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ORIGINAL ARTICLE

Evaluation of an imaging biomarker, Dixon quantitative chemical shift imaging, in Gaucher disease: lessons learned

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Abstract Gaucher disease (GD) is the first lysosomal storage disorder for which specific therapy became available. The infiltration of bone marrow by storage cells plays an important part in the pathophysiology of skeletal complications and can be quantified by measurements of bone marrow fat fraction (Ff). Ff measurements by Dixon quantitative chemical shift imaging (QCSI) are standard for the follow-up care of GD patients at the Academic Medical Center. Several criteria should be met in order for these measurements to qualify as an imaging biomarker. These include: 1) The presence of the imaging biomarker is closely coupled or linked to the presence of the target disease or condition; 2) The detection and/or quantitative measurement of the biomarker is accurate, reproducible, and feasible over time, and; 3) The changes measured over time in the imaging biomarker are closely coupled, or linked, to the success or failure of the therapeutic effect and the true end point for the medical therapy being evaluated. This review assesses the use of Ff measurements by QCSI as a biomarker for GD in light of these criteria. In addition potential pitfalls are discussed including: degenerative disc disease; vertebral collapse and infection; haematological malignancies; focal fatty deposits; age; menopause; phase and repositioning errors, and; fat surrounding the basivertebral vein.

QCSI measurements of Ff can be used as an imaging biomarker for GD taking these pitfalls into account. It is one of the first biomarkers, in particular imaging biomarkers, for GD that has been systematically evaluated and could be a valuable tool in clinical trials comparing different treatments or dosing regimens.

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Introduction

Gaucher disease (GD) is an autosomal recessively inherited lysosomal storage disorder resulting from a deficiency in the enzyme beta–glucerebrosidase. This deficiency leads to storage of glucocerebroside in macrophages, which in turn accumulate in the bone marrow, spleen and liver. Symptoms may include cytopenia, hepatosplenomegaly and skeletal complications ranging from osteopenia to avascular necrosis (Grabowski et al 2010). Although one of the more common lysosomal storage disorders, GD remains a rare and heterogeneous condition with a birth prevalence in the Netherlands of 1.16 in 100,000 (Poorthuis et al 1999). Given the rarity and heterogeneity of GD, therapeutic trials might be considered challenging. Nonetheless, specific therapy for GD has been available since the early 1990s. Initially, the available treatment options consisted of one enzyme preparation for enzyme replacement therapy (ERT) (alglucerase/imiglucerase, Genzyme Corp) followed in 2002 by an oral compound for substrate reduction therapy (SRT) (miglustat, Actelion Pharmaceuticals). Finally, two additional enzymes recently became available (taliglucerase alfa, Protalix Biotherapeutics, and velaglucerase alfa, Shire HGT). In addition, a new oral compound is currently under investigation in phase III clinical trials (eliglustat, Genzyme Corp). Both ERT and SRT are extremely costly ranging from € 90,000 to more than € 200,000 per patient, per year. The use of individual dosing is recommended as responses to these treatments vary

(Zimran 2011). This, therefore, necessitates an appropriate monitoring of responses.

A commentary by Smith et al focused on the necessity of having appropriate biomarkers available, especially in rare conditions such as GD. Smith et al specifically addressed the imaging community, urging radiologists to take their responsibility in providing imaging biomarkers (Smith et al 2003). A *biomarker* is defined as, a detectable biologic parameter that reflects the activity of a disease process (Katz 2004; Smith et al 2003). Biomarkers, and especially *surrogate* markers, are of interest in the development of new drugs. They may enable more efficient trials at lower costs to take place by allowing an earlier detection of safety and/or efficacy compared to the use of traditional clinical endpoints such as mortality and morbidity. An imaging method discussed as a biomarker during the 2010 Gaucher Disease Biomarker Qualification Workshop held by the U.S. Food and Drug Administration (FDA), is Dixon's quantitative chemical shift imaging (QCSI).

This is a magnetic resonance (MR)–based technique that can be used to separate the signals from water and fat molecules, firstly described by Dixon (Dixon 1984). The quantitative use of the Dixon technique enables calculation of the fat signal fraction (Ff) in a given tissue. More qualitative use of the Dixon technique provides homogeneous fat suppression in MRI evaluation (Maas et al 1999).

Several studies have focused on the evaluation of the bone marrow compartment by QCSI, focusing for instance on its use in haematological malignancies (Rosen et al 1988; Wismer et al 1985). QCSI has subsequently been studied in GD patients (Hollak et al 2001; Johnson et al 1992; Maas et al 2002; Rosenthal et al 1995; van Dussen et al 2013). In GD patients, bone marrow fat and haematopoietic cells are replaced by glucocerebroside–filled macrophages (Gaucher cells). This results in a low fat fraction as compared to healthy controls. At present, QCSI measurements are the only quantitative imaging method to assess bone marrow involvement being used for the follow–up of GD patients. An alternative, closely–related *semi*–quantitative score, is the bone marrow burden score (BMB) which has been shown to correlate with QCSI measurements (Maas et al 2003). BMB scores are being used as a secondary endpoint in a current clinical trial (EDGE, clinicaltrials.gov identifier NCT01074944). Nonetheless, BMB scores are considered to be less sensitive than Ff measurements. Maas et al showed that Ff measurements were able to detect a response to treatment in 92 % of the included patients, while a response was observed in 75 % of the patients using the BMB scoring system (Maas et al 2003).

The Academic Medical Center is a national referral center for patients with GD. It has used Ff measurements by QCSI as the standard of care for the follow–up of GD patients for nearly 20 years. During this time 86 patients have undergone at least one Ff measurement. Experience with these measurements has mostly been satisfactory. Nonetheless, Ff measurements by QCSI have not been systematically evaluated as a biomarker. Moreover, several *pitfalls* which have the potential to hamper its use have been encountered by us during clinical practice.

The purpose of this paper is to assess the use of Ff measurements by QCSI as a biomarker for GD. The critical evaluation of pitfalls can be used to assist in improved utility of QCSI in clinical practice. In addition, many of the pitfalls discussed here will also apply to the later developed and more widely used BMB score. Our experiences in validating QCSI have contributed importantly to the robustness of the BMB score. A separate discussion of BMB scores was beyond the scope of this article, but the identified pitfalls in Ff measurements will hopefully equally benefit clinicians using this semi–quantitative scoring system.

Three criteria for an imaging biomarker, as previously stated by Smith et al, will be presented, followed by evidence in support of the use of QCSI measurements as a biomarker. In addition, the delineation of each criterion will be completed by a discussion on the potential pitfalls affecting this criterion and their interpretation as supported by the literature.

Criterion 1) The presence of the imaging biomarker is closely coupled or linked to the presence of the target disease or condition

The evaluation of the use of Ff measurement by QCSI in GD was extended following the initial report of the use of QCSI in a patient with Gaucher disease by Wismer et al (Wismer et al 1985). As mentioned in the introduction, it was established that the average vertebral marrow fat fraction was lower in patients with this disorder as compared to healthy adults (mean Ff measured by Dixon QCSI in GD patients 10 % versus 29 % in normal adults age 21–47) (Johnson et al 1992). In addition, it has previously been shown that Ff is associated with the occurrence of bone complications in GD patients. These bone complications occur primarily in patients with an Ff of less than 0.23 (Maas et al 2002). Thus the use of Ff in GD shows the severity of disease of the bone marrow. Three pitfalls encountered with regard to this criterion should be discussed.

Pitfall 1) Degenerative disc disease (DDD), vertebral collapse and infection

DDD is relatively common both in the general population as well as in GD patients. In addition, vertebral collapse is a known complication in GD patients. Both entities can lead to the destruction of bone trabeculae and subsequent fatty degeneration (De Roos et al 1987; Modic et al 1988). Vertebral collapse can occur both as an isolated complication or as a complication of the progression of a monoclonal gammopathy

of undetermined significance (MGUS) to a multiple myeloma (*see pitfall 2*). Alternatively, Wismer et al discussed a 22 year-old woman who developed a spondylodiscitis of L5-S1 shortly after treatment for a dental abscess. This resulted in a hyperintense signal on the phase-contrast image. Spondylodiscitis is accompanied by oedema resulting in an increase in the water fraction with a reciprocal reduction in Ff in the acute phase. However, as is the case with discopathy and vertebral collapse, osteomyelitis will lead to a destruction of bone trabeculae and the subsequent fatty degeneration of the vertebrae involved. Spondylodiscitis, bone crises and vertebral collapse accompanied by oedema in the acute stages of the event will result in a reduction of Ff, followed by fatty infiltration and/or degeneration and subsequent increase in Ff.

Fatty degeneration, however, was not observed in all Dutch GD patients after a vertebral collapse. Ff remains low in some patients with a long-standing history of crises caused by vertebral collapse possibly as a result of fibrosis and/or sclerosis (not shown).

Figure 1a-b illustrate respectively: discopathy associated with accompanying changes in the vertebrae; vertebral collapse with subsequent fatty degeneration; vertebral collapse without subsequent fatty degeneration; and fatty degeneration after a spondylodiscitis of L2-L3.

In clinical practice, collapsed vertebrae are usually excluded from the Ff measurements, which is also common in dual-energy x-ray absorptiometry (DXA) measurements.

The areas affected by fatty degeneration are excluded from the region of interest used to calculate the Ff (see also pitfall 8).

Pitfall 2) Multiple myeloma and haematological malignancies

Several studies have focused on the application of QCSI in haematological malignancies. Rosen et al showed that QCSI could readily distinguish patients with aplastic anaemia or acute leukaemia from healthy subjects (Rosen et al 1988). While leukaemia was associated with a reduction in Ff, aplastic anaemia resulted in a increased Ff. This can be explained by the fact that haematologic cells contribute to the water fraction and thus result in a decrease in Ff. GD is associated with an increased risk of several haematological malignancies, most notably an increasing risk of multiple myeloma. The occurrence of these malignancies will have an impact upon Ff and the interpretation thereof. Ff can no longer be used reliably as a parameter to assess the burden of GD in the bone marrow in patients affected by haematological malignancies and non-haematological malignancies affecting the spine (including metastases). Alternatively, in clinical practice, these diagnoses should be considered in a GD patient displaying a sudden decrease in Ff without other accompanying signs of the progression of GD and give rise to the appropriate diagnostic work-up.

Pitfall 3) Focal fatty deposits

Focal fatty deposits are a common phenomenon in spinal bone marrow. They appear as local, well defined, lesions of increased signal intensity when compared to the surrounding vertebrae on both T1, as well as T2, weighted images. A study evaluating vertebral MRI examinations (cervical, thoracic and/or lumbar spine) of 120 patients reported a prevalence of 60 % of focal fatty replacement of haematopoietic bone marrow (Hajek et al 1987).

Focal fatty deposits should not be considered as a pathological condition. However, their prevalence could hamper the interpretation of Ff results since their presence would underestimate GD associated bone marrow infiltration and lead to falsely high Ff results. Similar to the situation for pitfall 1, areas of focal fatty deposits should be excluded from the region of interest used to calculate Ff or else the vertebra involved may be excluded. These recommendations are taken into account in our daily use of QCSI.

Criterion 2) The detection and/or quantitative measurement of the biomarker is, over a period of time, accurate, reproducible, and feasible

To the best of our knowledge, no studies have been performed in which Ff values are directly correlated to histological data. However, papers by Miller and Johnson have reported results of biochemical analysis of marrow specimens from patients with Gaucher disease and their respective controls. Their studies showed that triglyceride constituted the major lipid component in GD and normal marrow, but triglyceride content was reduced in the marrow of GD patients as compared to controls. The authors addressed the paradox of finding a reduced Ff in a lipid storage disorder, speculating that changes in triglyceride content are primarily responsible for this observation. The triglyceride content was inversely correlated with glucocerebroside content, supporting the assumption that the decreased Ff in GD was the result of the displacement of normal marrow adipocytes by lipid-laden Gaucher cells (Johnson et al 1992; Miller et al 1996). The reproducibility of QCSI measurements has been reported in two studies. Rosen et al reported reproducibility among healthy volunteers within an error of 0.05 for fat fraction (Rosen et al 1988). Maas et al later concluded that QCSI had an excellent reproducibility (Maas et al 2001). In this 2001 paper reporting on the reproducibility of Ff measurements by QCSI, a cohort consisting of 16 *healthy volunteers* (eight men with a mean age of 39 years, range 24–60 years and eight women with a mean age of 38 years, range 26–55 years) was studied (Maas et al 2001). Based on the original data of this cohort, we performed a Bland-Altman analysis of the two repeated

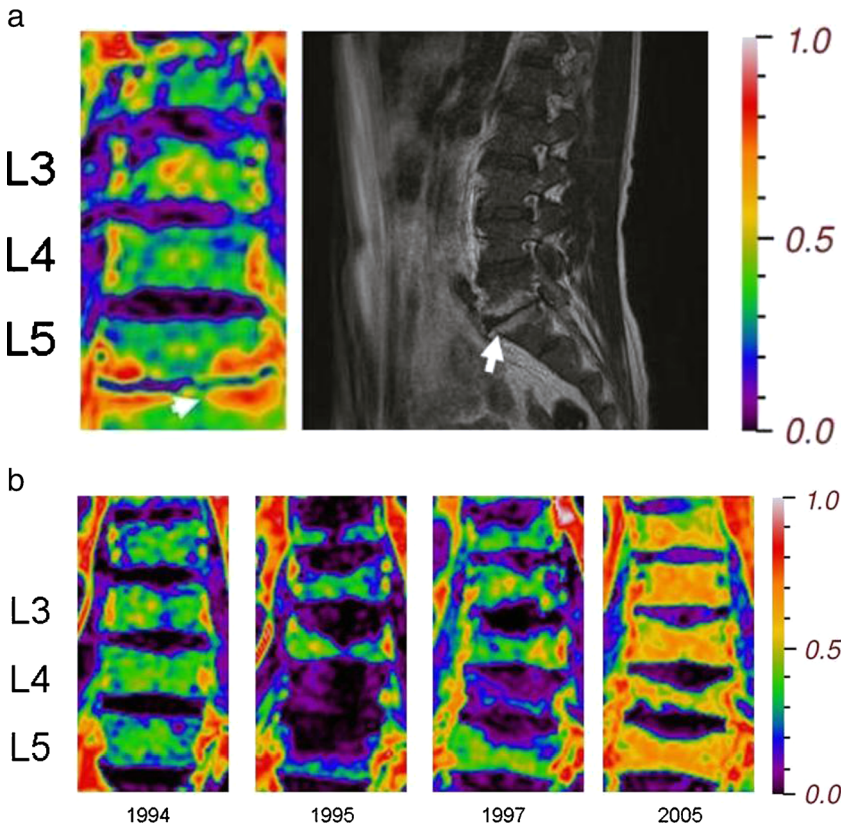

Fig. 1 Pitfall 1) Degenerative disc disease (DDD), vertebral collapse and infection **a**. Ff and T1 weighted images of a patient with discopathy at the level of L5-S1 (hyperintense are *arrows*). **b**. Follow-up of Ff in a patient who suffered a vertebral collapse of L4 in 1995, ERT was started in 1993. These images illustrate the different stages of the event. Vertebral collapse accompanied by oedema will result in a reduction of Ff, followed by fatty infiltration and/or degeneration and subsequent increases in Ff

measurements of mean Ff for L3-L5. The differences between the two measurements of Ff were plotted against the mean of the two measurements. The mean difference was −0.011, mean standard deviation (SD) was 0.038 and limits of agreement −0.063–0.085 (mean difference +/ −1.96*SD). The intraclass correlation coefficient was 0.93.

Our center has probably the most experience with longitudinal follow-up of Ff measurements. We assessed whether conspicuous changes or outliers in the measurements, (a) coincided with a scanner switch, and (b) could be explained by clinical events (change in therapy or change in other disease parameters), or technical events other than a scanner switch (bad positioning, phase artefacts, etc.). A rising trend in the graphs is common since successful therapy causes the Ff to increase in time. Analysing data from all patients with at least five measurements (n=63) covered 120 scanner switches in total and revealed that 13 out of 120 scanner switch periods showed a concurrent outlier. Nine of these could be explained by clinical or other technical events. In total we found 39 outliers including those coinciding with scanner switch periods, 24 of which could be explained by clinical or other technical events. Figure 2 shows the Ff curve of nine patients who underwent measurements on four different scanners, with scanner changes illustrated by dotted vertical lines. No systematic influence of scanner changes can be discerned. Thus, from a technical point of view, Ff measurements by QCSI are feasible over time. However, there are three important pitfalls to consider regarding this criterion.

Pitfall 4) Age

It has long been recognised that the volume of bone marrow occupied by adipose tissue increases with age. For example, Meunier et al studied the bone marrow biopsies of 34 healthy individuals showing a decrease in trabecular bone volume from 26 to 16 % between the ages of 20 and 65 with reciprocal changes in adipose tissue (Meunier et al 1971). In addition, they show that the percentage of adipose tissue in 50 osteoporotic subjects was more than 35 %, irrespective of age. More recently, Justesen et al postulated that this was the result of an increase in the differentiation of mesenchymal stem cells to adipocytes instead of osteoblasts (Justesen et al 2001). Their study found no difference between male and female volunteers. By contrast, a difference between male and female subjects was observed by Ishijima, who used chemical shift imaging to measure the water fraction of lumbar vertebral bone marrow. Their study concluded that the water fraction decreased in male patients until the age of 25, remaining relatively stable thereafter. In contrast, water fraction was relatively stable in female patients between the ages of five and 44, with a rapid decrease thereafter (Ishijima et al 1996). Measurements in a cohort of 16 healthy volunteers have

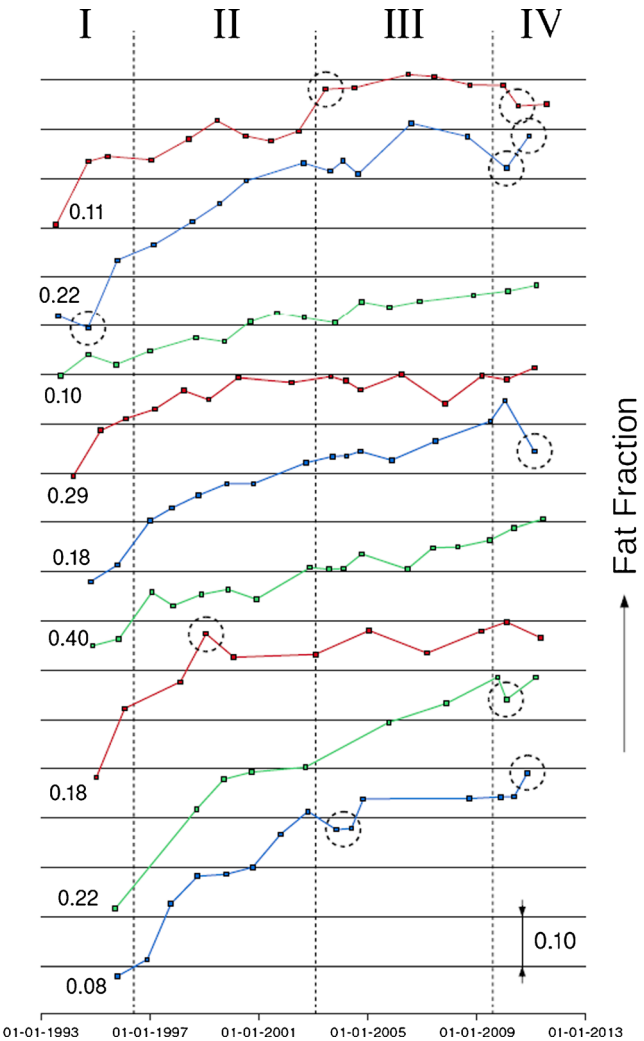

Fig. 2 This is the data from nine patients out of 63 who underwent measurements on all four scanners. The dates when the scanner changed have been indicated with dotted vertical lines. Four different 1.5 T MRI scanners have been used since 1993: (I) a Siemens SP until July 1996, (II) a Siemens Vision until February 2001, (III) a General Electric Signa Horizon until August 2009, and (IV) a Siemens Avanto since then. For clarity the individual graphs have been offset, or separated, in the Y-direction. The starting Ff-values are given for each patient. The difference between the two horizontal lines is always 0.10. Possible outliers have been marked with a circle. We see no systematic influence of scanner changes in the graphs

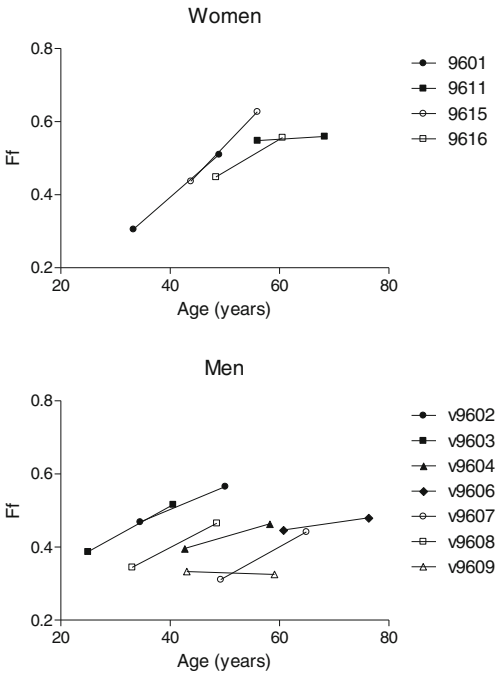

Fig. 3 Pitfall 4) Age. Ethical approval was obtained by the institutional review board of the Academic Medical Center, Amsterdam, in order to re-evaluate the cohort of 16 healthy volunteers studied by Maas et al (2001). A repeat Ff measurement was performed in 11 of the 16 subjects from the original cohort in order to assess the effect of age on Ff. Of the five volunteers who were not scanned, one volunteer refused to undergo a repeat measurement, two volunteers could not be traced, and in two volunteers, a repeated measurement could not be performed because of logistical reasons. Median Ff in 11 volunteers increased from 0.396 (range 0.305–0.548) to 0.510 (range 0.463–0.627) over a median of 15.55 years (range 12.17–16.58). However, as is shown in Figs. 4a and b, Ff increased with age in six out of seven men and in all women. **a**: Ff in relation to age in four women who underwent a repeat Ff measurement **b**: Ff in relation to age in seven men who underwent a repeat Ff measurement

previously been performed at our center to test reproducibility (Maas et al 2001). As demonstrated in that study, Ff showed a strong correlation with age in women, while no correlation was shown for men. The effect of age on Ff measurements is further demonstrated in Fig. 3a and b. These represent the results of a re-evaluation of the cohort of 16 healthy volunteers studied by Maas et al (2001) for which ethical approval was obtained by the institutional review board of the Academic Medical Center.

Age should be taken into account in clinical practice when interpreting Ff results. Given the particularly strong correlation between Ff and age in women, the use of age-adjusted reference values for Ff could be an option.

Pitfall 5) Menopause

As mentioned previously, Ishijima et al reported that bone marrow composition was both age and sex dependent. It was hypothesised that the differences observed between the sexes resulted from the influence of sex hormones and menstrual blood loss on bone marrow constitution as water fraction was significantly higher in women during the menstrual years (Ishijima et al 1996). In addition, it was suggested that osteoporosis might add to the decrease in water fraction in women over 45 years of age. Recently, Tang et al investigated bone marrow changes in osteoporotic post-menopausal women as compared to post-menopausal women with normal or osteopenic bone mineral density values using MR spectroscopy and diffusion-weighted MRI. This study confirmed the inverse relation between marrow fat content and bone density,

suggesting that estrogen might be responsible for the increase in marrow fat content by promoting adipocytic differentiation of mesenchymal stem cells (Tang et al 2010). The positive effect of estrogen on bone mineral density is underscored in a recent review which outlined the multiple potential mechanisms for its effects (Clarke and Khosla 2010). Estrogen's effect on bone, and inversely on bone marrow fat fraction, might influence Ff as measured in peri-menopausal women. Within the Dutch cohort of GD patients, 20 women had had more than one Ff measurement between the ages of 40 and 60 years. Four women were untreated during follow-up, three had both an untreated and a treated period during follow-up and 13 women received treatment (either SRT or ERT). Of the four untreated women, two display a remarkable increase in Ff between the ages of 45 and 50.

Our data set is too small to draw any definite conclusions on the effect of the menopause on Ff. However, we do feel that a sudden increase in Ff in peri-menopausal women should not automatically be attributed to an effect of treatment, nor is it necessarily a desirable response given the inverse relationship between marrow fat and bone density and the common occurrence of osteoporosis in post-menopausal women. Information regarding the onset of menopause in women with GD is important for a correct interpretation of Ff results and the presence of osteoporosis should be evaluated and treated accordingly.

Pitfall 6) Phase errors

We used the so-called *two-point Dixon* method, in which two MR-acquisitions are performed, for our QCSI measurements. We use one acquisition in which the water and the fat signals add up, and one acquisition in which the signal is the difference of the water and fat signals. In order to determine appropriately the amount of water and fat signal in the two acquisitions, we must know the *sign* of the difference, i.e. whether the water signal is stronger than the fat signal, or the other way round. For this, we use the *phase* of the difference acquisition, which is related to the direction in which the MR-magnetisation points when the signal is collected. In regions where the water and fat signals are nearly equal, however, the difference in the signal is small and any kind of interference, for example by noise or by motion artefacts, will cause large variations in the phase of the signal. This will render it impossible to determine the sign of the signal, or even cause a wrong sign to be determined.

Occasionally, these phase errors hamper the analysis. This mostly happens in L3, due to respiration or due to blood flow in nearby large vessels. We choose to exclude such a vertebra from the measurement when the resulting errors in Ff are considered too large.

Pitfall 7) Repositioning errors

Ff is measured in lumbar vertebrae L3, L4 and L5 in a coronal plane. Measurement acquisition slices are positioned on a midsagittal localiser image with special effort taken to position, and reposition, as accurately as possible, the imaging slice in the same location each time, and to obtain regions of interest of L3, L4 and L5 in a standardised manner. Maas et al previously studied the effects of repositioning errors on Ff measurements by performing measurements in three parallel slices, 3–4 mm apart, thereby deliberately creating a substantial repositioning error. The resulting variation in Ff results was of the same magnitude as the variation obtained when Ff measurements were performed on two different days without a deliberate variation in the repositioning of the acquisition slices. A direct comparison of results is difficult, however, as they stem from two separate experiments. Nonetheless, reproducibility of Ff measurements is determined to a certain extent by repositioning errors and will benefit from accurate repositioning. Accurate repositioning is promoted by carefully instructing technicians performing the MRI scans combined with availability of individual localiser images per patient for comparison.

Criterion 3) The measured changes over time in the imaging biomarker are closely linked to the success or failure of the therapy and the true endpoint sought for the medical therapy being evaluated

Three studies have established that treatment with enzyme replacement therapy resulted in increases in Ff. They concluded that Ff measurements can be used to study the skeletal response to treatment with enzyme replacement therapy for GD (Hollak et al 2001; Rosenthal et al 1995; van Dussen et al 2013).

An increase of Ff in response to treatment in GD is thought to reflect a reversal of bone marrow infiltration by Gaucher cells and as such is a desirable effect. Bone complications during treatment in patients with a baseline Ff measurement were observed irrespective of Ff in patients with other pathological conditions (multiple myeloma (n=1)), or in patients with a history of bone disease of the lumbar spine prior to the initiation of therapy (n=2). However, in those patients without a history of bone complications in the lumbar spine and an initial Ff below 0.23 (N=27), new bone complications almost invariably developed only when Ff was still below 0.23 (four cases). There was only one exception, who developed an AVN with an Ff greater than 0.50 (unpublished results). Physicians involved in the treatment of osteoporosis will interpret fatty transformation of the bone marrow as an unwanted phenomenon since it is related to a reduction in bone volume.

Increases in Ff values in GD patients above the normal range should thus be interpreted with caution as osteoporosis might contribute to this phenomenon.

Pitfall 8) Fat surrounding the basivertebral vein

Several MRI parameters have been used in the evaluation of bone marrow disorders including the follow-up of these disorders after treatment. Stevens et al studied MRI as an alternative to serial bone marrow biopsies and aspirations in 15 patients who received bone marrow transplantations after preparatory chemotherapy and/or total body radiation (TBI) for several haematological disorders (Stevens et al 1990). On post-transplant T1 weighted images of the vertebrae, they noted a "band pattern" with bright signal intensity centrally and a peripheral zone displaying intermediate signal intensity. They hypothesised that this pattern was determined by the vascularisation of the vertebrae with the return of haematologic cells peripherally and marrow fat surrounding the basivertebral vein centrally.

The presence of fat surrounding the basivertebral vein is evident in a substantial number of GD patients. Moreover, its quantity can change over time. As this pattern was observed, both in patients with a low overall Ff as well as patients with a higher Ff, a general pattern of bone marrow infiltration by Gaucher cells could not be distinguished and might be confounded by fibrotic changes in the vertebral body.

An example illustrating the significance of this pitfall is given in Fig. 4. The central part of the vertebra is included in the region of interest used to calculate the Ff. The presence of fat surrounding the basivertebral vein might, as a consequence, lead to an overestimation of the Ff and, in the evaluation of GD patients, an underestimation of the bone marrow infiltration by Gaucher cells. The interpretation of Ff results should, therefore, always be combined with evaluation of sagittal T1 and T2 images of the lumbar spine in order to uncover potential discrepancies between Ff results and vertebral bone marrow infiltration as a result of this phenomenon.

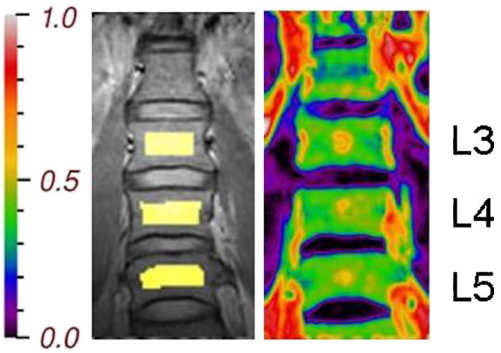

Fig. 4 Pitfall 8) Fat surrounding the basivertebral vein. *Left*: drawing of the regions of interest for which the Ff is calculated. *Right*: presence of fat surrounding the basivertebral vein in a GD patient

An overview of the pitfalls discussed is presented in supplementary Fig. 1.

Discussion

Ff measurements by Dixon QCSI have been part of the follow-up of GD patients at our center for nearly 20 years. During this time, it has proven to be a valuable tool which has supported numerous clinical decisions. One example would be that a bone marrow fat fraction below 0.23 is one of the indications to start treatment (Maas et al 2002). This article provides a justification for the use of QCSI measurements as an imaging biomarker for GD by assessing this modality in the light of several criteria put forward for such a biomarker.

Several pitfalls were identified in this review that might interfere with the interpretation of Ff results. In our 20 years use of QCSI we have incorporated solutions, in order to strengthen QCSI as an imaging biomarker. These may assist centers looking to implement these measurements in the follow-up of GD or other disorders.

Several alternative imaging techniques, some of which are quantitative, can be used to assess bone disease in GD. For example, dual-energy X-ray absorptiometry is relatively common in the follow-up of GD patients. An important advantage as compared to Ff measurements is its worldwide availability. Nonetheless, two important pitfalls should be taken into account with regard to this modality. Firstly, the bone marrow density (BMD) results may vary between scanners from different manufacturers and between different models from the same manufacturer. As a result, longitudinal follow-up is difficult without appropriate cross-calibration of densitometers (Official positions of the International Society for Clinical Densitometry 2007, ISCD.org). A 2012 study from the International Collaborative Gaucher Group Gaucher registry reports that low bone mineral density of the lumbar spine is a strong risk factor for fractures of the spine and femur (Khan et al 2012). However, data included in this registry are derived from different scanners and no cross-calibration was performed. While suggestive of a correlation between BMD and the occurrence of bone complications, this should be confirmed in a population where proper cross-calibration has taken place. Another pitfall of this technique is the fact that measured BMD is often high in patients having experienced bone complications due to sclerotic changes in the bone (van Dussen et al 2011).

Alternative imaging techniques to assess Ff include MR spectroscopy

Promising data on 1H spectroscopy in GD patients have been presented in the past, (Tran et al 2000) but there have been no

follow-up data reported in the literature. There is, therefore, little experience with this technique in GD. However, a new clinical trial studying the difference in fat fraction quantified by MRS in paediatric GD patients versus healthy controls is currently being conducted (ClinicalTrials.gov identifier NCT1397435). The correlation between MRS and bone marrow burden (BMB) scores (Maas et al 2003) and Spanish MRI scores S-MRI (Roca et al 2007) are part of this trial.

Gradient-echo MR imaging (GRE) can also be employed to measure Ff. The difference between GRE and spin-echo imaging is that GRE does not use a second radio frequency pulse. Instead it uses gradient reversal to realise a re-phasing of the signal. While GRE enables faster imaging, magnetic field homogeneity can influence signal formation (Elster 1993; Markl 2007). Gradient-echo sequencing has never been applied quantitatively in GD patients.

Several additional considerations also deserve note. We enhanced the reliability of QCSI as a biomarker through the assessment of each pitfall, since each of the aforementioned pitfalls has been overcome. This can be achieved, for example, either by adjusting the region of interest, or simply by enhancing the awareness of a particular pitfall thereby preventing incorrect interpretation of the results. A good example of this is age. An Ff result below 0.23 has been used as an indication to start treatment based on the findings that bone complications occurred primarily in patients in whom Ff results were below this value (Maas et al 2002). Perhaps this criterion should be adjusted given our growing understanding of the effect of ageing on Ff. An Ff of 0.25 might be considered normal in a 25 year-old woman, but is indicative of significant marrow infiltration in a 60 year-old woman.

Furthermore, in a 2006 commentary discussing evidence-based recommendations for monitoring bone disease in GD, Ff measurements by QCSI were said to be the most sensitive method for assessing bone marrow infiltration (vom Dahl et al 2006). Nonetheless, this article went on to state that: "the technical complexity of QCSI does not allow this method to be practically used for routine clinical practice." One might question the relevance of the current article given the limited use of QCSI measurements in routine clinical practice. However, QCSI measurements have recently been incorporated successfully as an exploratory parameter in a phase III trial with taliglucerase alfa (van Dussen et al 2013). It should be stated that patients were sent to the AMC for Ff measurements for this trial.

In addition, several MRI vendors have SE Dixon techniques available in their product sequences, enabling a wider use of the technique. For instance, the technique was recently used to assess the degree of fat infiltration in duchenne musculuar dystrophy patients (Wokke et al 2013), thereby increasing the relevance of this review.

Efforts to increase availability of Ff measurements will become more relevant given the recent registration of two new enzyme preparations for treatment of Gaucher disease and ongoing trials studying the effects of a new oral substrate inhibitor, eliglustat tartrate and the subsequent need for sensitive biomarkers to compare treatments.

Lastly, a widely used alternative imaging method to assess bone marrow infiltration by GD is the bone marrow burden (BMB) score (Maas et al 2003). This semi-quantitative scoring system, like many others of its kind, is based on the signal intensity changes on T1- and T2-weighted images caused by bone marrow infiltration by Gaucher cells. The main difference with other semi-quantitative scores is the fact that its correlation with Dixon QCSI has been documented. All of the pitfalls discussed in this article will, with the exception of phase errors, affect the signal intensity of bone marrow on T1- and T2-weighted images and have the potential to influence BMB-scores and related scoring systems. This underscores the importance of the identification of these pitfalls. Development of QCSI preceded the development of the closely related BMB scores by 10 years and our experience using this technique is therefore more advanced. This review is meant to assist in bridging this gap in experience.

In conclusion, QCSI measurements of bone marrow Ff can be used as an imaging biomarker for GD, taking a number of pitfalls into account. It is one of the first imaging biomarkers for GD to have been evaluated systematically and could be a valuable tool in clinical trails when comparing different treatments or dosing regimens.

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Informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

Animal rights This article does not contain any studies with animal subjects performed by the any of the authors.

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