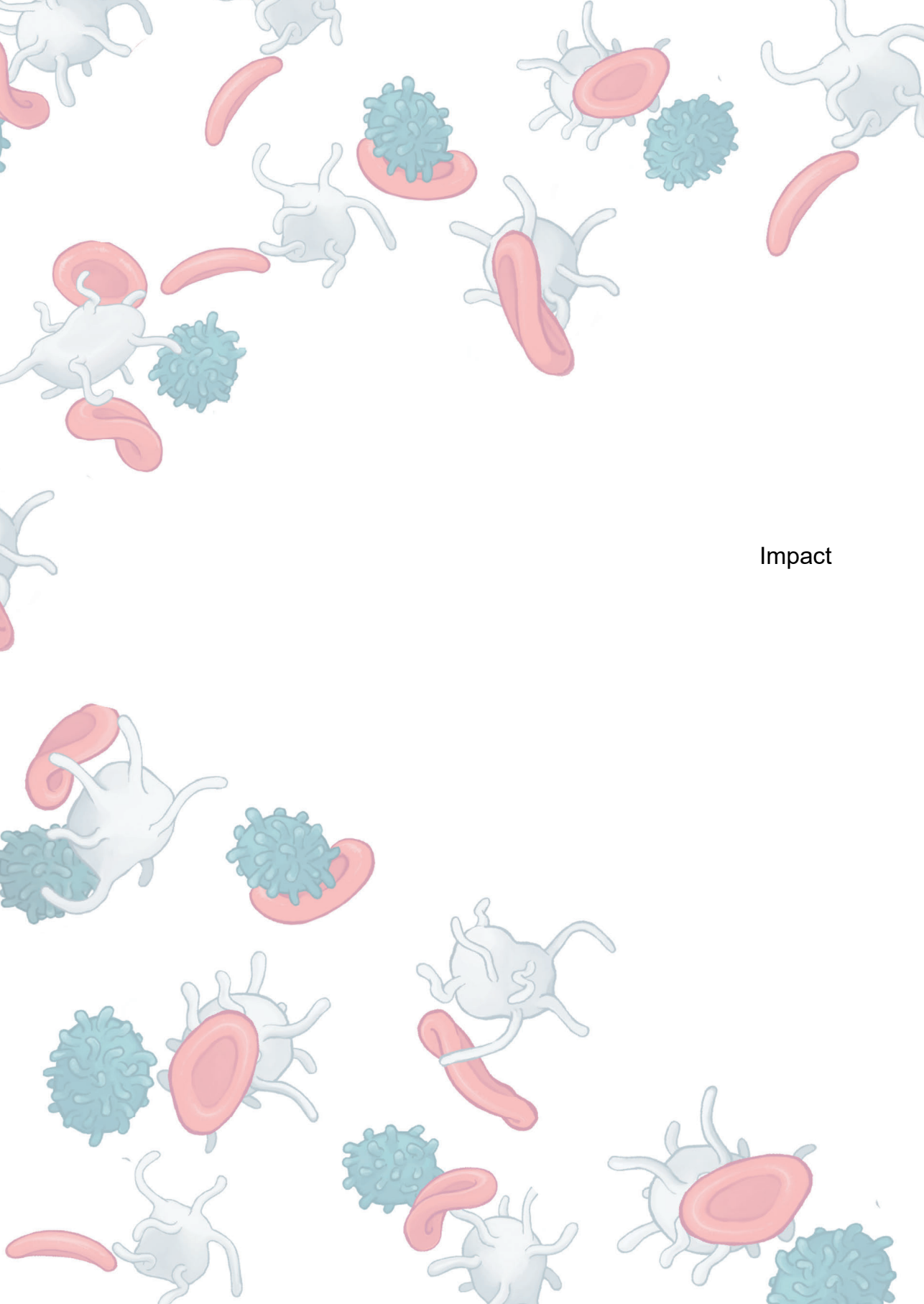
Unraveling platelet function in inflammation and thrombosis



Impact

Research, what is that exactly? What are you achieving and what does it mean? These questions are often asked the moment I discuss research with a non-scientific public. In general, research aims to deliver significant impact to science and the society. The way this is achieved and the extent to which depends on the scientific area and research subject.

With the research depicted in this thesis, we aimed to obtain novel mechanistic insights in the function of platelets in thrombosis and vascular inflammation by making use of emerging technologies. Current treatment guidelines are mainly directed against pro-thrombotic platelet activation,[1,2] however, the pathophysiology of arterial and venous thrombosis comprises both thrombosis and inflammation. We suggested to investigate pro-inflammatory platelet responses, specifically platelet-endothelial and platelet-leukocyte interactions. We provided a thorough overview of platelet-endothelial interactions in *Chapter 2*, and extended this to a translational perspective in disease pathophysiology (*Chapters 3, 6* and *7*) and therapeutic activities (*Chapters 4* and *5*). Highly advanced and state-of-the-art techniques have been developed to investigate the great complexity of thrombo-inflammatory diseases such as endothelial-lined microfluidics and vessel-on-a-chip models (*Chapter 2* and references [3,4]). These *in vitro* models allow a good representation of the *in vivo* situation when implementing a number of essential variables, including chamber dimensions, shear stress, and cell type, passage, and confluency, as reviewed in *Chapter 2*. We have attempted to gain more knowledge about the underlying pathophysiology of heart failure with preserved ejection fraction (HFpEF). This major health issue without effective treatment presents an inflammatory environment with leukocyte activation and endothelial dysfunction,[5,6] but the value of platelets in this is still unknown. With our study (*Chapter 6*) being the first to investigate this, we wanted to obtain novel mechanistic insights in vascular (dys)function and interactions in HFpEF. Our HFpEF cohort showed endothelial activation, but interestingly, platelet activation markers in plasma were decreased, in particular in patients with concomitant type 2 diabetes mellitus. Platelet activation correlated positively with leukocyte count, although neutrophil degranulation was unaffected. These results are a foundation in unravelling the mechanisms underlying HFpEF and improving therapeutic efficacy. We also found a contributing role for platelets in inflammation-driven venous thrombosis (VT) in mice. This is absent in mice deficient for SLC44A2, a gene recently linked to a potential increased risk of VT. Our study and other recent studies gave a better understanding in the role of SLC44A2 in VT which possibly involves platelets, neutrophils, and endothelial-derived von Willebrand factor (*Chapter 6* and references [7,8]), creating more awareness in VT and the genetics behind this. Throughout this thesis, we have extensively examined and reported on the interactions of platelets with endothelial cells and leukocytes. A fundamental aspect in these interactions is the secretion of granules, vesicles and soluble molecules, predominantly from platelets. We explored whether these can be a potential target in thrombo-inflammatory diseases. We

focused on more common antiplatelet medication (aspirin, $P2Y_{12}$ antagonists and $\alpha_{IIb}\beta_3$ antagonists) but also investigated if and how phosphodiesterase (PDE) inhibitors interfere with pro-inflammatory platelet function (*Chapters 4 and 5*). The respective PDE3 and -5 inhibitors cilostazol and tadalafil, both already prescribed in other diseases, dampened direct platelet interactions with endothelial cells and leukocytes as well as platelet-derived chemokine and (pro-coagulant) extracellular vesicle release. We added functional observations substantiating a beneficial effect of especially cilostazol in thrombo-inflammatory diseases, possibly in combination with aspirin or a $P2Y_{12}$ antagonist. In this way, we connected basic platelet and vascular research with the cardiovascular clinic.

Technologies are evolving, allowing people to target research from a different angle in order to discover new, yet unrecognized disease targets or mechanisms. One of the consequences is that datasets become larger and more complicated. With the emergence of advanced technologies and big data, we provided an easy and broadly applicable method to analyse these large datasets. In *Chapter 8*, we pointed out that network biology is not only a method to describe pathways and connections between genes and proteins, but can also be used to include more content such as information about phosphorylation sites and the likelihood proteins are being phosphorylated by certain kinases. In this way, new information can be obtained about signalling pathways in platelets and other cells and new candidate proteins to target in the treatment of (thrombotic) diseases can be detected. We used both new technologies creating big data as well as analytical tools to structure and interpret such datasets. We were able to deeper explore disease mechanisms and signalling pathways downstream of cAMP-protein kinase A (PKA) and to gain more insights in the processes they control and the proteins involved. Vesicle-mediated transport, regulation of cyclin-dependent protein kinases, and regulation of small GTPases were identified as being important processes in platelet activation. We suggested several proteins downstream cAMP-PKA (PPP1R14A, Stonin-2, ABLIM3, KARLN) as being potential switches in platelet activation and possible new targets. This method is suited to re-evaluate large existing datasets in order to come across new perspectives of the research one is performing. The use of network biology is not only applicable to the field of cardiovascular research, but can be implemented in a broad variety of research areas. Therefore, the impact and importance of our research ranges from absolute basic to completely clinical science as well as from health sciences to social sciences. Hereby, this thesis connects basic, clinical and computational research, depicting diversity and association in science.

The general population does not only have questions about research, but also understands how important (laboratory) science is. Especially in times of health crisis and distress, such as during the COVID-19 pandemic, I feel that the value of research is increased and tends to be appreciated even more by the society. The fact that people are more aware of the importance of research makes it essential to

involve the general public and inform them about research projects and the progress and outcomes, particularly in translational and clinical studies.[9] In addition, it is important that clinicians, in this case in particular cardiologists and vascular biologists, are continuously updated and informed about the latest scientific insights in order to compose the best possible treatment plan for their patients. Information transfer from researchers to clinicians and to society can be carried out directly by presenting the results on patient information days or indirectly via the clinician or charities such as the Heart Foundation and the Thrombosis Foundation. Researchers and clinicians can be educated and advised via on-site and online conferences, symposia and lectures, but also via the accessibility of published research in recognized journals and via collaborations with each other. The availability of open access journals and data makes research more accessible to the scientific and non-scientific public. I think that with the activities mentioned above, the relevance of research can be transmitted to broad and diverse audiences, by which the interest in and understanding for research will grow and the divergence between the scientific and non-scientific population will become smaller.

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