Molecular signatures of myocardial infarction

Chapter 9

Impact

Cardiovascular diseases, especially myocardial infarction (MI), are still the number one cause of death globally, with approximately 18.6 million deaths in 2019 [1]. They are also a challenge for the health care system due to their high morbidity [301, 302]. To address this problem, research into MI and cardiac ischemia/reperfusion (I/R) injury is needed to improve our knowledge on the different aspects of the diseases and the molecular pathophysiology in particular [303, 304].

This thesis focuses on the molecular signatures observed after MI, by addressing on-tissue spatial distributions using MALDI-MSI, and the composition of cardiac troponins (cTn) in MI and non-MI patients' circulation. The described research is of interest to a broader audience, from the MSI community that can benefit from the spatial-omics workflow (**chapter 3**) to healthcare professionals and diagnostic companies who will be interested in the molecular composition of cardiac troponins (**chapters 6** and **7**). This is a direct result of the multidisciplinary approach chosen to conduct these studies. Additionally, the publication of most of the work in open-access scientific journals and the availability of the proteomics data in public data repositories enables other researchers to benefit from it and use it to enhance their research.

The optimized workflow including MALDI-MSI and laser capture microdissection (LMD), as described in **chapter 3**, enables the use of a single section for a spatially resolved proteomics analysis. This innovative method has been used as the basis for a patent filed in the course of this research as part of a continuous effort to increase the economic impact of academic research. The implementation of this workflow, more specifically the use of conductive non-membrane slides for LMD, is of great value to the scientific MSI community and its related industries. First of all because it reduces the amount of tissue needed, hence multiple datasets (e.g. MALDI-MSI and omics) could be generated from a single tissue section. Moreover, section-to-section variability is avoided, which improves the co-registration and is of great importance now the MSI field is moving towards single cell analysis. Next, the entire MSI community can benefit as the workflow is most likely not limited to the use of MALDI-MSI and/or proteomics. And undoubtedly the protocol could also be considered in other (non)-cardiovascular studies, where distinct molecular profiles are targeted to classify tissue and provide complementary information used in biomarker discovery. Eventually, future collaborations with industrial partners would further optimize the workflow providing a more advanced, and high throughput platform, which could result in increased revenues for these partners if marketed.

In addition to the optimization of the workflow, our data in **chapter 4** demonstrates the potential of using MALDI-MSI as a screening technology for LMD tissue selection over traditional histology. Here, we used the on-tissue protein signature to guide the in-depth proteomics analysis and identified altered proteins and corresponding pathways that can be used to guide future research to gain more understanding of the processes in cardiac I/R. Also, these proteins could be targeted in functional and intervention studies for validation and to study their potential role as therapeutics or in decision-making.

The combination of MALDI-MSI, lipidomics and immunohistochemistry, as described in **chapter 5**, enabled us to evaluate the lipid signature of cardiac I/R on a spatial and temporal level. This multilevel data provides the cardiovascular research field with more insights in the on-tissue lipid alterations and their correlations with immune cell infiltration, e.g. macrophages. This could impact the establishment of cell specific molecular profiles, which can be deployed in future studies to monitor the different processes during the I/R process, leading to a better understanding of the inflammatory process. Moreover, further validation of these lipid signatures might guide the development of improved diagnostics or treatments for patients suffering from MI.

The second part of this thesis focusses on cardiac troponins (cTnT and cTnI), the gold standard cardiac biomarkers for the clinical diagnosis of MI and crucial for patients without persistent ST-elevation (NSTEMI). The introduction of the high-sensitivity assays led to improvements in the diagnosis of earlier and smaller NSTEMI infarctions, and subsequently to an improved diagnosis in women, who in general present with vague symptoms and lower cTn concentrations. Unfortunately, this increased sensitivity also led to a decrease in the specificity for MI, which complicates clinical decision making. Our research group hypothesized, based on previous work, that the molecular composition of cTn in the blood can differentiate between MI and non-MI conditions. Therefore, we compared the composition in study populations, both MI as non-MI. These samples were available due to longstanding collaborations within Maastricht University Medical Center and with Radboud University Medical Center.

Altogether, in NSTEMI patients [23] we observed cTnT as part of the ternary cTn T-I-C complex, as free cTnT (intact, 40 kDa) and cTnT fragments (29 kDa and 15-18 kDa), while in patients with end-stage renal disease (ESRD) [25] and in serum of marathon runners (**chapter 6**) we only observed the smaller cTnT fragments. It is therefore suggested that a novel cTnT assay should target cTnT forms ≥29 kDa to be more specific for the acute phase of MI. Additionally, for cTnI we mainly observed the binary cTn I-C complex. No clear differences were found between the populations (**chapter 7**), though the ternary cTn T-I-C complex was only seen for

the early NSTEMI patient, suggesting this might be used as an indication of the age of the infarct. Although further validation of the molecular cTn compositions is needed, it could drive the development of novel diagnostic cTn assays.

While these results are of interest for healthcare professionals, the industrial parties that produce the clinical assays will also benefit from it. The collaboration with companies like Roche Diagnostics and Abbott Laboratories, provided valuable resources that helped us investigate the conformation of cTnT (**chapter 6**) and cTnI (**chapter 7**). In return, we could provide the knowledge and insights presented in the data, which allows them to change, improve and potentially patent a new hs-cTn immunoassay. These developments have a positive economic impact on these companies if the assays become clinically available. Moreover, the implementation of more specific assays could lead to improved clinical guidelines for MI diagnosis and treatment.

Ultimately, these new, optimized diagnostic assays should improve clinical decision making for patients with suspected MI, by discriminating between MI and non-MI causes of cTn elevations. For approximately 90% of the patients with acute chest pain presenting at the emergency department cTn measurements are required, and for 60% a serial cTn assessment for the 0h/1h- or 0h/3h-algorithm is needed. It is speculated that new, more specific cTn assays could reduce the number of patients that require additional hs-cTn measurements for diagnosis, as the measurement at presentation (0h) would make it possible to rule out patients with non-MI causes of cTn elevations. In this case, where serial measurements are not needed, the time at the emergency department would be shortened especially in those hospitals using the 0h/3h-algorithm. All in all, it would reduce the hospitalization duration and improve the quality of patient's care, as treatment can therefore be started earlier, and/or patients could be referred to another department or discharged faster, eventually resulting in more cost-effective patient care.

Lastly, this PhD trajectory started within a newly founded collaboration between the Maastricht MultiModal Molecular Imaging Institute (M4i) at Maastricht University and the Central Diagnostic Laboratory of the Maastricht University Medical Center. Also, other strong collaborations were established throughout this PhD project, with pathologists, cardiologists, and research groups within and outside Maastricht University as well as abroad (France and Singapore). These collaborations strongly impact the scientific community at the Brightlands Maastricht Health Campus. The interdisciplinary scientific collaborations have impacted my personal development as I could benefit from different expertises, use the available infrastructures and samples/patient cohorts. Moreover, these collaborations are the basis for good translational research, where knowledge is transferred, and cross-border ideas are stimulated by the interdisciplinarity.