Eindhoven dissertation prizes 2011

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For the 23rd time in a row, starting in 1990, the Netherlands Society of Cardiology (NVVC) and the Interuniversity Cardiology Institute of the Netherlands (ICIN-Netherlands Heart Institute) supported the competition for the best three cardiovascular PhD theses published in the year 2011. The dissertation prize carries the name of one of the greatest Dutchmen in the history of cardiovascular medicine: Willem Einthoven, who in 1902 for the first time recorded the human ECG, for which he received the Nobel Prize in 1924.

This time the jury received a total of 24 PhD dissertations. The jury members were very much impressed by the high scientific quality of the PhD fellows. Based on originality, the number of articles in first-rate journals, the contribution as a first author (or shared first author) to the papers, and the number of citations, the jury finally selected three nominees: Dr. Fatih Arslan (UMCU, Utrecht), Dr. Moniek Cox (UMCU, Utrecht), and Dr. Nina Ajmone Marsan (LUMC, Leiden). The three candidates presented their PhD theses at the annual Spring Congress of the NVVC in Noordwijkerhout on 12 April. The ultimate winners of the first, second, and third prize were chosen by the audience. Summaries of the three nominated PhD theses are given below.

Chairmen of the jury

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Enhancing cardiac repair—targeting myocardial I/R injury and adverse remodelling

More patients survive the initial myocardial infarction, thanks to pharmacological advances (e.g. anti-thrombotics) and technical breakthroughs (e.g. stent technology). Unfortunately, the surviving patients do suffer from progressive deterioration of cardiac function. For this reason, the incidence of heart failure and other MI-related morbidity is increasing. Hence, novel adjunctive therapeutics are necessary to further reduce cardiac injury and/or prevent the development of infarct-related complications. In this thesis, we studied two major determinants for progressive deterioration of heart function after myocardial infarction: 1) myocardial ischaemia/reperfusion (I/R) injury and 2) adverse ventricular remodelling. The ‘danger model’ served as a theoretical framework to design our experiments and interpret our data (Fig. 1). Briefly, the danger model states that every single molecule associated with cell death and/or matrix degradation triggers immune responses. The immune response can either be constructive or destructive, depending on the danger signal.

Myocardial I/R injury is a very complex process causing additional post-ischaemic injury. Mediators responsible for cell death during ischaemia are different from those during the reperfusion phase. Myocardial salvage from reperfusion injury may result in infarct size reduction and improved cardiac function. In Part I of this thesis, we provide evidence that activated Toll-like receptor (TLR) 2 expressed by circulating cells mediates infarct size increase after I/R. By using novel anti-TLR2 antibodies in mice and pigs, we were able to reduce infarct size by 40 % and improve cardiac function. The humanised TLR2 antibody is currently being tested in phase 1 clinical trials. Next, we identified exosomes as small particles within conditioned media of mesenchymal stem cells to have cardioprotective properties. Exosomes appeared to directly interact with the ischaemic/reperfused myocardium in order to reduce infarct size after I/R. In Part II, we focus on the reparative processes in the

infarcted heart, referred to as remodelling. Remodelling is characterised by inflammation, matrix turnover and subsequently scar formation and maturation. Enhanced inflammation may result in adverse remodelling and subsequently heart failure after MI. First, we showed that TLR2 expressed by circulating cells mediates adverse remodelling. Knocking out TLR2 in bone marrow-derived cells improved cardiac function and geometry in mice after infarction. Second, we identified for the first time fibronectin-EDA as a critical mediator of adverse remodelling. It appeared that fibronectin-EDA in the myocardium serves as a danger signal that activates and recruits cells to the infarcted myocardium. The absence of fibronectin-EDA in the myocardium reduced inflammation, fibroblast activation, dilatation and fibrosis of the remote myocardium. Finally, we used haptoglobin knock-out mice to increase oxidative stress after infarction. Haptoglobin knock-out mice exhibited a dramatic phenotype characterised by increased intramural

haemorrhage, cardiac ruptures and leaky vessel formation after infarction.

In conclusion, the ‘danger model’ is a useful theoretic framework to understand and explore immune responses and repair processes after MI. So far, TLR2 inhibition and exosome treatment are potential candidates as adjunctive therapeutics to enhance cardiac repair and function in patients suffering from acute MI. Future studies are required to confirm the efficacy of exosome treatment in large animals and to further explore the role of fibronectin-EDA in infarct-related inflammation.

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Fig. 1 Schematic overview of determinants of cardiac repair and function, as described in this thesis

Arrhythmogenic right ventricular dysplasia/cardiomyopathy Activation delay, diagnosis and DNA

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is classically characterised by progressive fibrofatty replacement of myocardium, primarily of the right ventricle. Typically, first arrhythmic events occur between the second and fourth decade of life, usually being right ventricular (RV) monomorphic tachycardias. However, phenotypic expression is highly variable, and particularly ventricular fibrillation and sudden death may be first manifestations.

Task Force Criteria (TFC), proposed in 1994, were universally used for clinical diagnosis. After the discovery of mutations in genes encoding desmosomal proteins, involved in mechanical coupling of cardiac myocytes, ARVD/C is considered an inherited desmosomal disease. The desmosomal changes, resultant electrical and cellular uncoupling and surviving myocardial bundles embedded in fibrofatty tissue form the substrate for activation delay. This RV activation delay is crucial for reentrant tachycardias, and is the hallmark of ARVD/C.

The first part of this thesis focused on development and validation of clinically applicable diagnostic electrocardiographic (ECG) criteria, based on the hallmarks of ARVD/C. Firstly, prolonged terminal activation duration (TAD; nadir of S wave to end of depolarisation >55 ms in V1-3), a marker of RV activation delay, was studied in initial ECGs, while off drugs, in 42 ARVD/C patients. Results were compared with 27 controls with idiopathic ventricular tachycardia (VT) originating from the RV outflow tract and related to outcome of DNA analysis on *PKP2*. In ARVD/C patients, prolonged TAD was observed more often than the 1994 TFC on activation delay: epsilon waves and QRS duration >110 ms.

Since ARVD/C may affect different parts of the right ventricle, VTs originating from affected areas may show left bundle branch block (LBBB) morphology with different axes, including a superior axis. LBBB VT with a superior axis (-30° to -150°) was recorded in 67 % of ARVD/C patients. Besides, >1 different VT morphology was recorded in 88 %. All three newly proposed diagnostic criteria were present in the majority of ARVD/C patients and seemed disease-specific. Thereafter, 33

patients highly suspected of ARVD/C, but not fulfilling the 1994 TFC, were similarly evaluated. In total, 23 of 33 (70 %) probable ARVD/C patients met ≥ 1 of the newly proposed criteria: 14/33 prolonged TAD, 12/33 VT with LBBB morphology and superior axis, 9/33 multiple VT morphologies. These patients would thereby fulfil the ARVD/C diagnosis if these criteria were applied in addition to 1994 TFC.

Results were not different between mutation carriers and non-carriers.

We participated in the new international Task Force that revised the 1994 TFC. Two of our newly proposed ECG criteria were incorporated in the revised 2010 TFC: prolonged TAD and VT with LBBB morphology and superior axis.

The second part of this thesis involves different aspects of DNA analysis in ARVD/C: both benefits and pitfalls. After multiple studies on evaluation of genetic changes concerning desmosomal genes, among others in collaboration with the Mayo Clinic, the final chapter reports on the large long-term Dutch genotype-phenotype correlation study. A total of 149 ARVD/C patients, diagnosed according to the 2010 TFC, and 302 relatives from 93 families underwent comprehensive clinical and genetic evaluation (*PKP2*, *DSC2*, *DSP*, *DSG2*, *JUP*). Of index patients, 58 % carried pathogenic mutations, mainly truncating *PKP2*, the highest percentage reported thus far (Fig. 1). Pathogenic mutations were identified in as many as 90 % of familial cases. Although 282/302 family members were initially asymptomatic, screening revealed familial cases identified in 45 % of families. ARVD/C was diagnosed in 31 % of relatives with mutations and in 5 % of relatives of index patients without mutations. Thus, discovering a pathogenic mutation in index patients enables identification of relatives with a sixfold increased risk for ARVD/C diagnosis. Prolonged TAD was the criterion observed most and already at a young age. In conclusion, this study underscored the importance of comprehensive genetic testing in patients diagnosed with ARVD/C and consequences for family screening.

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Fig. 1 Schematic representation of family members, demonstrating distribution of ARVD/C signs and symptoms as well as mutations. Numbers between brackets indicate the number of different families

Incremental value of advanced cardiac imaging modalities for diagnosis and patient management *Focus on real-time three-dimensional echocardiography and magnetic resonance imaging*

The objectives of this thesis were to investigate the incremental value of advanced cardiac imaging modalities, and in particular of real-time three-dimensional echocardiography (RT3DE), myocardial deformation imaging, contrast-enhanced echocardiography and cardiac magnetic resonance imaging (CMR), for diagnosis and patient management in different cardiac diseases. In Part I, the use of RT3DE for quantification of left ventricular (LV) volumes and function and for the assessment of LV dyssynchrony was explored in heart failure patients undergoing cardiac resynchronisation therapy (CRT, Part IA). Different 3D measures of LV dyssynchrony, based on the full volume approach (Fig 1.) or on the tri-plane approach, were applied to improve candidate selection for CRT and to predict favourable response after implantation. In Part IB, RT3DE was applied in patients with atrial fibrillation or heart failure for the quantification of left atrium

volume and for the assessment of different left atrial functions (conduit, active and reservoir function).

Part II evaluated the additional diagnostic and prognostic value of contrast-enhanced echocardiography, particularly in patients within 24 h after acute myocardial infarction. Safety and accuracy (in combination with RT3DE) of this modality in this specific group were demonstrated. The administration of contrast agents in these patients improved endocardial border visualisation and reproducibility of LV function assessment. In addition, the incremental value of myocardial infarction extension, as assessed by myocardial perfusion analysis, to predict LV remodelling after infarction was explored together with novel measures of LV performance.

The use of CMR for a comprehensive assessment of patients with various cardiac diseases was the focus of Part III. Novel CMR approaches for an accurate and reproducible measure of LV dyssynchrony were proposed and applied in heart failure patients undergoing CRT (Part IIIA). The standard deviation of 16 segments time-to-maximum wall thickness showed to be an important predictor of significant LV reverse remodelling after CRT, together with the extension of myocardial infarction. In

addition, the value of CMR for the assessment of myocardial viability was evaluated in different groups of patients (heart failure, LV aneurysm, ischaemic cardiomyopathy).

Part IIIB explored the value of CMR in valvular heart disease, and particularly as a reference technique for the assessment of mitral regurgitation (MR) severity. A strong correlation was observed between RT3DE and CMR for the measurement of MR volume, with no significant bias between these two techniques; conversely, conventional 2D echocardiography significantly underestimated regurgitant volume.

Finally in Part IV, novel physiopathological aspects in CRT patients were studied using advanced echocardiographic imaging modalities. In particular, the effect of

CRT was evaluated on: 1) LV rotational mechanics, as assessed by speckle tracking strain analysis; 2) cerebral blood flow, as measured by transcranial Doppler; 3) severity of MR. In this specific study, it was demonstrated that CRT might also be considered a potential therapeutic option in patients with severe functional MR, who are not referred for or are denied surgery due to their high operative risk. In fact, a significant improvement of MR was observed after CRT in approximately 50 % of patients, and the improvement in MR resulted in superior long-term survival after CRT.

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Fig. 1 Example of the 3D LV model generated by post-processing of an RT3DE dataset and subdivided by the software in 17 sub-volumes (left panel). Right lower panel: for each volumetric segment, it is possible to derive time-volume curves over the cardiac cycle and assess the time needed to reach the minimum systolic volume (Tmsv, red dots). In this example, LV contraction is dyssynchronous and the standard deviation

of 16 segment Tmsv, expressed in percentage of the cardiac cycle (systolic dyssynchrony index SDI) is 11.7 %. Right upper panel: example of parametric image, which employs colour coding (blue indicating early mechanical activation and orange-red is late activation) to represent Tmsv. In this heart failure patient, the infero-postero-lateral wall (segments 4, 5, 6, 10 and 11) is clearly the latest activated region

Moniek Cox won the first prize, Fatih Arslan the second prize, and Nina Ajmone Marsan the third prize.