The role of magnetic resonance imaging in the evaluation of thalassemic syndromes: current practice and future perspectives

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Abstract

Iron can be deposited in all internal organs, leading to different types of functional abnormalities. However, myocardial iron overload that contributes to heart failure remains one of the main causes of death in thalassemia major. Using magnetic resonance imaging, tissue iron is detected indirectly by the effects on relaxation times of ferritin and hemosiderin iron interacting with hydrogen nuclei. The presence of iron in the human body results in marked alterations of tissue relaxation times. Currently, cardiovascular magnetic resonance using T2* is routinely used in many countries to identify patients with myocardial iron loading and guide chelation therapy, specifically tailored to the heart. Myocardial T2* is the only clinically validated non-invasive measure of myocardial iron loading and is superior to surrogates such as serum ferritin, liver iron, ventricular ejection fraction and tissue Doppler parameters. Finally, the substantial amelioration of patients' survival, allows the detection of other organs' abnormalities due to iron overload, apart from the heart, missed in the past. Recent studies revealed that iron deposition has a different pattern in various parenchymal organs, which is independent from serum ferritin and follows an individual way after chelation treatment application. This new upcoming reality orders a closer monitoring of all organs of the body in order to detect preclinical lesions and early apply adequate treatment.

Introduction

Thalassemias are relatively common genetic disorders and constitute a major problem for patients, health providers and society. The term β -thalassemia denotes a significant shortage or complete absence of β globin chains as a result of decreased or absent function of one (heterozygous carrier) or both β

genes (homozygous form). The latter conditions result in an excess of α -chains, which continue to be normally synthesized, but cannot remain in solution; instead, they precipitate intracellularly, causing premature erythroid cell death. As a result, patients suffer from severe anemia (thalassaemia major or Cooley's anemia) and frequent transfusions are necessary throughout their lifespan.¹

Over 300,000 children are born each year with a hemoglobinopathy, of whom, more than 25,000 have thalassemia major (TM) and need regular transfusions to survive beyond early childhood. Unfortunately, only less than 50% of transfused TM patients are taking adequate iron chelation therapy; as a consequence approximately 3000 TM die each year, due to iron overload in their mid-20s.²

Iron in the human body

Iron can be deposited in all internal organs, causing different types of functional abnormalities. However, myocardial iron overload, contributing to heart failure, remains one of the main causes of death in TM. The onset of ventricular dysfunction is induced by iron toxicity, including oxidative damage to membrane lipids and enzymes in the mitochondrial respiratory chain,³ which occurs after a prolonged period of iron loading.⁴ It is impossible to early predict those at high risk to die from iron-related heart failure, using indirect indices, such as serum ferritin, liver biopsy, electrocardiographic and echocardiographic criteria. The measurement of plasma ferritin provides an indirect index for the total body iron stores, but its usefulness is limited by many common clinical conditions, such as inflammation, fever or liver disease.⁵ It also fails to reflect myocardial iron overload.^{6,9} Liver biopsy suffers from the same problems, mostly owing to the fact that iron deposition occurs in the liver prior to and at a greater scale than that of the myocardium.^{6,9} Furthermore, it is an invasive procedure that cannot be repeated for routine follow up. A previous study suggested that maintenance of serum ferritin levels below 2500 $\mu\text{g/L}$ decreased the risk for cardiac death in these patients,⁷ but many others with ferritin below this threshold have died from heart failure. Echocardiography is not an effective indicator of heart involvement in β -thalassaemia, as it reveals only the cases where impaired heart function is already present.^{9,10} The addition of two-dimensional speckle tracking could be potentially a promising indirect index of myocardial iron; however, further studies are needed for documentation.¹¹ Additionally, some recent publications¹² claim that the hyperdensity on a computed tomographic scan, though not specific for iron, was

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correlated strongly with heart T2*. However, more studies are needed before final conclusions can be drawn. The superconducting quantum interference device, a non-invasive technique for the measurement of tissue iron stores, has been confined exclusively to liver evaluation in clinical studies. Furthermore, the lack of availability, cost, technical demands, unsatisfactory correlation with biopsy, lack of heart-relevant data and suboptimal reproducibility have restricted the clinical use of the method.¹³ Therefore, it is clear that there is an emerging need for a non-invasive, easily reproducible index, capable of accurate detection of iron in an individual organ and in an individual patient.

The role of magnetic resonance imaging in iron evaluation

Magnetic resonance imaging (MRI) uses the magnetic properties of the human body to provide pictures of any tissue. Hydrogen nuclei are a principal constituent of body tissues in water and lipid molecules. A hydrogen nucleus produces a dipole moment (magnetic field) that can interact with an external magnetic field. MRI machines generate a strong, homogeneous magnetic field by using a large magnet, made by passing an electric field through superconducting coils of wire. When patients are exposed to the magnetic field, hydrogen nuclei in the body, which normally have randomly oriented spins, align in a direction parallel to the magnetic field. The MRI machine applies short electromagnetic pulses at a specific radio frequency (RF). The hydrogen nuclei absorb the RF energy and precess away from equilibrium. When the RF pulse is turned off, the precessing nuclei release the absorbed energy and return to normal. The strength of

the signal varies, depending on the RF magnetic fields applied. A tissue that is examined returns to normal in the longitudinal plane over a characteristic interval called the T1 relaxation time. In the transverse plane, the return to normal occurs over a characteristic interval called the T2 relaxation time. These values may also be expressed as relaxation rates, R1 (1/T1) and R2 (1/T2). Using MRI, tissue iron is detected indirectly by the effects on relaxation times of ferritin and hemosiderin iron that interact with hydrogen nuclei. The presence of iron in the human body results in marked alterations of tissue relaxation times.¹⁴⁻¹⁸ While T1 decreases only moderately, T2 demonstrates a substantial decrease.¹⁹⁻²¹ Myocardial T2, a parameter measured by spin-echo techniques, has been shown in experimental animals to have an inverse correlation with myocardial iron content.²² In a study, published by our group, that compared myocardial T2 with iron content in heart biopsy, an agreement was found between myocardial biopsy and the MRI results.²³ Unfortunately, the MRI signal is affected by multiple acquisition variables. Although T2 is relatively independent of field strength, there is an exception in the case of iron overload. In these patients, there is the linear dependence of T2 relaxivity (1/T2) on field strength.²³ Most reports have measured T2 at relatively lower magnetic fields of 0.5 T, where the field effect is somewhat diminished.¹⁵ Using 1.5 T, the T2 relaxation time was not measurable in heavily iron overloaded patients, because the signal intensity approximated to background noise.²⁴

In a recent study, it was documented that although recent work has demonstrated clinically valid estimates of human liver iron using T2 relaxation time, the calibration varies with MRI sequence, field strength, iron chelation therapy, and organ imaged, forcing recalibration in these patients.²⁵

Current cardiovascular magnetic resonance protocols for iron overload evaluation

Currently, cardiovascular magnetic resonance (CMR) using T2* is routinely used in many countries to identify patients with myocardial iron loading and guide chelation therapy, tailored to the heart. Myocardial T2* is calibrated to the myocardial iron concentration²⁶ and has been shown to improve with intensive iron chelation in parallel with the ejection fraction.²⁷⁻²⁹ Myocardial T2* is the only validated non-invasive measure of myocardial iron loading in the clinical practice and is superior to surrogates such as serum ferritin, liver iron, ventricular ejection fraction and tis-

sue Doppler parameters.^{4,26-31} This finding is of tremendous clinical importance, because i) it allows the individualization of iron assessment in different organs, which is not always in agreement with the total body iron load, ii) it allows the individualization of chelation protocols, according to iron overload of each organ.

Although worldwide survival is still poor,³⁰ life expectancy is increasing in countries with regular blood transfusions and well-managed iron chelation therapy.³³⁻³⁵ In most cases, chronic myocardial siderosis is both preventable and reversible with modern chelation regimes.³⁶⁻⁴⁰ Progress has also been made in treatment of acute heart failure and LV systolic dysfunction.^{37,38} A substantial 71% decrease in deaths has been observed in the UK thalassemia cohort since the introduction of T2* CMR and improved iron chelation.^{7,41} Other countries have also reported important amelioration in management of cardiac iron using T2* CMR.^{39,42-48} The use of the threshold of T2*=10 ms, below which the risk of cardiac complications rises significantly, has been already confirmed and alternative explanations for heart failure apart from myocardial iron loading seem unusual. The findings from independent centers worldwide indicate that myocardial T2* is a robust clinical tool with potential for further expansion to guide chelation regimes, which are tailored to prevent the development of heart failure and prolong survival.⁴⁹ T2* multislice multiecho CMR allows: i) the quantification of the segmental distribution of myocardial iron overload;⁵⁰ and ii) the detection of preferential patterns of myocardial iron overload in thalassemia major.⁵¹

The first T2* sequence described was a multi breath-hold, mid-ventricular short axis acquisition with end-diastolic gating.²⁴ A single breath-hold sequence that reduced scan time, improved image registration between images and had good reproducibility, was later developed.⁵² This technique has become the mainstay of clinical evaluation and follow-up of TM. It has been installed on CMR scanners from different vendors at multiple sites throughout the world with reproducible results⁴⁹⁻⁵⁴ and has been already used to investigate the cardiac efficacy of chelating agents.^{39,55-57}

Consequences of iron deposition in other organs apart from the heart

Liver-pancreas

Iron accumulates initially in the reticuloendothelial system (bone marrow, spleen, and liver), then in the hepatocytes, later in the

heart (myocytes) and the endocrine glands.¹ Chelation therapy has been extensively used to eliminate its deposition in different organs.^{58,59}

Liver is the primary site for iron storage in hemochromatosis or transfusion-dependent anemia; therefore, liver iron concentration (LIC), obtained by liver biopsy, accurately reflects total body iron stores⁶⁰ and is a proven prognostic indicator,⁶¹ but remains an invasive procedure that carries a 0.5% complication risk.⁶² Additionally, liver iron was not correlated with heart iron, as it was proven after multiple MRI studies and therefore cannot be used as an index for myocardial iron status.²¹⁻²⁴

Impairment of the endocrine and exocrine functions of the pancreas is a common complication in TM.⁶³ The incidence of impaired glucose tolerance and diabetes in TM varies from 9% to 15%, depending on the age of assessment, the intensity of chelation, the transfusions' number and the patients' compliance.⁶⁴ The etiology of diabetes in TM includes increased peripheral resistance to insulin and direct toxic effect of excess iron in the acinar and β cells of pancreas, which causes insulin deficiency.⁶⁵ Pancreatic iron is the strongest predictor of β cell toxicity, but total body iron burden, age and body habitus may also influence glucose regulation. MRI and fasting glucose/insulin are complementary screening tools, reducing the need for oral glucose tolerance testing. They can also identify high-risk patients, before irreversible pancreatic damage takes place,^{66,67} but do not correlate with siderosis in other organs.⁶⁸ Finally, iron loading is accelerated in splenectomized TM, due to decreased extrahepatic iron buffering capacity in these patients.⁶⁹

Hypothalamic-pituitary function

According to a recent MRI study in young TM, comparing iron accumulation in liver, myocardium, and pituitary gland, a significant negative correlation was observed between liver MRI and LIC determined by biopsy. However, pituitary to liver MRI and liver to myocardial MRI values were only moderately correlated and pituitary MRI was not correlated to myocardial MRI values. It seems that iron accumulation in TM is progressing with age and presents an independent behavior in different organs.⁷⁰

Growth failure in TM has been early recognized and still persists, despite major therapeutic advances. TM children have a particular growth pattern, which is relatively normal until the age 9-10 years; however, after this age, a slowing down of growth rate and reduced or absent pubertal growth spurt are usually observed. The pathogenesis of growth failure is multi-factorial. The main reason is free iron and iron deposition damage of the endocrine glands. The anterior pituitary gland is particularly sensitive to free radical stresses. It has

been reported that the GH deficiency (GHD) may be secondary to either pituitary or hypothalamic dysfunction. The duration of the disease, the patient's age and the severity of iron overload are the most important factors responsible for the malfunction of growth hormone (GH) secretion. Recent reports have documented that the frequency of growth hormone deficiency in TM is 13%-32%. Additional factors that may contribute to growth delay include chronic anemia, hypoxia, chronic liver disease, zinc, folic acid and nutritional deficiencies, intensive use of chelating agents, emotional factors, endocrinopathies (hypogonadism, delayed puberty, hypothyroidism, disturbed calcium homeostasis and bone disease) and last but not least dysregulation of growth hormone-insulin like growth factor 1 (GH-IGF-1) axis. Hypogonadism is the most common morbidity in patients with transfusion-dependent anemias, such as TM. There is evidence that pituitary iron overload and volume loss were independently predictive of hypogonadism. However, hypogonadal patients showed substantial improvements in pituitary function after intensive chelation therapy.⁷¹

Three phases of growth disturbances according to age of presentation have been already recognized in TM. In the first phase, growth disturbance is mainly due to hypoxia, anemia, ineffective erythropoiesis and nutritional factors. In the second phase that takes place during late childhood, growth retardation is mainly due to iron overload affecting GH-IGF-1 axis and other potential endocrine complications. Although appropriate iron chelation therapy can improve growth and development, TM children and adolescents, treated intensively with desferrioxamine, remain short, showing body disproportion between the upper and lower body segment. In the third phase, after the age of 10-11 years, delayed or arrested puberty is an important contributing factor to growth failure in adolescent TM, who do not exhibit a normal growth spurt. Successful therapeutic advances and bone marrow transplantation contributed in prolonged survival of TM. However, growth retardation remains a significant challenge in TM, often affecting their social adjustment and quality of life.⁷² Recently, pituitary MRI indices, as measured on T2*-weighted images, proved to reflect pituitary iron overload and could, therefore, be used for a preclinical detection of patients, who are in greater danger of developing hypogonadism.⁷³

Body disproportion in TM is independent of pubertal or pre-pubertal period of greater height gain. Treatment with recombinant GH (rhGH) is recommended when GH deficiency is established; however, the therapeutic response is often non satisfactory. Growth acceleration is mostly promoted with sex steroids in children with associated pubertal

delay. Sexual complications in TM, include delayed puberty, arrested puberty-hypogonadism and are the commonest endocrine complications. Iron deposition on gonadotroph cells of the pituitary leads to disruption of gonadotrophin production, proven by the poor response of follicle-stimulating hormone and luteinizing hormone to gonadotropin-releasing hormone (GnRH) stimulation. The damage to the hypothalamus and pituitary is progressive, even if intensive chelating therapy is given and the hypogonadism in both sexes is often unavoidable. Secondary hypogonadism appears later in life, and is manifested in women as secondary amenorrhea and in men as decline in sexual drive and azoospermia. Early recognition of growth disturbance and prevention of hypo-gonadism by early chelation therapy is essential for patients' improvement.⁷³ MRI in conjunction with a GnRH stimulation test may be useful in predicting future pituitary impairment; however, further studies are needed to assess the effect of chelation on glands' iron.⁷⁴

Gonadal function

Iron overload in TM usually results to low gonadotropin secretion, conducting in reduced ovarian antral follicle count and ovarian volume, but levels of anti-müllerian hormone (AMH), a sensitive marker for ovarian reserve which is independent of gonadotropin effect, are usually normal. AMH correlates with non-transferrin-bound iron (NTBI), suggesting a role of labile iron in the pathogenesis of decreased reproductive capacity, possibly occurring in parallel to cardiac iron toxicity, as cardiac iron was associated with the presence of amenorrhea and with NTBI levels. AMH is an important biomarker for assessment of reproductive capacity in TM, demonstrating that fertility is preserved in the majority of TM younger than 30 to 35 years. It can be also used to evaluate chelation regimes adequate for fertility preservation, whereas NTBI and labile plasma iron may be valuable for monitoring iron effect on the reproductive system.^{75,76}

In the majority of TM, gonadal function is normal, as most women with amenorrhea are capable of achieving pregnancy with hormonal treatment;⁷⁷ however, paternity is less common in thalassemic men, even those with normal or near normal spermatogenesis. The exact cause for this discrepancy is unclear. Given the milieu of iron overload in which spermatogenesis is taking place, this may be due to sperm dysfunction associated with variable degrees of hypogonadotrophic hypogonadism⁷⁸ and/or reactive oxygen species-inflicted damage on sperm nuclear DNA.⁷⁹ Advances in assisted reproductive techniques such as intracytoplasmic sperm injection (ICSI) in which a single spermatozoon is injected into the cytoplasm of an oocyte, have improved the prospects of

childbearing in oligozoospermic thalassemic patients.⁸⁰ Although little is known about sperm quality, damage in sperm DNA and correlation between sperm motility and DNA damage, it seems that iron overload in TM predisposes sperm to oxidative injury. These findings have important implications in assisted reproductive procedures such as ICSI, where there is increased risk of transmitting defective DNA to the offspring. Therefore, all TM should have semen cryopreserved as early as possible, before oxidative injury takes place. Fertility counseling offered to all patients prior to cryopreservation of sperm should include information about the potential of fetotoxicity and reproductive failure.⁸¹

Kidneys

The improvement of survival in TM has allowed several clinical morbidities to manifest, including renal complications. Thus, TM may experience proximal tubular dysfunctions and abnormalities in glomerular filtration rate. Hypoxia may lead to renal damage with resulting proximal tubular epithelial cell dysfunction and interstitial fibrosis, while anemia induces renal hemodynamic changes. Iron overload can also result in an increase in oxidative stress and direct cytotoxicity to the kidney. Moreover, the use of certain iron-chelating agents is associated with a transient, non-progressive increase in serum creatinine levels. However, most available evidence comes from small, cross-sectional studies. Longitudinal follow-up is needed to better understand the mechanisms of renal abnormalities in these patients.⁸²

Bones

In patients with TM, genetic and acquired risk factors lead to osteoporosis, pathologic fractures of the spine and back pain. Osteoporosis in TM is progressive; thus, early diagnosis and treatment are recommended. Bisphosphonates are relatively safe and effective. Characteristic intervertebral disc degeneration has been also observed in TM with severe iron overload or those who receive deferoxamine. Spinal asymmetry and overt scoliosis are common in TM. The prognosis seems favorable with many patients showing spontaneous resolution without need for intervention. In thalassemia intermedia, ineffective erythropoiesis drives extramedullary, hematopoietic tissue formation, which is mostly evident on magnetic resonance imaging.⁸³ Paraspinal involvement is of greatest concern, because of the associated spinal cord compression. Several treatment options have been described, including transfusion therapy, laminectomy, radiotherapy and the use of fetal hemoglobin-inducing agents that decrease the hematopoietic drive.⁸⁴

Current and future perspectives

Despite the fact that the single breath-hold GRE T2* sequence has a good reproducibility⁵² and has become the mainstay of clinical assessment and follow-up of TM, it also has some undesirable characteristics: i) the contrast between blood pool and myocardium may be suboptimal, and ii) flow compensation, artifacts from motion and blood flow may compromise the accuracy of measurements. More recently, a double inversion recovery *black blood* sequence has been reported, which suppresses the blood signal and a comparison with white blood T2* imaging suggested good reproducibility.⁸⁵

Recently, myocardial T2 and T1 were compared in a TM population and it was documented that they were correlated with T2*, but in patients with low or normal myocardial iron concentration, other factors were dominant in affecting T2* values, as it was shown by scattered T2* data. However, myocardial T1 correlated linearly with T2 in all patients, suggesting that these two relaxation parameters avoid extrinsic magnetic field inhomogeneity effects and may potentially provide better myocardial tissue characterization.⁸⁶

Finally, data about the quantification of iron overload at 3 T have been already published. These data suggest that the iron-dependent component of R2* scales linearly with field strength over a wide range of tissue iron concentrations. The incidence of susceptibility artifacts may, however, also increase with field strength.⁸⁷ In another study aimed to determine the feasibility, reproducibility, and reliability of the multiecho T*(2) Magnetic resonance imaging technique at 3 T for myocardial and liver iron burden quantification and the relationship between T*(2) values at 3 and 1.5 T, a good diagnostic reliability for T*(2) assessment at 3 T was demonstrated; T*(2) quantification of iron burden in the mid-ventricular septum, global heart, and no heavy-moderate livers resulted to be feasible, reproducible, and reliable at 3 T. However, segmental heart T*(2) analysis at 3 T may be challenging, due to significantly higher susceptibility artifacts.⁸⁸

Conclusions

MRI, using T2*, can detect iron overload in different organs early on and be used as a reliable follow up index to evaluate different chelation protocols, not only in the heart but also in other organs. Additionally, the amelioration of patient survival, allows the detection of abnormalities in other organs, apart from the heart, missed in the past. Recent studies

revealed that iron deposition has a different pattern in various parenchymal organs, which is independent of serum ferritin and follows an individual way after chelation treatment. This new upcoming reality orders a closer monitoring of all organs in order to detect preclinical lesions and apply adequate treatment; additionally, collaboration between MRI specialists and clinicians of different subspecialties of Internal Medicine is of paramount importance for the best possible care of TM patients.

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