# Biomarkers in Duchenne and Becker muscular dystrophy

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# Summary, discussion and future perspectives

# Summary

The overall aim of this thesis was to improve and facilitate trial design for Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) by studying different types of biomarkers. To this end, the national registry for dystrophinopathy patients in the Netherlands was restructured to better support recruitment of investigator-initiated studies and pharmaceutical trials, and enable post-marketing surveillance. Next, three types of biomarkers (physiological, cardiac ultrasound and muscle MRI) and a biomarker group (circulating) were investigated for different types of context in DMD and BMD.

In **chapter one** the concept and new structure of the third version of the Dutch Dystrophinopathy Database (DDD), the national registry in the Netherlands, is described. This third version was created to serve as a platform to study epidemiology, liaise directly between clinical investigators and eligible study candidates, assess the feasibility to recruit for specific clinical trials, perform post-market surveillance, and collaborate in (inter)national data sharing initiatives. To achieve this, different levels of participation were created, patient and clinician reported data were included, and changes were made to comply with regulatory requirements on data handling, to optimize transparency in use of data, and to provide regular feedback to participants. Five registration levels were created to make registration as accessible as possible. The only obligatory level was to provide name and contact details and the clinical diagnosis. Other registration levels included the response to a yearly questionnaire covering disease milestones and medication, the storage of clinical data acquired at one of the involved academic centres and finally, the consent to exchange individual coded data with non-commercial and commercial partners. A system-independent information level was used in order to ensure interoperability. A total of 742 patients were included between February 2020 and November 2023. Among these were 366 re-registered patients and 75 non-responders from the previous versions of the DDD, 116 newly registered patients, and 185 deceased patients. A total of 524 patients had been diagnosed with DMD, 174 with BMD and 44 participants registered as female carriers. Only the 482 actively registered patients (366 + 116 patients) consented to different levels of the registry. 451 patients (93.6%) provided consent for yearly questionnaires. 227 (47.1%) patients had their regular outpatient visits at Radboudumc or LUMC. Among these, 218 (96.0%) consented to store clinical data. A total of 432 (89.6%) and 296 (61.4%) provided consent for data sharing with non-commercial and commercial partners respectively. Between the renewal of the registry in February 2020 and November 2023, it was used to inform participants about nine pharmaceutical trials and five investigator-initiated studies, to collect and report standardized clinical assessments for the conditional reimbursement of ataluren in the Netherlands, and to conduct seven feasibility assessments through TREAT-NMD. The description of the

reconstruction of the DDD thus provides an example of how trial readiness, post-marketing surveillance, effective use and interoperability of data can be achieved with minimal burden for both patient and healthcare providers.

Increased blood pressure and body mass index (BMI) as possible modifiable physiological characteristics for progression of left ventricular (LV) dysfunction were studied retrospectively in 273 visits of 65 DMD patients and are described in **chapter two**. We showed that mean systolic blood pressure (SBP) z-score was significantly increased in age groups <14 years. Z-SBP increased with higher BMI and decreased with use of cardiac medication over time. In a subset of younger patients, reduced LV global longitudinal strain (GLS) was associated with higher BMI cross-sectionally. Thus, BMI was the only independent determinant in this study, related to both increased SBP in the whole cohort, and to decreased GLS in the DMD patients <11 years. Prospective studies are needed to determine the ability of BMI and SBP to function as monitoring biomarkers for progression of cardiomyopathy in DMD.

**Chapter three** assesses the value of LV GLS as a potentially more accurate echocardiographic parameter than LV ejection fraction (EF) to detect and monitor LV dysfunction in BMD. For this purpose, we measured 40 adult BMD patients at baseline and 29 patients after two years. In seven BMD patients LV GLS was reduced while LVEF was within normal limits at baseline. Furthermore, LV GLS worsened significantly (-1.3±0.8%, p=0.002, SRM=0.70, annually=0.60%) while LVEF remained stable. LV GLS could thus be considered as a more sensitive biomarker compared to LVEF to identify and monitor LV dysfunction in regular clinical assessments and clinical trials.

**Chapter four** covers changes of circulating biomarkers creatine kinase (CK), creatine/creatinine$_{ratio}$ and myostatin over 4 years-time and their associations with disease severity and disease progression in 34 adult BMD patients with a total of 123 visits. Both creatine/creatinine$_{ratio}$ and myostatin showed a high intraclass correlation coefficient, indicating that these are strongly patient-specific, and highly heterogeneous across patients. These biomarkers showed no correlation with age. CK was less patient specific and declined with increasing age (adjusted p = 0.0002). In ambulant patients, the North Star Ambulatory assessment (NSAA), ten meter run velocity (TMRv) and the 6-minute walking test (6MWT) showed strong negative correlations with the creatine/creatinine$_{ratio}$ (rho <-0.80) and strong positive correlations with myostatin (rho > 0.79), while these tests did not correlate with CK levels. Creatine/creatinine$_{ratio}$ and myostatin correlated moderately with the average yearly change of the 6MWT (rho = -0.53 and 0.56 respectively). The explained variation of concurrent functional performance by these biomarkers together with age was up to 75%. Creatine/creatinine$_{ratio}$, and myostatin may be used as monitoring biomarkers in BMD as higher

creatine/creatinine$_{ratio}$ and lower myostatin were associated with patients' performance after correction for age, and they improved the prediction of concurrent functional performance when combined with age. However, both of these biomarkers and the functional scales only showed small changes over time. This currently limits the possibility to more closely define the context of use.

Finally, in **chapter five** the selection of an optimal biomarker using quantitative MRI of the thigh and lower extremity and functional measures in BMD are described. We included 24 adult patients at baseline and 20 of these patients had a follow-up visit after a mean of 1.95±0.23 years. We developed a stepwise selection approach analyzing standardized response means (SRMs), correlation to baseline function and interobserver variability. We examined a total of 71 potential MRI parameters, i.e. fat fraction (FF) of three center slices and of the whole muscle, and contractile cross-sectional area of the center slice for a total of 19 individual muscles and 6 muscle groups. Whole muscle FF increased significantly over 24 months in the thigh (median +1.9%, range -0.7–5.4, $p$=0.01) and lower leg (median +0.7%, range -1.0–6.4, $p$=0.02). Moreover, median FF of all individual muscles increased over 24 months. Eight FF parameters of the thigh were selected as potential biomarker for disease progression based on standardized response mean (≥0.8) and correlation with three ambulatory tests (NSAA, TMRv and 6MWT, ρ≤0.8). We considered whole thigh three center slices FF as the best biomarker in our cohort because of small interobserver differences and the advantages of imaging only a small part of the muscle. This biomarker potentially lowered the sample size by 39 patients (40% reduction) compared to the NSAA in a clinical trial with an intervention with an assumed reduction of disease progression by 50% over 24 months.

# General discussion

There is currently no cure for Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD). Disease modifying options for these diseases include treatment with glucocorticoids, while disease supporting therapies include physiotherapy, respiratory support, and proactive management of reduced cardiac function. Development of new drugs has moved at high speed in the past years. In a small subset of DMD patients, and also depending on the continent they are living, other disease modifying drugs may be available (e.g. antisense oligonucleotides eteplirsen, godolirsen, vitolarsen and a gene therapy called delandistrogene moxeparvovec which are currently only available in the United States, and ataluren which is conditionally approved by EMA)(48). Very recently, histone deacetylase inhibitor givinostat, received FDA approval for DMD patients aged 6

years and older. For BMD, there are no disease modifying drugs that received conditional or accelerated approval by the EMA / FDA respectively and givinostat failed to reach the primary endpoint in a clinical trial(61). There are, however, more drugs being investigated like vamorolone and sevasemten (clinical trial identifiers NCT05166109, NCT05160415 and NCT05291091).

Alongside development of new drugs, clinical trial design and conduction is still highly complicated due to the rarity of the disorders, slow disease progression and disease heterogeneity. To this end, well-designed national patient registries are needed to aid patient inclusion into clinical trials and natural history studies, as well as robust biomarkers to, for example, measure disease progression and therapy response. As outlined in the introduction, there are seven categories of biomarkers according to the U.S. Food and Drug Administration (FDA) its definitions; diagnostic, monitoring, response, predictive, prognostic, safety and susceptibility(78). When investigating or using one of these types of biomarkers, it is essential to describe its context of use. This includes two components namely its category (see above) and intended use. An example of the latter includes the absence of dystrophin expression to confirm DMD (diagnostic biomarker). Monitoring biomarkers are assessed serially over time and may provide evidence of effect of an intervention by focusing on change of a value over time. When biomarkers are not assessed sequentially, they could for example function as prognostic biomarkers as these indicate an increased or decreased likelihood of a future event (such as disease progression). Based on the results of this thesis, three promising biomarkers and a biomarker group will be evaluated for which category they belong to, what their context of use could be and the possible benefits compared to currently available biomarkers for trial design and conduction.

**National registries**

In the past decade, insight in the importance of national registries grew rapidly. National registries for DMD and BMD exist now in many countries. Data from national registries on other diseases such as rheumatoid arthritis and breast cancer have already been used to identify new biomarkers(272, 273). For DMD and BMD however, extensive international collaboration and long follow-up is required due to the rarity and heterogeneity of the disorders. To this end, the Dutch Dystrophinopathy Database (DDD) was designed in such a way that threshold for registration is low, and that synchronization with other international dystrophinopathy registries is facilitated. Item selection was based on the expanded dataset of TREAT-NMD(134). Furthermore, reduced patient burden was achieved by using data from the outpatient visits from regular clinical care. Since the implementation of the updated version of the DDD in February 2020 (chapter one), it has been used for several TREAT-NMD enquiries. In addition, various requests from researchers within Duchenne Center

Netherlands (DCN) have been approved to use coded data from the DDD for quality, epidemiology and natural history studies. An example of how registries can be used to aid in drug development is ataluren in DMD patients with nonsense mutations. Strategic Targeting of Registries and International Database of Excellence (STRIDE) is an international, multicenter registry of real-world ataluren use in DMD patients with a nonsense mutation. In a 2022 interim analysis, it was shown that standard of care treatment plus ataluren use delayed loss of ambulation by 4 years compared to standard of care treatment alone(274). The EMA granted conditional market authorization for ataluren. Since 2020, the DDD registry has been used for the conditional reimbursement program of ataluren by the National Health Care Institute in the Netherlands. Based on recent results, the European Medicines Agency (EMA) has repeatedly recommended a non-renewal of the authorization of the drug, although as of September 2024, a final decision by the European Commission is still pending(52). Registries such as the DDD may also be used to select eligible patients for the first gene therapy in the near future, for example for delandistrogene moxeparvovec (Elevidys ®) as the FDA recently granted approval for pediatric patients aged 4 years and older, although use in non-ambulatory people was approved under accelerated approval (275). The DDD demonstrates the enormous efforts that have to be taken to create, maintain and develop a sustainable registry, as well as the hurdles that still exist. For example, the DDD currently does not capture a large number of older DMD patients who do not visit the DCN nor does it include patient-reported outcome measures.

**Biomarkers**

The selection of a biomarker to use for clinical trial design and/or conduction depends highly on the goal of the study, mechanism of action of the drug, and the targeted population amongst others. In addition, as described in the introduction, loss of dystrophin in DMD and BMD leads to membrane fragility in both skeletal myocytes and cardiomyocytes. As these are different tissues, the three biomarkers and biomarker group that were studied in this thesis will be divided in two main groups; biomarkers for cardiac and skeletal muscle tissue.

*Biomarkers for cardiac function*

There are different methods to assess left ventricular (LV) function. The most commonly used method in clinical routine is measurement of ejection fraction (EF) with conventional echocardiography. Another method that can be used is LV global longitudinal strain (GLS) measured by more advanced echocardiographic techniques like speckle tracking echocardiography (STE). A third method is measurement of LV function by cardiac magnetic resonance imaging (CMR). Using CMR, both LVEF and LV GLS can be quantified. However, CMR is not easily accessible, may be hard to analyse by unexperienced interpreters, has a

long scanning duration, and is expensive to use. In our data, LV GLS, measured by STE, was more sensitive than LVEF to detect LV dysfunction in BMD (chapter three) which is in line with several studies in other neuromuscular diseases like DMD, limb-girdle muscular dystrophy type 2B and myotonic dystrophy(90, 276-278). As already shown in DMD, a recent retrospective study in a large cohort of 183 BMD patients indicated that timely treatment with ACEi improved long-term cardiac outcome(207, 279, 280). Early detection of LV dysfunction with GLS could therefore have important implications for the timing of ACEi treatment. Although CMR is generally considered as the gold standard to measure LV function, the incremental value of CMR over (advanced) echocardiography is modest when the acoustic window is good(281). Worsening of the acoustic window can be due to for example obesity or scoliosis. In chapter two, we showed with serial measurements that the physiological characteristics increased body mass index (BMI) elevated systolic blood pressure (SBP) were often present in (pediatric) DMD patients. Here, we also showed that reduced GLS was associated with higher BMI in a subset of younger patients cross-sectionally (less than 11 years). A retrospective study by Houwen et al. showed that the transition to the non-ambulatory phase may be crucial in the development of excessive weight gain in DMD patients(83). This transition usually takes place around the age of 10-12 years. The first signs of echocardiographic LV dysfunction in DMD usually start in the early second decade of life. On the contrary, preliminary data from a natural history study in adult BMD patients at our institution showed no increase in BMI (unpublished data) and there is no known relation between age and onset of LV dysfunction. Thus, LV GLS measured by advanced echocardiography may be used as diagnostic biomarker for LV dysfunction in BMD (chapter two) and DMD when the acoustic window is still expected to be good.

Our data from chapter three shows that LV GLS may be suitable as monitoring biomarker in BMD, as LV GLS declined over two years while LVEF remained stable. The predicted sample size of 133 patients per study arm for LV GLS is smaller than for LVEF, which demonstrates the beneficial sensitivity to change of LV GLS. Abundant evidence from other studies showed that LV GLS is a more powerful predictor of cardiovascular events (e.g. death, heart failure development or need for implementation of a cardiac device) compared with LVEF in patients with heart failure, valvular heart diseases and different cardiomyopathies(282, 283). Using CMR, prognostic value for cardiac events has been shown for myocardial fibrosis, detected by LGE, in several neuromuscular diseases(284). While LGE has been suggested to correlate to the extent of fibrosis in DMD, prognostic value of LV GLS measured by echocardiography itself has not been shown in neuromuscular diseases(285). Next to the disadvantages of CMR mentioned earlier, other challenges may include the burden for patients, especially if non-ambulant, and specific contra-indications like implantable cardiac devices(130). Thus, when the prognostic value has been shown, LV GLS seems suitable as monitoring biomarker

in BMD and young DMD patients, while close monitoring of LV function in older, more severely affected, DMD patients is challenging.

We provide first evidence that increased values of BMI and SBP could function as prognostic biomarkers for cardiac disease progression in DMD (chapter two). The relation of increased values of these physiological characteristics and worsening of cardiovascular disease in non-DMD children and adults is already well known(286). In a retrospective study of 324 DMD patients with a median age of 14.9 (IQR 12.3 – 17.9), it was demonstrated that whole body fat mass indexed to height was independently associated with cardiac dysfunction in a multivariable analysis that was corrected for age and corticosteroid use amongst others(287). One important limitation of our study was the analysis with retrospective, single blood pressure measurements. To validate our results from chapter two, the blood pressure measurement protocol in our institution was improved meaning that measurements were performed repeatedly in DMD patients on the same day from 2017 onwards. Preliminary analysis of these results confirm that mean Z-SBP is elevated in younger DMD patients compared to the healthy reference population, although z-numbers seemed overall a bit lower compared to the results from chapter two. Furthermore, longitudinal SBP increased with higher BMI. Definite implementation of increased BMI and SBP as prognostic biomarkers in DMD requires demonstration of prognostic value over time (e.g. a relation with worsening of LV function) and also analytical and clinical validation (e.g. reproducibility in other cohorts). After this, BMI and SBP could then for example be used to stratify patients in clinical trials that are of higher risk for progression of cardiac dysfunction, or not.

*Biomarkers for skeletal muscle tissue*

We studied two types of biomarkers that reflect muscle mass in this thesis. The first are circulating biomarkers. Circulating biomarkers may provide a representation of the overall condition of patients.

Research into candidate protein circulating biomarkers in BMD has been limited while the list of potential biomarkers in DMD is quite extensive as recently outlined in a review by Al-Khalili Szigyarto(82, 96, 215, 218, 233, 288). We showed that creatine/creatinine$_{ratio}$ and myostatin highly correlated with disease severity but not with progression (chapter four) after correction for age in BMD, in agreement with studies in DMD(96, 98). This could well be due to a power issue as disease progression in BMD is slow and heterogeneous. The relation between disease progression and creatine/creatinine$_{ratio}$ and myostatin should be clarified in order for these biomarkers to be used as monitoring biomarkers in clinical trials. Whenever demonstrated, it is still unlikely that solely creatine/creatinine$_{ratio}$ and myostatin would be used to monitor disease progression in clinical trials with BMD as with the current available knowledge, large numbers of BMD patients, highly homogeneous groups, long-

lasting clinical trials or highly efficacious treatments would be needed. However, patients could be stratified in more homogeneous groups based on these biomarker levels instead of stratification based on functional measures as has been used a recent clinical trial with givinostat in BMD that unfortunately failed to reach its primary endpoint (NCT03238235). This would require the ability to define discrete biomarker values for the grouping of patients and associated prognostic ability.

A combination of creatine/creatinine$_{ratio}$ and myostatin did improve prediction accuracy of concurrent functional performance in our study. This indicates that these biomarkers may be used as prognostic biomarkers, although for this it is essential to show an increased or decreased likelihood of a future event (e.g. disease progression). Other circulating biomarkers have already been used for this purpose in other neuromuscular diseases, such as in amyotrophic lateral sclerosis (ALS) and Charcot-Marie-Tooth disease type 1A (CMT1A) (289, 290). In one study with ALS, use of a single biomarker from plasma or cerebral spine fluid resulted in goodness-of-fit R2 ranging between 0.0270 and 0.4237 for prediction of ALS diseases progression while biomarkers combined achieved R2 values of 0.769, 0.617, and 0.962(289). Thus, the currently available data on creatine/creatinine$_{ratio}$ and myostatin in BMD limits their sole utility as biomarkers in clinical trials for any purpose. To clarify their potential as prognostic and/or monitoring biomarkers, a longer follow-up, increasing the sample size and/or focusing on patients with a more similar rate of functional decline are needed.

In conclusion, although the data in this thesis on creatine/creatinine$_{ratio}$ and myostatin is promising, it is not enough to more closely define the context of use for these biomarkers. It would be interesting to include creatine/creatinine$_{ratio}$ and myostatin in trials (for example as secondary endpoints) to collect more longitudinal data.

The second type of biomarker for skeletal muscle tissue studied in this thesis is muscle fat fraction (FF) measured by quantitative MRI (qMRI) in BMD patients. As described in the introduction, skeletal muscle tissue is gradually replaced with fibrofatty tissue as disease progresses in both DMD and BMD amongst others. In contrary to circulating biomarkers, qMRI FF has the ability to study specific muscles. Extensive studies have investigated the relation between FF of several muscles and/or muscle groups of the lower extremity and tests of muscle function in neuromuscular diseases such as DMD, BMD several of the LGMDs, and FSHD(113, 254, 256, 259, 291, 292). In addition, the ability of qMRI FF to measure disease progression has been demonstrated in DMD and various neuromuscular disorders other than BMD(103, 104, 197, 259, 261). However, most of the studies did not have standardized methods to determine which muscle and/or muscle group is the best to use to monitor disease progression. Chapter five added to the existing literature by showing that qMRI can also detect disease progression in BMD. We included single muscles and

muscle groups of the lower extremity as well as functional measures in a flowchart and assessed them for sensitivity to disease progression, correlation to functional measurements at baseline and inter observer variability. The sensitivity to disease progression, as assessed by the standardized response mean (SRM), was in general highest for muscle groups followed by single muscles and functional outcome measures. FF of whole thigh three center slices was the optimal biomarker in our cohort of 24 BMD patients. This biomarker potentially lowered the sample size of a clinical trial over 24 months by 39 patients compared to the North Star Ambulatory Assessment, which is equivalent to a reduction of approximately 40%. A recent study also described higher sensitivity to change for FF measures of whole muscle groups compared to individual muscles in several other neuromuscular diseases(293). In DMD, FF of the vastus lateralis is currently often used to monitor disease progression, although at the time of writing this thesis no extensive studies exist that compare single muscles and muscle groups to monitor disease progression(104, 110, 294, 295).
Thus, qMRI FF may be used as monitoring biomarker to detect treatment effects using smaller sample sizes than functional measures in BMD. The flowchart we designed may help in selecting the optimal biomarker to monitor disease progression in neuromuscular diseases. Although definite implementation requires validation in an external cohort, FF at baseline has been included as monitoring biomarker in several clinical trials with DMD and BMD (e.g. NCT02851797 and NCT03238235) as secondary endpoints. A drawback of FF as monitoring biomarker is that fat replacement of muscle tissue is considered irreversible, e.g. this muscle tissue is lost. Most therapies that are currently under development aim to slow disease progression rather than reverse pathology. Therefore, evaluating the rate at which fat replacement of muscles takes place, could be a potential monitoring biomarker for disease progression but only if the tested drug is expected to have an effect on this measure within the measured window. Thus, depending on the drugs mechanism of action, other pharmacodynamic biomarkers may be necessary to show biological response on a short time window.

## Future perspectives

The ultimate goal to optimize trial design and facilitate conduction would be to discover response biomarkers that could serve as surrogate endpoint. As per definition, a biomarker may only qualify as surrogate endpoint when it is shown to predict a clinical meaningful effect. As such, it is not enough to show that biomarker levels differ between patients and controls, but information is necessary on how the biomarker levels correlate with future functional changes and/or clinical endpoints.
The number of cardiac specific clinical trials in DMD and BMD is limited. With the

implementation of new therapies aiming to restore or support skeletal muscle function, the impact of cardiac disease on (long-term) outcome has become more evident. As a result, the interest in assessing cardiac function is growing. While clinical trials investigating cardiac drugs in non-neuromuscular patients have focused on endpoints such as development as heart failure or mortality, this approach seems not useful in dystrophinopathy patients as (cardiac) disease progression may be too slow for the typical clinical trial duration of one or two years. Also, skeletal muscle weakness (in DMD) often prevents overt clinical symptoms of heart failure and mortality is multifactorial (e.g. respiratory and cardiac weakness). In addition, the pathophysiology in DMD and BMD is clearly different from most other patients with primary heart failure. Therefore, other cardiac endpoints are necessary. In DMD and BMD, long-term studies should be performed that assess the relation between deteriorating LV GLS and increasing amount of fibrofatty replacement (measured by LGE using CMR), next to cardiac events such as development of systolic dysfunction or heart failure. The data of these natural history studies could be used whenever drug has been found that could delay cardiac disease progression (placebo control) and to investigate prognostic value. Once the prognostic value has been proven, LV GLS could be used as prognostic biomarker to classify DMD and BMD patients according to their risk of progression of LV dysfunction. Its feasibility is demonstrated by the current use of LV GLS to monitor cancer therapy related cardiac dysfunction in early stages. Development of cardiotoxicity is associated with poor prognosis, and its early detection could lead to instituting interventions to prevent it(296). Considering that the disease progression (both cardiac and skeletal) is usually stretched over years, especially in BMD, it would be very useful to find circulating biomarkers that are associated with onset and/or progression of cardiac fibrosis, or to identify composite endpoints that examine the totality of disease burden. For example, this could be achieved by a model that includes functional data, qMRI FF data, circulating biomarkers (creatine/creatinine$_{ratio}$, myostatin) as well as cardiac biomarkers. Potential new drugs could demonstrate a clinical meaningful effect on motor endpoints like loss of ambulation or loss of hand-to-mouth function or, more likely, a surrogate endpoint such as qMRI FF in combination with slowing down development of myocardial fibrosis. To this end, the (prognostic) relation between qMRI FF and a clinical milestone in BMD should be shown as we showed that qMRI FF reduced the sample size by 40% compared to the NSAA (chapter five). In DMD, qMRI FF in DMD was able to predict change in function and increase the risk of instantaneous loss of ambulation when obtained in the VL and to loss of hand-to-mouth function when determined in the elbow flexors(110, 111). For both diseases, the minimal clinical important difference (MCID) remains to be determined. MCID is the smallest change that is considered important by patients and/or their parents. Also, continuous collection of natural history data (both functional data and circulating biomarkers), as well as qMRI data and cardiac imaging data should be collected. This is especially true for BMD patients as only very little (longitudinal)

natural history data exists(297-299). Part of this gap in data may be filled by the set-up of a national biobank in the Netherlands that enables collection of blood and urine samples next to clinical data. Other solutions include to make qMRI standard of care, and to intensify international collaborations between registries and investigator-initiated studies.

To conclude, in this thesis we studied different types of biomarkers with the overall aim to improve and facilitate trial design. Also, we showed that national registries are increasingly important for trial design by collection of real-world data. Future research in BMD should focus finding composite surrogate endpoints (cardiac and skeletal muscle function), as well as the creation of prediction models that include several types of biomarkers. For this, studies with more homogeneous groups and a longer follow-up are needed. In both DMD and BMD, trials should start focusing on cardiac-specific endpoints and, in general, more longitudinal data on cardiac specific measures is needed.