Heartbeat-to-heartbeat cardiac tissue characterization

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Chapter 9

General discussion and future perspectives

Early detection of cardiovascular diseases remains key for effective treatment after diagnosis. While multiple quantitative cardiac magnetic resonance imaging (MRI) techniques, such as T_1-, T_2- and T_2^*-mapping, are getting standardized for improved implementation into clinical protocols (Messroghli et al. 2017), the curent use of these techniques is still limited. Reference values for healthy myocardium and several cardiovascular diseases can help making these techniques better interpretable, but it remains unclear whether they could be used as a marker prior to the onset of cardiac remodeling (Chapter 2 and 3). Therefore, multiple additional cardiac MRI techniques have been developed that could offer an early marker for cardiac remodeling that is potentially induced by common risk factors such as obesity, hypertension (HT) or type 2 diabetes mellitus (DM).

Since microvascular dysfunction is currently an expected underlying mechanism of several cardiomyopathies resulting in heart failure (HF) (Haddad et al. 2015), there is an increasing interest in imaging techniques that enable the assessment of the cardiac microvasculature (Petersen and Pepine 2015). However, limitations in the use of contrast agents in humans and the burden of an injection or long acquisitions on the MRI workflow asks for a radically different approach to assess vascular function (Friedrich 2020). The introduction of a new cardiac blood oxygenation level dependent (BOLD) MRI technique might offer such readout (Part III), although it needs further validation to answer remaining technical questions. Furthermore, other vascular imaging approaches such as assessment of endothelial function with dynamic contrast enhanced (DCE) (Chapter 4) and vessel architectural imaging (VAI) (Chapter 8) do require contrast agents, but should still be further explored in animal models until new intravascular contrast agents make their way into clinical applications (Gale et al. 2018). Even though these new techniques might provide new detailed tissue characteristics and advance beyond the standard

cardiac magnetic resonance (MR) assessment of cardiac function of late gadolinium enhancement (LGE), the main question remains if they can help with earlier and more accurate diagnosis of cardiomyopathies.

Fibrosis as early cardiac MRI tissue marker

Starting with T_1 mapping, which shows significantly increased T_1-values in myocardium that is affected by either hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM) or myocarditis (MC) (van den Boomen et al. 2018), and can even distinguish between HT with or without left-ventricle hypertrophy (LVH) (Hinojar et al. 2015a). This sensitivity of T_1-mapping to the diffuse fibrosis is for all evaluated non-ischemic cardiomyopathies (NICM) the same, but distinction between the diseases could still be made as long as diagnosis also includes other MRI readouts such as left ventricular (LV) mass and ejection fraction (EF), and in some cases blood markers. Furthermore, it is also clarified in Chapter 2 that any meaningful interpretation of T_1-values needs healthy reference values that have to be center, scanner and sequence specific. Also, the fact that HT without LVH does not show an increased T_1 implies that fibrosis co-occurs with cardiac remodeling, which limits its applicability as an early marker. Lastly, it should be kept in mind that there are two different forms of fibrosis (Krenning et al. 2010), either replacing dead cardiomyocytes or increasing the interstitial space without losing cardiomyocytes, and T_1 cannot differentiate between those. Therefore, diagnosis and decisions on treatment approaches still need other readouts, aside from T_1 mapping.

It is interesting to note that only limited studies were done in populations with cardiovascular risk, since from the studies in HT it is now known that reactive fibrosis co-occurs with cardiac remodeling (Biernacka and Frangogiannis 2011). Where HT shows signs of T_1 increase when the tissue attemptes to retain the needed cardiac function, obesity and DM could cause a similar increasing stress on the heart (Levelt et al. 2015), which could also result in an increase in fibrosis and therefore the T_1. In these populations cardiac MRI could be helpful to determine the timing when remodeling becomes irreversible in order that the treatment plan can be reassessed accordingly.

An earlier marker for myocardial tissue and function alterations might be the change of strain distribution in the heart, causing reactive fibrosis to compensate the altered load (Cheng et al. 2013). Myocardial tagging MRI, used in Chapter 4, is one of the existing imaging approaches that is sensitive to such strain changes. The

major drawback of that technique is the tag fading over the time of a cardiac cycle (Pai and Axel 2006), but also the time needed to obtain a full 3D tagging volume of the heart (Scatteia et al. 2017). Therefore, other techniques, including feature tracking, are being explored and slowly find their way into clinical practice (Romano et al. 2018). Evaluation of such techniques in populations with increased cardiovascular risk, might offer an earlier marker than the assessment for fibrotic tissue deposition, since they are expected to mainly manifest reactive fibrosis.

In addition to the change in strain distributions that can cause fibrosis, earlier alterations in the tissue on the micro-anatomical level could already be present prior to this. A technique that can assess the changes in myofiber orientation, defining the contractility of the heart, is cardiac diffusion tensor imaging (DTI) (Froeling et al. 2014). This technique has the potential to provide an early marker for cardiac remodeling but might also only show a change parallel to LVH (Mekkaoui et al. 2017). Nevertheless, DTI has already been evaluated in HCM and DCM, and showed compromised helix angel transmurality in both (Nguyen et al. 2016). It this point, it is only natural to evaluate this technique for its capability to determine early disease progression, which could provide a new early marker for remodeling onset.

An animal model with controllable cardiomyopathy progression would be best suitable for the evaluation of strain and DTI as early markers for cardiac remodeling. In the year 1990 an trachycardia-induced HF model has been developed and thoroughly described (Spinale 1996). Such model slowly induces DCM be forcing a continues increased heart rate by use of a pacemaker. Furthermore, the combination of such high heartrate model with a high fat diet would mimic the current western lifestyle even more accurately, which is expected to be related to the increase in prevalence of HF (Benjamin et al. 2017, Lam et al. 2011). Longitudinal follow up from the start of this increased pacing with both strain and DTI readouts in combination with T_1-mapping could answer the question of whether they can serve as early markers of irreversible fibrosis.

Eventually the conformation that T_1-values increase due to the presence of fibrosis, which takes place in parallel with cardiac remodeling, provides a quantitative measure for tissue remodeling. This can particularly be useful for comparison of other new emerging techniques that might provide an earlier readout that could advance cardiac cardiomyopathy diagnosis and eventually treatment.

Inflammation as early cardiac MRI tissue marker

Where fibrosis is known to occur after myocardial tissue is damaged, other quantitative mapping techniques, such as T_2- and T_2^*-mapping, might be able to serve as an earlier marker for tissue changes prior to fibrosis. The meta-analysis in Chapter 3 shows that T_2 indeed changes for myocardial infarction (MI) due to edema formation and that this also can be seen in cardiac transplants, MC, DCM and HCM. However, for these last two populations, the exclusion of LGE hypo-enhanced patients resulted in no significant T_2 increase anymore. The presence of LGE hypo-enhancement already indicates significant myocardial scarring, which can also be detected with T_1-mapping. Furthermore T_2^* decreases due to the infiltration of iron in the myocardium and also due to hemorrhage formation after a MI, which are both post-damage measures. However, for T_2^* only a few studies were performed in at-risk patients with HCM, DCM or HT and they all showed a decrease in T_2^*. While this decrease was not significant in either HCM nor DCM, the HT population did show significantly changed T_2^*-values in both HT with and without LVH (Chen et al. 2018). This single study gave a first indication that T_2^* could be sensitive to tissue alteration prior to the cardiac remodeling, but there is more research needed to confirm this.

It should be noted that this single study that evaluated T_2^*-mapping in HT patients used a BOLD sensitive MRI sequence (Chen et al. 2018). While they split the acquisition of multiple echoes for the T_2^* mapping over several breath-holds, they still see a significant decrease that also correlated with the extra cellular volume (ECV) calculated from post-contrast T_1-mapping and not with native T_1 itself. Their conclusion was that a decrease in oxygenation was seen in the HT patients, which decreased even further when LVH progresses. This statement was based on the fact that BOLD MRI detects changes in deoxygenated hemoglobin, which can be measured using either T_2 or T_2^* (Friedrich and Karamitsos 2013). Interestingly this decrease in T_2^* and therefore increase deoxygenated hemoglobin are not seen in HCM anymore (Chapter 3), which could mean that other tissue alterations are taking over at that point in the disease progression (Seferovic et al. 2019). Furthermore, the additional presence of ECV indicates tissue remodeling which apparently has not advanced into LVH yet and therefore lacks the tissue characteristics that are detectable with T_1 or T_2 (van den Boomen et al. 2018, Amano et al. 2017, Park et al. 2018). This makes assessment of ECV another potential early marker of cardiac remodeling, in addition to the BOLD based T_2^* assessment (Chen et al. 2018) presents.

A study including the evaluation of T_1, ECV and T_2^* changes in a disease progression model would be appropriate to understand how these values are linked together. A hypertension model that progresses into a hypertrophic disease needs gene editing and has only been done in small animal models (Camacho et al. 2016), which is known for having limitations in the translation to humans. Therefore, the same disease progression model of DCM as described in the previous section might be more applicable, since that approach enables a longitudinal follow-up with MRI scans. In addition to these MRI readouts a potential correlation of disease progression and blood inflammation markers such as monocytes or N-terminal pro–B-type natriuretic peptide, should be studied, since those factors are known to accelerate vascular risk (Haig et al. 2019).

In conclusion, inflammation could be used as a marker for both disease progression and recovery. T_2 and T_2^* both give independent information on the severeness of an inflammation but could also be sensitive to tissue oxygenation in addition to inflammation. With the search to an early marker for cardiovascular risk, tissue oxygenation changes might be a better characteristic to look at than inflammation. This is mainly because of the complexity of the inflammatory response in tissue remodeling, that is described in more detail in the next section, which makes it difficult to determine appropriate treatment even if it is possible to detect the presence of inflammation (Prabhu and Frangogiannis 2016).

Vascular endothelial function as early cardiac MRI marker

Heart failure knows various pathways within different cardiomyopathies (Seferovic et al. 2019), but one of the most excessively studied ones would be ischemia reperfusion (I/R) injury. The vascular occlusion initiating I/R provokes a cascade including cell death, inflammation, and fibrosis, which occur in two clearly defined phases described as the inflammatory phase followed by a reparative phase (Prabhu and Frangogiannis 2016). Since I/R models can be highly reproducible and the different components of the injury also occur in most NICM, I/R is a very popular first validation of any newly developed imaging technique. However, the fact that there are still no successful translations of therapeutic strategies to treat the imbalance of the cardiac repair phases after I/R, indicates a general lack of understanding the remodeling process (Prabhu and Frangogiannis 2016). Longitudinal follow-up research with non-invasive imaging techniques could offer new insights by detecting the onset of fibrosis, edema, and hemorrhage formation, with techniques such as the T_1-, T_2- and T_2^*-mapping that were discussed above. However, these approaches might

not offer sufficient details on the refined complexity of inflammatory and reparative processes (Prabhu and Frangogiannis 2016).

The vasculature plays a central role in both the inflammatory and reparative phase of myocardial tissue healing. Initially hypoxia is the main cause of vascular impairments, whether it is by complete vascular occlusion or by a prolonged reduction of oxygen supply to the tissue (Olivotto et al. 2006). As a result, the endothelial barrier of the vasculature becomes more permeable and leukocytes can infiltrate into the myocardial tissue. In case of perfusion recovery, an abrupt oxygenation of the tissue causes a burst release of oxygen related free radicals (Timmers et al. 2012). Furthermore, damaged tissue, either by necrosis or an increase in stress, also causes the release of danger-associated molecular patters (DAMPs), which induce an inflammatory response (Timmers et al. 2012) that are detectable by either T_2- or T_2^*-mapping (Chapter 3). Looking for an early marker for cardiac tissue remodeling the vascular endothelial cell dysfunction could probably serve as a predictor of such inflammatory response. Furthermore, the endothelial cells show a similar dysfunction when vascular sprouting occurs in the reparatove phase due to upregulations of vascular endothelial growth factor (VEGF) (Weis and Cheresh 2005). Only later in the reparative phase the new vessels mature due to the recruitment of smooth muscle cells and pericytes, which facilitates collagen deposition (Prabhu and Frangogiannis 2016) detectable by T_1-mapping (Chapter 2).

In the brain, DCE MRI is often used to determine (micro)vascular dysfunction by the assessment of the vascular volume and permeability (Kalpathy-Cramer et al. 2014), but in contrast with the cardiac vasculature, healthy brain vessels include a non-permeable blood-brain-barrier (BBB). This BBB prevents the regularly used low-molecular-weight intravascular contrast agents, such as gadolinium diethylenetriaminepentacetate (GdDTPA), from extravasating from healthy vasculature, while it will slowly leak from dysfunctional vasculature (Wang et al. 2006). Here, analysis of the signal intensity (SI) changes after injection of the contrast agent provides an initial peak change and the following slow change over the time are convertible into an fractional blood volume (fBV) and permeability x surface area product (PS). For a similar vascular fBV and PS analysis of cardiac vasculature a larger molecule contrast agent, such as albumin linked GdDTPA, is needed that remains intravascular in healthy cardiac capillary (Engel et al. 2019). Such blood pool agent can offer a valuable readout for vascular density and endothelial permeability (Vandoorne et al. 2016), which showed to be sensitive to the therapeutic effects of statins and a regenerative therapy on I/R (Leenders et al. 2018, van den Boomen et al. 2019a). Since both of these studies were animal studies and included a his-

tological confirmation of the DCE MRI results with a fluorescent labeled version of albumin, this technique offers a great opportunity for non-invasive longitudinal studies.

The major drawback for cardiac DCE MRI is that intravascular larger molecule contrast agents are generally not clinically permitted because of some associated risks (Gale et al. 2017). Especially *Gadofosveset* itself is currently not even produced, since clinical translation was determined to be unacceptable (European Medicines Agency 2017). Therefore, other contrast agents need to be investigated if translaton of the DCE based cardiac vascular volume and permeability detection is desired in humans. The new intra-vascular manganese contrast agent could be an appropriate alternative but will need some significant upscaling and clinically validation to enable production for humans (Gale et al. 2018). Nevertheless, in the near future this DCE based vascular volume and permeability technique could be translatable to humans for more detailed knowledge on vascular alteration in ischemic and potentially also non-ischemic cardiovascular diseases.

Oxygenation as early cardiac MR tissue marker

As described in the previous paragraphs, cardiac MRI offers several tools to determined tissue characteristics. However, most of these techniques only measure surrogate markers of ischemia, which misses the actual target of unbalanced myocardial oxygenation (Friedrich 2010). Fortunately, by making use of the difference between the paramagnetic properties of oxygenated and deoxygenated hemoglobin a BOLD change can be measured during an induced activity (Pauling and Coryell 1936, Ogawa et al. 1990). While several approaches, including T_2- and T_2^*-mapping and weighted imaging, have been explored for their cardiac applicability (Friedrich and Karamitsos 2013), the one broader explored cardiac BOLD technique is based on a T_2-prepared oxygen sensitive steady state free precession (SSFP) sequence (Fieno et al. 2004, Dharmakumar et al. 2005). This is because of its sensitivity to BOLD changes but probably also because of the easy implementation and dynamic capacity of the sequence. However, to be able to detect a BOLD respond differences between healthy and ischemic tissue this technique needs either an endogenous stressor or excessive hyperventilation followed by an maximum extended breath-hold (Arnold et al. 2012, Fischer et al. 2015, Fischer et al. 2018).

This thesis explores a radically new approach for BOLD imaging combining the ease and speed of an single breath-hold acquisition with the sensitivity of T_2- and

T_2^*-mapping into a dynamic heartbeat-to-heartbeat acquisition (van den Boomen et al. 2020) (Part III). Since a T_2^* SI based approach already showed a reduced BOLD response in HT patients (Beache et al. 2001) it is not surprising that both, the dynamic T_2- and T_2^*-maps, provided by the GESE-EPI sequence confirm this. However, the major advantage of the GESE-EPI approach is that it only needs one single breath-hold to identify this attenuated vascular response without involving any endogenous stressors. Furthermore, the extension of this technique to an acquisition of multiple simultaneous slices (Chapter 7) makes it diagnostically interesting to identify areas with reduced oxygenation with only limited effect on the MRI workflow (Friedrich 2020). Nevertheless, even though there are indications that tissue oxygenation spatially varies in the myocardium, further research in MI models should determine the ability of gradient-echo spin-echo echo-planar-imaging (GESE-EPI) based BOLD to the assessment of tissue viability.

Again an animal model with an I/R injury would be suitable for the validation of the sensitive of the GESE-EPI BOLD sequence to injury and tissue viability, since three interesting altered tissue types will be present. These tissue types include the infarcted core, damage by the coronary occlusion and containing necrotic cells, the infarct periphery, inflamed due to inflammatory markers but without necrotic tissue, and the remote area, often opposite to the infarcted area which needs to compensate for the new load distribution (Prabhu and Frangogiannis 2016). The reperfusion could reestablish the regular blood flow in the infarcted myocardium, but changes in the vasculature have already reduced the vasodynamics, as described in the previous sections. Any change in the vasculature's ability to vasodilate or vasoconstrict could influence the BOLD readouts over the time of a breath-hold, and can be used to determine the presence of tissue alterations (van den Boomen et al. 2020).

While identification of the different tissue types and their viability would be highly valuable, solely the ability to detect MI affected tissue using GESE-EPI BOLD without the need of a contrast agent would already have a substantial effect on clinical cardiac MRI (Friedrich 2020, Friedrich 2010). To determine whether the detection of MI would be feasible for GESE-EPI BOLD first a comparison with other MR based readouts should be performed for validation. Tissue that indicates a reduced GESE-EPI BOLD responsiveness to a breath-hold perturbation should be compared to golden standard LGE readouts, and also to T_1-, T_2- and T_2^*-mapping to determine BOLD correlations with fibrosis, edema and hemorrhage formation. Furthermore, aortic and pulmonary flow assessment with phase contrast MRI could be performed to give an indication of potentially compromised flow due to the breath-holding (Sakuma et al. 2001). Additionally, another oxygen sensitive sequence could be in-

cluded in such evaluation, such as T_2prep-SSFP, which has already proven its ability to detect coronary artery disease (CAD) under supervision of well controlled hyperventilation and breath-hold maneuvers (Fischer et al. 2015, Fischer et al. 2016, Fischer et al. 2018).

Since this GESE-EPI BOLD imaging method was inspired by brain BOLD imaging, a closer look at those imaging approaches could help provide insights in the mechanisms behind the cardiac BOLD responses. For example the repetitive task schemes that exist of alternating active and resting states provides intrasubject statistics for brain BOLD analysis (Aguirre and D'Esposito 1999). Cardiac BOLD imaging could benefit from such approaches by potentially improving its sensitivity to smaller alterations. Furthermore, quickly following repetitions of the active events, in the case of cardiac BOLD the breath-hold perturbation, could also result in the saturation of the BOLD response. A first indication of this cardiac BOLD saturation has already been detected in some preliminary testing in animals with controlled breath-holds, which could provide insights on the oxygenation recovery time (van den Boomen, unpublished data). Lastly, reversing of the breath-hold perturbation by performing hyperventilation could provide an additional readout by increasing the O_2 saturation in the blood. Previously it has been shown that hyperventilation can induce a detectable SI change when using the T_2prep-SSFP BOLD approach (Fischer et al. 2015). Combining these different triggers for BOLD assessement could hypothetically provide additional information on the vascular responsiveness, either by looking at the minimal recovery time, the maximum oxygen enhancement, or the vasodilating response to oxygen reduction.

Other modalities could also be used to validate this newly developed cardiac GESE-EPI BOLD approach. For example, in a previous study computed tomography (CT) angiography was used to detect the location of an occlusion prior to a BOLD MR scan (Fischer et al. 2018), but simultaneous evaluation would be preferred. Fortunately, hybrid positron emission tomography (PET)-MR could offer such simultaneous readout of both the uptake or distribution of a PET-tracer and a BOLD response. (Yang et al. 2017) already validated the vasodilating effectiveness of $PaCO_2$ by using PET-MR and showed from ^{13}N-ammonia PET scans that the myocardial blood flow increased similarly to inducing stress with adenosine, while the myocardial perfusion response remained the same. However, to evaluate potential tissue oxygenation with cardiac BOLD MRI the use of ^{15}O-gas PET would be more appropriate (Yamamoto et al. 1996, Iida et al. 1996). The uptake of ^{15}O-gas via the lungs and its distribution afterwards throughout the body into the cells that consume the oxygen can provide a direct validation of the sensitivity of

GESE-EPI BOLD to tissue oxygenation or vasoreactivity. The major challenge using this PET technique for such oxygenation comparison would be that the additional detection of the remaining vascular percentage of the tracer and the change in blood flow using ^{15}O-water and carbon-^{15}O-gas are needed (Yamamoto et al. 1996, Iida et al. 1996), of which the later one remains trapped in the red blood cells. In addition to the use of multiple tracers, the half-life of 2.06 minutes of ^{15}O either in the form of water, oxygen- or carbon monoxigen-gas makes such PET based evaluation study highly challenging but probably the only direct method to evaluate the MRI based BOLD.

Another multimodal way to determine if the cardiac BOLD based changes correspond with tissue alterations is by indirectly comparing oxygenation with metabolic changes. The latter can be influenced in a MI reperfusion model by either inflammatory cells, that upregulate local myocardial metabolism, or by cell death, that downregulates local myocardial metabolism (Anselm et al. 2011). Regular ^{18}F-FDG with a half-life of 109.8min is seen as an accurate indicator of glucose uptake (Quail and Sinusas 2017). However, this glucose-based tracer is non-specific and can indicate a decrease in cell viability or an increase in infectious or inflammatory processes. The detection of inflammatory tissue with ^{18}F-FDG can be improved by fasting in combination with a heparin injection prior to administration of ^{18}F-FDG. This increases the free fatty acids in the blood and suppresses the physiological ^{18}F-FDG consumption in normal myocardium, but it will remain increased in inflamed myocardium (Nensa et al. 2015, Nensa et al. 2017). Eventually this ^{18}F-FDG PET approach might not offer the direct validation of cardiac BOLD, but it can definitely help with the interpretation of BOLD changes due to MI or I/R injury.

An more direct approach to validate the cardiac MR BOLD results with PET approach would be to determine the presence of hypoxia with the ^{18}F-fluoromisonidazole (MISO) tracer. This PET based validation approach relies on the accumulation of ^{18}F-MISO in hypoxic cells that contain low oxygen levels compared to healthy myocytes (Davidson et al. 2018). However, the uptake period of this tracer is long, the clearance from the blood circulation is low and on top of that the total accumulation is only small. Also, ^{18}F-MISO only enables detection of hypoxic tissue with reduced blood flow but does not show necrotic with normal perfused myocardium (Martin et al. 1992). Lastly, since ^{18}F-MISO based PET imaging has mainly been evaluated in cancer related studies and is has not widely been investigated for cardiac applicability yet (Davidson et al. 2018), using it as validation of a new cardiac MR based BOLD imaging technique might not be the first choice. The other, potentially more indirect, measures described in the previous paragraphs

can provide more reliable readouts and therefore more accurate interpretation of the cardiac MR BOLD readout.

In conclusion, the GESE-EPI BOLD imaging approach could offer clinically highly valuable readouts but needs further evaluation. This is particularly needed to determine the clinical applicability of the technique but in parallel also useful to expand general knowledge on cardiovascular mechanisms involved in the development and recovery of cardiovascular diseases.

Vessel architecture as early cardiac MR tissue marker

From the previous paragraphs it already becomes clear that the myocardial vasculature plays an important role in cardiovascular disease progression and recovery. While the vascular condition and responsiveness could be assessed with DCE and cardiac BOLD imaging, they do not provide insights in the underlaying vascular structure. Since cardiac VAI has now shown to be able to identify different vascular structures in the healthy heart (Chapter 8), new opportunities emerge for the assessment of microvascular dysfunction.

One of the opportunities for VAI lays in the longitudinal follow-up of populations with increased cardiovascular risk. Since tissue oxygenation impairments might form the base of cardiac fibrosis (Olivotto et al. 2006, Galati et al. 2016) and the evaluation of GESE-EPI BOLD in HT patients already showed changes in the vascular responsiveness (van den Boomen et al. 2020, Beache et al. 2001), alteration in the vascular architecture could either be the initiator or the result of the tissue remodeling. Changes in either vascular volume, caliber or density could be different in conditions such as HT, DM or obesity and might eventually have a unique role in progression of cardiovascular disease (Camici et al. 2015). However, understanding the role of vascular architecture in cardiovascular disease progression is currently limited to the effect of arterial pressure and flow on tissue remodeling (Mayet 2003). This knowledge could be further extended with the use of VAI imaging in longitudinal follow-up studies, in addition to the other cardiac MRI tissue characterization techniques that have shown to be valuable in detection of tissue remodeling (Messroghli et al. 2017).

Even thought the cardiovascular risk populations would be the primary group of interest to evaluate VAI, studies including cardiovascular animal models of these risk factors are also recommended. This is particularly important because additional histology assessments can provide information on the accuracy and coherency of

VAI with expressed vascular alterations (Farrar et al. 2010). Different cardiovascular models could be used, as already described previously in this chapter. Furthermore, aside from histological evaluation other techniques and imaging modalities could be used to determine their correlation with the VAI based vascular structure. Some interesting approaches for such evaluations are described in the following paragraphs.

MRI based validation of the VAI based vascular structure and indices could be done with regular first pass perfusion in rest and stress, which provides a readout for the fractional flow reserve used as indicator for cardiovascular disease progression (Nagel et al. 2019, Raman et al. 2019). Furthermore the use of aortic and pulmonary flow assessment with phase contrast MRI could be correlated with VAI to give an indication of a possible increased or compromised blood flow alongside the changes in vascular structure. Lastly, a comparison with the GESE-EPI or T_2prep-SSFP based BOLD response acquisitions should be used to determine the effect of the vascular structure on its responsiveness (Fischer et al. 2018).

Unfortunately, the assessment of VAI also requires the use of a contrast agent, which raising the same concerns as described for the DCE imaging techniques (Gale et al. 2017). However, the use of an animal model could help to determine the optimal contrast agent for the technique before translating it to human clinical practice. Such ideal contrast agent should provide a maximum change in R_2 and R_2^* relaxation and preferable remains intravascular to enable the extraction of a full VAI vortex, as seen in the brain (Emblem et al. 2013). Potential contrast agents that could be evaluated are ultrasmall superparamagnetic iron oxide (USPIO), which provides a greater T_2 and T_2^* change (Yilmaz et al. 2013), GdDTPA labeled albumin, which is known to remain intravascular (Vandoorne et al. 2010), or Manganese, which discharges the potential concerns on contrast agent toxicity and retention (Gale et al. 2018). Furthermore, the development of other potential contrast agents should be closely followed and evaluated for their potential applicability for VAI.

In the case of evaluation of VAI with a multimodal approach such as simultaneous PET-MR several perfusion radiotracers would be available. An advantage of such simultaneous approach is the possibility to assess flow, perfusion and VAI readouts within the same breath-hold. Examples of the most widely used PET tracers for perfusion are ^{82}Rb (half-life 1.27min) and $^{13}NH_3$ (half life 9.96min) (Nakazato et al. 2013). However, theoretically ^{15}O-water (half-life 2.06min) is ideal for quantitative flow measurements by PET (Nakazato et al. 2013) but the noisy low counts and short half-life result to this tracer being a less routinely accessible technique. Furthermore, it is also common practice to perform a rest and stress acquisition

with these PET tracers, were CO_2 can potentially also be used as indigenous stressor (Yang et al. 2017). Nevertheless, quantitative interpretation of PET perfusion imaging could help determine the influence of flow and blood volume changes on the VAI readouts, which eventually might push the need of a free-breathing version of VAI.

Lastly, aside from the already performed 2D histological assessment of endothelial and smooth muscle cells, a 3D structural assessment would be a more appropriate way to validate the vascular readouts of VAI. Such 3D assessment could for example be performed using MRI, which requires the injection of an intravascular tracer prior to Euthanasia and organ harvesting. The ex vivo heart could afterwards be scanned with a (ultra-high field) MR system providing a stack of 2D images of the macro and even microvascular structure (Rasmussen et al. 2010). In addition, the use of vascular tracking would enable 3D assessment of the vasculature, which has previously already been performed in the brain (Bernier et al. 2019). Aside from using MRI, another histological approach termed tissue CLARITY has been developed, which allows imaging of molecular phenotyping in intact tissues (Chung et al. 2013). This technique has already been shown to enable assessment of the 3D cardiac microstructure for validation with for example DTI MRI (Lee et al. 2018). However, immunofluorescence staining of the endothelial and smooth muscle cells could be added to this 3D structure (Chung et al. 2013, Pratumvinit et al. 2013), which could enable the assessment of the 3D micro- and microvasculature. Both of these 3D imaging approaches would provide a more accurate correlation of the MR VAI readouts with the present vasculature than the currently used 2D based histology techniques.

In conclusion, the GESE-EPI VAI imaging technique will need excessive validation by histology and other imaging techniques, but holds great potential for eventual translation to clinical applications. Where populations with increase cardiovascular risk might be the first group of interest for evaluation, simple tissue viability assessment could also improve clinical decision making on treatment in for example MI and ischemic reperfusion (IR) injury. However, for now VAI should only be seen as an experimental approach that could provide more insight is cardiovascular disease progression.

Conclusion

Where the T_1, T_2 and T_2^* mapping techniques for fibrosis, edema and hemorrhage identification are already pushing into clinical applications, there is still a lot unknown about the mechanisms behind those targeted tissue alterations. Insights in these mechanisms will eventually improve the diagnostic value of these T_1, T_2 and T_2^*. However, as early markers for cardiac remodeling they all seem to be lacking, but fortunately other techniques are emerging to potentially provide insights into the early stages of cardiovascular risk. Initially cardiac blood oxygenation level dependent (BOLD) imaging should be studied for its potential replacement of the use of late gadolinium enhancement (LGE) to determine tissue viability but in addition to this the vessel architectural imaging (VAI) might provide more tissue characteristically readouts. While applicability in humans is already shown for both of these techniques, evaluation in cardiovascular disease models is still needed. Especially since interpretation of detected changes in their readouts still have to be established. Nevertheless, each described tissue characterization technique has their diagnostic potential in different phases of cardiac remodeling and an optimal combination could provide future improvements for both diagnosis and treatment.