PRESS RELEASE

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Finding needles in the haystack: Clinician-scientists discover new biomarkers to predict heart failure after a heart attack

Singapore, 11 September 2020 - Besides being a traumatic event, a heart attack (also known as a myocardial infarction) may damage or stiffen the heart, leading to a condition called heart failure.[1] The occurrence of heart failure after a heart attack is truly a double tragedy for the affected patient. Just as the patient is starting to get back on his feet after a severe illness, another major wave washes over him. In heart failure, the heart stops pumping blood properly causing severe breathlessness, markedly reduced stamina and reduction of quality-of-life.[2] HF results in frequent hospitalisations and one in five patients will die in five years.[2] HF is rising in prevalence worldwide, with 4.5% of people in Singapore affected by the condition.[3]

Identifying MI patients who are at high risk of developing HF would enable doctors to intervene earlier and perhaps prevent HF from occurring. While hundreds of proteins have been found to be associated with the development of HF after an MI, researchers still have to conduct extensive experimentation of each protein to determine if it can be a useful biomarker or possible drug target in heart failure after a heart attack. This is in essence a massive undertaking to look for the 'needle in the haystack'. In a new study by Associate Professor Mark Chan from the NUS Yong Loo Lin School of Medicine and Senior Consultant of the Department of Cardiology at the National University Heart Centre, Singapore ,

[1] Mayo Clinic. Heart Failure. https://www.mayoclinic.org/diseases-conditions/heart-failure/symptoms-causes/syc-20373142. Updated 2020. Accessed August 28, 2020
[2] Gerber Y, Weston SA, Enriquez-Sarano M, et al. Mortality associated with heart failure after myocardial infarction: a contemporary community perspective. *Circ Heart Fail*. 2016;9:e002460
[3] Lam CSP. Heart failure in Southeast Asia: facts and numbers. *ESC Heart Failure*. 2015;2:46-49.

Professor A. Mark Richards, NUS Yong Loo Lin School of Medicine and Senior Consultant of the Department of Cardiology and Deputy Director at the National University Heart Centre, Singapore, and colleagues combined two powerful new technologies to help accelerate this process of discovering new biomarkers and potential treatment targets to prevent heart failure after a heart attack.[4]

This study, the results of which were published online ahead-of-print in the journal *Circulation*, involved patients from two countries who had experienced a MI: 1) the Coronary Disease Cohort Study (CDCS), consisting of 500 patients in New Zealand, and 2) the Improving Outcomes in Myocardial Infarction through Reversal of Cardiac Remodelling (IMMACULATE) registry, comprising 200 patients in Singapore.

The first powerful discovery technology that A/Prof Chan and team applied was large-scale plasma proteomics which uses short DNA strands called aptamers to simultaneously measure more than a thousand proteins in blood.[5] The major advantage of this protein-profiling technology is that it is incredibly sensitive and can detect even minute quantities of individual proteins with high certainty. To ensure that the proteins they measured had actually increased because of changes in living cells, rather than through a non-specific release from dying heart muscle cells early after the MI, the researchers waited one month after the MI before taking blood and performing the assay.

A/Prof Chan and colleagues found more than 200 proteins that predicted the future onset of HF up to 7 years after the MI. They then cross-referenced the proteins of the greatest significance to the RNA fragments of more than 6,000 different individual cells from mice and humans with heart attacks and heart failure using the second powerful discovery technology called single-cell RNA sequencing. RNA is the product of our more than 20,000 genes (DNA). When genes are activated to produce a certain protein, cellular RNA production is increased. Because RNA are precursors of proteins, correlation between RNA from cells and proteins in the blood give greater assurance that the proteins discovered by the first technology are relevant to the disease process. By analysing the RNA content of single cells one-at-a-time, instead of the more conventional method of analysing RNA from a large groups of cells in bulk, the researchers are able to detect subtle but important changes in

[4] Chan MY, Motakis E, Tan S-H, et al. Prioritizing Candidates of Post-Myocardial Infarction Heart Failure Using Plasma Proteomics and Single-Cell Transcriptomics. Circulation…
[5] Rohloff JC, Gelinas AD, Jarvis TC, et al. Nucleic acid ligands with protein-like side chains: Modified aptamers and their use as diagnostic and therapeutic agents. *Mol Ther Nucleic Acids*. 2014;3:e201.

RNA expression in subpopulations of cells that would otherwise be missed with a bulk cell approach.

After hundreds of hours of bioinformatics analysis shifting through massive amounts of data, A/Prof Chan and colleagues found their 'needles in the haystack'—six "highest-priority" proteins for other investigators to focus their efforts on instead of testing several hundred proteins. Of these six proteins, two were already well-established biomarkers of heart failure after a heart attack - cardiac troponin T, and N-terminal B-type natriuretic peptide – that were secreted from heart muscle cells after a heart attack, giving them confidence about the other four proteins. The single cell RNA sequencing determined that 2 of these 6 proteins, thrombospondin 2 and latent transforming growth factor-β binding protein-4 originated not from heart muscle cells but from other cells within the scaffold in which heart muscle cells are embedded, also called the extracellular matrix. To the surprise of the investigators, the sixth key protein was not secreted by either heart muscle or extracellular matrix cells but the inner lining of blood vessels, also called the endothelium.

A/Prof Chan and colleagues are now collaborating with biomedical engineers to develop 'lab-on-chip' devices to measure these proteins for user-friendly detection. Prof Richards has also completed work on some of these proteins showing that modifying their effect can accelerate recovery of heart function after a heart attack.

"Strong and reliable signals, identifying those patients who are unfortunate enough to incur heart failure following their heart attack, remain an urgent need," said Prof Richards. "This work, the result of bilateral and inter-disciplinary collaboration, all skilfully coordinated by A/Prof Mark Chan, has taken us an important step closer to being able to step in post-heart attack and more fully protect patients from further harm."

Added A/Prof Chan: "As a cardiologist who has spent a lot of time looking after patients with heart attacks in the last 10 years, it is disheartening to see patients develop heart failure even after performing emergency angioplasty for them in double-quick time. Paradoxically, by saving more lives, emergency angioplasty has actually led to more patients having heart failure after surviving a heart attack. This research would not have been possible 10 years ago but recent technology has enabled us to measure thousands of blood proteins at once with large-scale plasma proteomics and sequence tens of thousands of RNA fragments one cell at a time with single-cell RNA sequencing, helping us to save time by focusing on the targets that matter most to predict and possibly find new treatments for heart failure after a

heart attack. A big thank you to the 19 colleagues who worked tirelessly with me to deliver this important discovery."

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Our multidisciplinary and real-world approach to education, research and entrepreneurship enables us to work closely with industry, governments and academia to address crucial and complex issues relevant to Asia and the world. Researchers in our faculties, 30 university-level research institutes, research centres of excellence and corporate labs focus on themes that include energy, environmental and urban sustainability; treatment and prevention of diseases common among Asians; active ageing; advanced materials; as well as risk management and resilience of financial systems. Our latest research focus is on the use of data science, operations research and cybersecurity to support Singapore's Smart Nation initiative.

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Through a dynamic and future-oriented five-year curriculum that is inter-disciplinary and inter-professional in nature, our students undergo a holistic learning experience that exposes them to multiple facets of healthcare and prepares them to become visionary leaders and compassionate doctors and nurses of tomorrow. Since the School's founding in 1905, more than 12,000 graduates have passed through its doors.

In our pursuit of health for all, our strategic research programmes focus on innovative, cutting-edge biomedical research with collaborators around the world to deliver high impact solutions to benefit human lives.

The School is the oldest institution of higher learning in the National University of Singapore and a founding institutional member of the National University Health System. It is Asia's leading medical school and ranks among the best in the world (Times Higher Education World University Rankings 2019 by subject and the Quacquarelli Symonds (QS) World University Rankings by Subject 2019).

For more information about NUS Medicine, please visit https://medicine.nus.edu.sg/

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With member institutions under a common governance structure, NUHS creates synergies for the advancement of health by integrating patient care, health science education and biomedical research.

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