg horses) and has only caused sporadic cases in humans. Considering its high sensitivity and specificity for *B. pseudomallei*, our assay is a valuable tool for the diagnosis of melioidosis. Further research is needed to validate and optimise the assay for use in various settings.

Conclusion

Our newly developed antigen capture assay offers simple, reliable, and rapid detection of melioidosis. Considering its high sensitivity and specificity, this assay has the potential to facilitate timely diagnosis and effective management of this potentially fatal disease.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#18170712). The full report is available from the Health and Medical Research Fund website (https://rfs2.healthbureau.gov.hk).

References

1. Choy JL, Mayo M, Janmaat A, Currie BJ. Animal melioidosis in Australia. Acta Trop 2000;74:153-8.
2. Godoy D, Randle G, Simpson AJ, et al. Multilocus sequence typing and evolutionary relationships among the causative agents of melioidosis and glanders, *Burkholderia pseudomallei* and *Burkholderia mallei*. J Clin Microbiol 2003;41:2068-79.

AUTHOR INDEX

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