



**CARDIAC SIDEROSIS SEVERITY ASSESSMENT USING CMR T2*
SEQUENCE AMONG TRANSFUSION-DEPENDENT
THALASSAEMIA PATIENTS: EXPERIENCE IN HOSPITAL
SEBERANG JAYA**

BY

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DECLARATION

I hereby declare that this research has been sent to Universiti Sains Malaysia for the degree of Master of Medicine in Transfusion Medicine. It is not to be sent to any other universities. With that, this research might be used for consultation and can be photocopied as reference.





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LIST OF ABBREVIATIONS

AABB	American Association of Blood Bank
AMDI	Advanced Medical and Dental Institute
CMR T2*	Cardiac Magnetic Resonance T2* Sequence
EF	Ejection Fraction
SF	Serum Ferritin
Hb	Haemoglobin
HK	Hospital Kulim
HKB	Hospital Kepala Batas
HPP	Hospital Pulau Pinang
HRPB	Hospital Raja Permaisuri Bainun
HSJ	Hospital Seberang Jaya
HTI	Hospital Sultan Abdul Halim
HSB	Hospital Sultanah Bahiyah
HTI	Hospital Teluk Intan
HTF	Hospital Taiping
LIC	Liver Iron Concentration
LV	Left Ventricle
LVEF	Left Ventricle Ejection Fraction
MLR	Multiple Logistic regression
MOH	Ministry of Health Malaysia
MRI	Magnetic Resonance Imaging
TIF	Thalassaemia International Federation
SLR	Single Logistic Regression
USM	Universiti Sains Malaysia

ABSTRAK

Latar belakang: Transfusi darah yang tetap sebagai rawatan untuk pesakit talasemia telah menyebabkan mereka terdedah kepada komplikasi siderosis jantung, penyebab utama kematian dalam populasi ini. Di Malaysia, kadar pembawa HBE dan β -talasemia adalah 3-5% dengan taburan yang lebih banyak di bahagian utara semenanjung dan juga Borneo Sabah. Prevalens siderosis jantung di Malaysia tidak pernah lagi diterbitkan setakat ini walaupun permintaan pemeriksaan bagi CMR T2* semakin meningkat di kebanyakan pusat penjagaan kesihatan. Tujuan kajian ini dijalankan adalah bagi menentukan prevalens dan keterukan siderosis jantung di kalangan pesakit talasemia bergantung kepada transfusi di bahagian utara Semenanjung Malaysia serta kolerasi antara kesemua faktor yang berkaitan; iaitu umur, jantina, ferritin serum, pemuatan besi hati dan pecahan luka ventrikel kiri (LVEF).

Kaedah: Kajian tinjauan kembali telah dijalankan dengan melibatkan 1187 pesakit talasemia yang bergantung kepada transfusi dan telah menjalani urutan pengimejan resonans magnetik (MRI) sebagai sebahagian daripada penilaian klinikal mereka. Kesemua pesakit yang menjalani pemeriksaan menjalani jantung, hati dan LVEF melalui urutan MRI CMR T2* antara Januari 2012 hingga Mei 2017. Data-data tersebut diperoleh dari pusat pendaftaran di Hospital Seberang Jaya yang mana kesemua kes-kes telah dirujuk. Semua data demografi dan klinikal yang berkaitan dengan status simpanan besi dan keadaan semasa penyakit telah direkod dan dipindahkan kajian performa sebelum dianalisa.

Keputusan: Prevalens siderosis jantung adalah 16.8%, 7.1% daripadanya tergolong dalam kumpulan teruk, 4.0% sederhana dan 5.7% pada tahap ringan. Umur min pesakit adalah 20.90 tahun dan thalassemia HBE Beta merupakan taburan yang paling banyak, 46.1% diikuti oleh major thalassemia Beta, 31.8%. Lelaki mempunyai kelaziman siderosis jantung yang lebih tinggi (56%) berbanding perempuan. Min serum ferritin dalam pesakit jantung siderosis adalah 6249.05 dengan 61.76% LVEF dan 2.21% nilai pemuatan besi hati T2 *. Prevalens siderosis hati adalah tinggi, 83%, namun hanya 15.84% dari mereka mempunyai siderosis jantung komorbid. Kegagalan jantung, ditentukan berdasarkan nilai LVEF <56%, didapati hanya sebanyak 9.8% daripada populasi kajian. Antara faktor-faktor tersebut, hanya nilai pemuatan besi hati yang terbukti secara statistik dalam menyumbang kepada pembangunan jantung siderosis dengan nilai 74.2% ROC.

Kesimpulan: Prevalens siderosis jantung adalah 16.8% dan prevalens beban besi hati adalah tinggi iaitu, 83%. Perkaitan statistik yang ketara antara pemuatan besi hati dengan siderosis jantung menekankan kepentingan mengesan siderosis jantung pada lebih awal melalui CMR T2 * terutama sekali dengan kelaziman kes-kes talasemia bergantung transfusi yang tinggi

Kata kunci: serum *ferritin*, *siderosis* jantung, pemuatan jantung besi, *siderosis*, pemuatan besi hati, thalassaemia tanggungan-transfusi, transfusi ketoksikan besi, CMR T2*, T2* hati.

ABSTRACT

Background: Regular blood transfusion as a treatment for thalassaemia patients had rendered them susceptible to the complication of cardiac siderosis, the main leading cause of death in this population. In Malaysia, HBE and Beta-thalassaemia carrier rate is 3-5% predominantly distributed at the northern part of peninsular and Borneo Sabah. The prevalence of cardiac siderosis in Malaysia has not been published despite the ever-increasing demand of CMR T2* sequence in most of the healthcare centers. The purpose of this study was to determine the prevalence and severity of cardiac siderosis in the northern part of peninsular Malaysia and all its associated factors; namely age, gender, serum ferritin, liver iron loading and left ventricular ejection fraction (LVEF).

Methods: A retrospective study was conducted involving 1187 transfusion-dependent thalassaemia patients who underwent magnetic resonance imaging (MRI) sequence as part of their clinical evaluation. All enrolled patients underwent cardiac, liver and LVEF CMR T2* sequence MRI between January 2012 to May 2017. Data were obtained from a single center registry in Hospital Seberang Jaya where all the cases were referred. All demographic and clinical data pertaining to iron storage status and disease current state were recorded and transferred to research performa.

Results: The prevalence of cardiac siderosis was 16.8%, of which 7.1% were severe, 4.0% moderate and 5.7% mild. The median age of patients was 20.90 years and HBE Beta thalassaemia constituted the most, 46.1% followed by Beta thalassaemia major, 31.8% of

the patients. Male has a higher prevalence (56%) of cardiac siderosis. The mean serum ferritin in cardiac siderosis patients was 6249.05 with 61.76% LVEF and 2.21% liver iron loading T2* value. Liver siderosis prevalence was high, 83%, however only 15.84% of them had a comorbid cardiac siderosis. Heart failure define as LVEF <56%, present in only 9.8% of the study population. Liver iron loading were the only statistically proven factors contributing towards the development of cardiac siderosis with 74.2% ROC value.

Conclusions: The prevalence of cardiac siderosis was 16.8% and the prevalence of liver iron overload was relatively high, 83%. The significant associations between liver iron loading and cardiac siderosis emphasized the importance of detecting cardiac siderosis early through CMR T2* especially in a state with high prevalence cases of transfusion dependent thalassaemia.

Keywords: serum ferritin, cardiac siderosis, cardiac iron overload, liver siderosis, liver iron overload, transfusion dependent thalassaemia, transfusion iron toxicity, CMR T2*, liver T2*.

CHAPTER 1- INTRODUCTION

1.1 OVERVIEW

1.1.1 Thalassaemia

Thalassaemia is an inherited blood disorder caused by defective in the synthesis of globins, a key component of red blood cells. Haemoglobin disorders are common in 71% of 229 countries affecting 89% of births. Around 7% of pregnant woman carry a significant variant of haemoglobinopathy globally (Bernadette Modell *et al.*, 2008). It was estimated that about 8,000 persons were afflicted with HbE β -Thalassaemia, and 8,000 with homozygous β -Thalassaemia (Kaur, 1995).

In Malaysia, the carrier rate for β -thalassaemia and HbE β -thalassaemia is estimated to be 3-5%. Based on an estimated population of 22.7 million, the number of cases afflicted with β -thalassaemia major among Malays and Chinese are 4,380, with 2,140 HbE β -thalassaemia cases among Malays. Each year there are an estimated 46 births of β -thalassaemia major. The peninsular states showed a distribution pattern that followed the population density but with a distinct pattern of HbE β -thalassaemia being more pronounced in the northern states bordering Thailand (MyThalassaemia Malaysia Thalassaemia portal 2010). A different scenario was seen in east Malaysia whereby Sabah appears to have a higher incidence of thalassaemia major. The projection of cases as of year 2010 showed 1,272 cases were registered under the Thalassaemia Association of Sabah. Out of these, Kadazan-Dusun ethnic group contributed almost half of the reported individuals in that state.

Once diagnosed, transfusion-dependent thalassaemic patients will be regularly transfused with the aim not only to resolve the anaemia symptoms but also to suppress the extramedullary haematopoiesis while minimising complications of under-transfusion and maintaining normal well-being. In Malaysia, regular transfusions will be given once major thalassaemia had been confirmed or when haemoglobin is unable to be maintained above the level of 7g/dL. Poor growth is also recommended as an indicator for regular transfusions under Level III clinical recommendation (Malaysian CPG guideline 2009).

As substantial health burden worldwide, only half of the diagnosed thalassaemia patients obtained adequate iron chelation therapy causing nearly 3000 deaths annually due to uncontrolled iron toxicities (Modell B *et al.*, 2008). It was found that mean survival of those not on chelation therapy was 17.4 years versus 31 years for those who received hypertransfusion and chelation therapy (Ehlers *et al.*, 1991). Another study stated that survival after a heart failure episode was 48% (Kremastinos *et al.*, 2001).

1.1.2 Thalassaemia and iron overload

Despite thorough assessment and established algorithm for iron chelation in transfusion-dependent thalassaemia, iron overload complications are inevitable on certain individuals due to poor compliance, logistic-topographic hurdles, and/or the disease burden itself. Pathophysiological occurrence of iron overload is contributed by either excess of gastrointestinal absorption or secondary to repeated blood transfusions. In advanced cases of thalassaemia disease, iron will be accumulated in parenchymal cells of the liver, heart, pancreas and endocrine organs which are sensitive to toxic effects of iron. When the iron binding capacity of transferrin is exhausted, free iron appears as non-transferrin bound iron (NTBI) which is toxic to the heart. Complications aroused from these include: heart failure (5.4-12%) (Kremastinos *et al.*, 2001; Aessopos *et al.*, 2001), heart disease/cardiac impairment (37-51%) (Sau *et al.*, 1995; Richardson *et al.*, 1993; Wolfe *et al.*, 1985), arrhythmias (5%) (Borgna-Pignatti, 1998), impaired left ventricular ejection fraction (LVEF) (<45%-13-37%) (Aldouri *et al.*, 1990; Kremastinos *et al.*, 1995; Hahalis *et al.*, 2001; Halilis & Monalis., 2002;), pulmonary hypertension (59.1%) (Aessopos *et al.*, 2001), and diastolic dysfunction (50-54%) (Vaccari *et al.*, 2002; Yaprak *et al.*, 1998; Hou *et al.*, 1994). Although iron overload-related cardiomyopathies are reversible, the delayed clinical presentations and investigations are the contributing factors which lead to the common death cause for patients suffering from thalassaemia (Bogna-Pignatti *et al.*, 2005).

Few investigations are therefore being suggested to monitor the progress of disease in thalassaemia and tailored treatment to be instituted as prompt as possible. Serum ferritin for example, is significantly correlated with the levels of myocardial iron grade and

content (Lombardo *et al.*, 1995). In view of the heterogenicity of iron distribution in the myocardial tissue, myocardial biopsy is considered as insensitive and invasive modality to detect myocardial iron overload (Fitchett *et al.*, 1980).

Cardiac Magnetic Resonance Imaging (CMR) is a non-invasive method for iron quantification in the heart. CMR T2* is the most sensitive technique to assess myocardial iron overload (Anderson *et al.*, 2001) and has the capability to rapidly assess iron content in the heart, examine functional parameters concurrently, allow longitudinal follow up, and be reproducible. T2* measures the local magnetic field heterogeneities of any tissue or substrate, and the value decreases with iron deposition. Iron overload causes signal loss in affected tissues because iron deposits become magnetized in the scanner, inducing local irregularities in the magnetic field, which causes water protons around these deposits to lose phase coherence. The normal value of T2* is 50 ± 16 ms and pathologic T2* value is <20 ms.

In addition, significant curvilinear, inverse correlation is seen between iron concentration measured by liver T2* and liver biopsy in transfusion-dependent patients. The measurement of liver iron loading includes the conventional liver biopsy or non-invasive MRI technique utilizing the principle of T1 and T2 relaxation times shortening due to the presence of ferritin iron, and thus reduction in signal intensity of spin echo image. Liver iron loading severity is classified according to T2* value and falls between a category of mild 2.8-6.29ms, moderate 1.4-2.79ms and severe ≤ 1.39 ms. T2* value of ≥ 6.3 ms is considered normal.

1.1 RESEARCH JUSTIFICATION AND BENEFITS

Cardiac complications namely heart failure and arrhythmias are the major causes of death in organ haemosiderosis (Sonakul *et al.* 2009, Ohene-Frempong *et al.* 2009). Serum ferritin is available and regularly monitored in a thalassaemia patients, however since serum ferritin is one of the serum reactive proteins, the measurement is limited by common clinical conditions such as inflammation, fever, and liver disease (false positive results).

Malaysian guidelines of Thalassaemia 2009 has suggested the commencement of iron chelation therapy to be initiated once the serum ferritin has reached above 1000 ug/L. In paediatric population, this is equivalent to the age of two to three years old. The first line iron chelators include Desferrioxamine and Deferiprone. Inadequate chelation is defined by presence of serum ferritin of more than >2500 ug/L and with such level, the higher risk of cardiac complications and death were to occur (Olivieri *et al.*, 1994) despite few reported cases of cardiac death at low ferritin levels (Shebl SS *et al.*, 1999).

Other than serum ferritin, another relatively inexpensive and readily available screening test that is regularly performed on thalassaemia patients is echocardiography. However due to its low sensitivity rate in comparison to CMR, echocardiography prediction for cardiac siderosis are restricted and possibly missed (Anthanossiois *et al.*, 2006). Despite being operator dependent, echocardiography is currently being used daily to determine the functional status of the heart due to its availability. However, the echocardiography parameters are only useful as late indicators, revealing cases where impaired heart function were already present in cardiac siderosis.

In Malaysia, CMR T2* sequences were not performed routinely on every patient as the availability of this modality is restricted. Up till now, there are no specific guidelines pertaining to the specific timing to refer a case for CMR T2* sequence. According to Malaysian 2009 guidelines, only children more than 10 years old are indicated for CMR T2* regardless of the iron chelation status. In comparison, the Thalassaemia International Federation (TIF) recommends the first assessment of CMR T2* is to be done at puberty for the well-chelated patients who received chelation therapy early and regularly. There are however, no specific indications for poorly chelated patients according to this guideline.

A study done in 2013 by Carpenter J.P and friends involving 27 worldwide centres using CMR T2* revealed that 96.3% of symptomatic heart failure patients under thalassaemia follow-ups had finding of T2*<10ms and 100% in those with T2*<20. This survey concluded that 43% of the thalassaemia major patients had moderate/severe myocardial iron loading with myocardial CMR T2* values of less than 10ms, a strong association with heart failure and death.

There are geographical differences in terms of T2*<20ms distribution throughout the world in which Egypt has the lowest prevalence (9.6%) while South East Asia has the highest prevalence of 29.1%. The possibility of genetic component for the resistance to cardiac iron loading in Egypt patients might be the reason of such low prevalence (Amal *et al.*, 2013). Presence of myocardial iron loading was also associated with occurrence of hypogonadism and diabetes mellitus (Ai *et al.*, 2013).

Despite being able to give the high predictive values of CMR T2* in detecting cardiac siderosis especially in asymptomatic thalassaemia major patient (Kremastinos DT *et al.*, 2010), previously published data by Maceira Am *et al.*, and Westwood MA *et al.* in the year 2007 and 2008 respectively, had revealed that other indices such as LV volumes and EF varies in non-cardiac iron load thalassaemia major patients from healthy non-anaemic controls. This is likely due to chronically increased cardiac output related to the anaemia. Despite that, the normal T2* value and a low serum ferritin level does not rule out the presence of heart disease, as diastolic cardiac function can be impaired before the occurrence of systolic dysfunction (Ozbek *et al.*, 2012). It is also worth mentioning that there is no statistical difference between the degree of CMR T2* abnormality and cardiac systolic function (Faridah *et al.*, 2014).

In Hospital Seberang Jaya (HSJ), CMR T2* examination has been performed since December 2009. Although the service of CMR T2* had started since 2009 in HSJ, 2010 has seen the steady rise in the number of referrals with five to eight cases performed each day. The referrals of patient for CMR T2* in HSJ came from centres such as Hospital Kulim, Hospital Pulau Pinang, Hospital Taiping, Hospital Kepala Batas, Hospital Raja Permaisuri Bainun, Hospital Sultan Abdul Halim and HSJ itself. The reports of the CMR T2* were compiled in a hard copy format and kept in Radiology department, HSJ, for safe keeping. Dual copy of such results were given to the respective hospitals for recording and subsequent managements.

1.2 LIST OF DEFINITIONS

- i. **Cardiac Siderosis:** An abnormal deposition of iron in the heart which can occur congenitally such as in hereditary haemochromatosis or acquired from transfusion regime in transfusion dependent thalassaemia.
- ii. **CMR:** Cardiac magnetic resonance also known as cardiac MRI which is a non-invasive imaging technique to assess the structure and function of the heart through a specialised MRI machine. It is considered as a gold standard for measurement of all left and right ventricular indices.
- iii. **T2*:** A relaxation parameter that arises on MRI principally from local magnetic field inhomogeneities that are increased with iron deposition.
- iv. **LVEF:** Measurement of the percentage of blood ejected during systole in relation to the total end diastolic volume. LVEF is calculated by dividing stroke volume by the end-diastolic volume $LVEF (\%) = \text{Stroke Volume} / (\text{LV end-diastolic volume}) \times 100\%$. CMR is considered the gold standard for assessment of LVEF due to its accuracy, reproducibility and minimal risk.
- v. **Serum Ferritin:** High-molecular-weight iron containing protein that functions in the body as an iron storage compound, and an acute phase protein.
- vi. **Liver iron loading:** Iron accumulation in the liver due to variety of conditions such as excessive iron supply (multiple transfusion), congenital or hepatitis.
- vii. **Myocardial iron loading:** Excess body iron which will then results in iron accumulation in the myocardium.
- viii. **Liver iron concentration:** Direct measurement of liver iron concentration (LIC) via absorption spectrophotometry, often performed when the specific diagnosis of iron overload is suspected or to monitor therapy with normal

ranges from 0.2 mgFe/g dry weight to 2mg Fe/g/dry weight. MRI can also accurately measure the LIC using techniques such as signal intensity ratio and relaxometry methods.

- ix. **Cardiac siderosis severity:** Cardiac iron toxicity or cardiac siderosis is classified into 3 different severity according to its T2* measurement namely; mild: 14-19.99ms, moderate: 10-13.99ms and severe: ≤ 9.99 ms.
- x. **Liver siderosis severity:** Also known as liver T2* which is a measurement of the relaxation times in ms. It shows the severity of liver iron overload, also measured via CMR T2* sequence. Grading of severity are mild: 2.8-6.29ms, moderate: 1.4-2.79ms and severe: ≤ 1.39 ms.

1.3 RESEARCH OBJECTIVES

1.3.3 General Objective

To study the prevalence of cardiac siderosis among transfusion-dependent thalassaemia patients by CMR T2* sequence and its associations.

1.3.4 Specific Objectives

- i) To determine the prevalence and severity of cardiac siderosis among transfusion-dependent thalassaemia patients based on the T2* value.
- ii) To determine the association of age, gender, diagnosis, serum ferritin, liver iron loading (T2* value) and left ventricular ejection fraction with cardiac siderosis.

1.4 STUDY HYPOTHESIS STATEMENT

- i) Null Hypothesis

There is no significant association between cardiac siderosis and age, gender, serum ferritin, left ventricular ejection fraction and liver iron loading (T2* value).

- ii) Alternative hypothesis

There is significant association between cardiac siderosis and age, gender, serum ferritin, left ventricular ejection fraction and liver iron loading (T2* value).

1.6 CONCEPTUAL FRAMEWORK

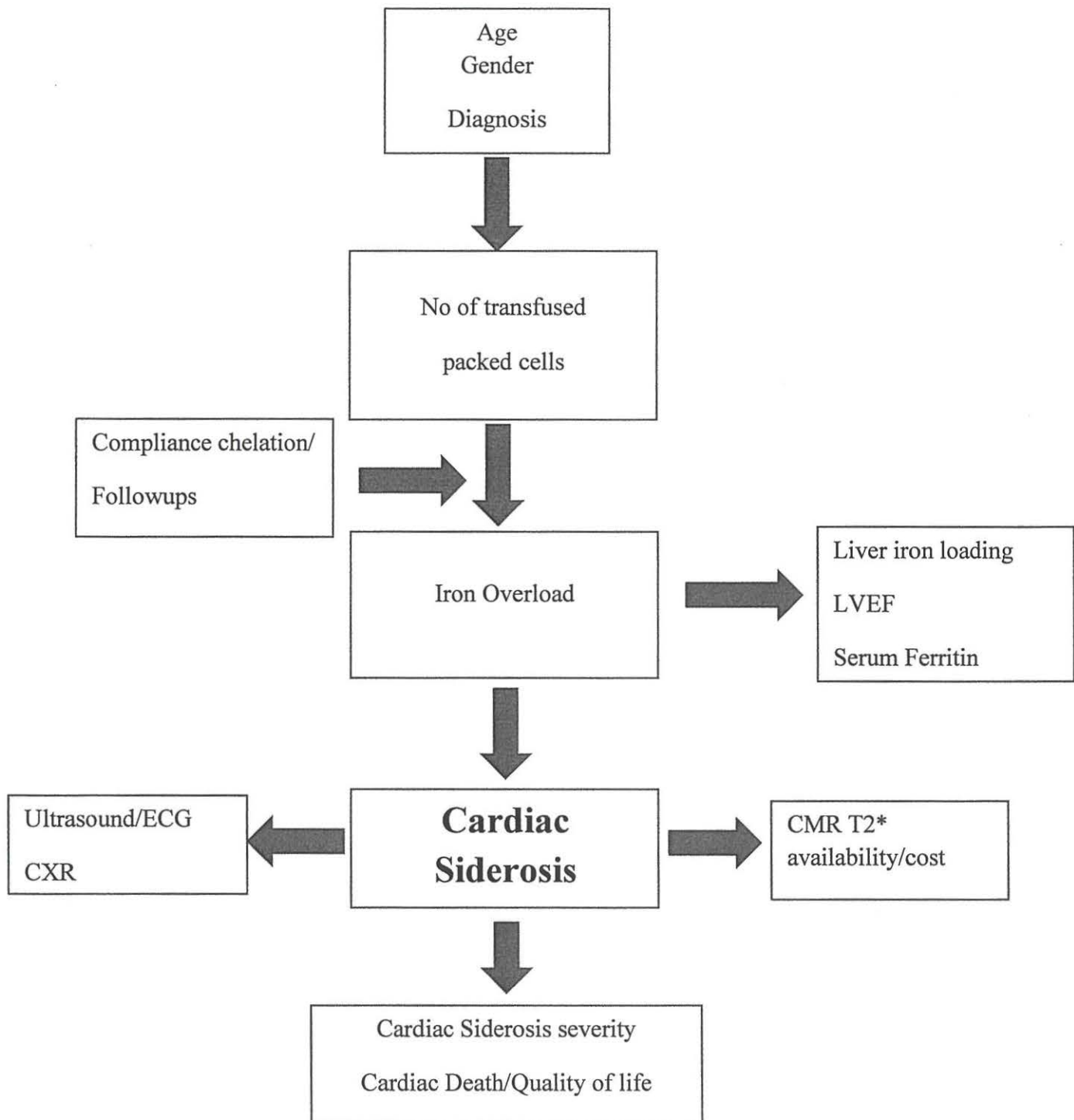


Figure 1.1 Conceptual Framework of Cardiac Siderosis with its associated

CHAPTER 2- LITERATURE REVIEW

2.1 Role of iron in human body

The importance of iron to human was acknowledged as early as the ancient Greeks era when iron had been given to the soldiers to improve muscle weakness following injury. During 18th century, haemoglobin was found to contain an iron element of 0.35% (Hughes, 1977). Chlorosis or green disease, a condition which we now identify as nutritional iron deficiency anaemia, (Shmidt, 1959) had been treated with iron salts since the 16th century by the alchemist and physicians (Marks *et al.*, 1975).

Iron uptake by enterocytes followed by transportation to the circulation and delivery to the cells for usage or storage, are the main processes of iron homeostasis (Nadadur *et al.*, 2008). Iron are absorbed passively by the apical membrane of enterocytes mainly in the intestinal lumen (Wang *et al.*, 2011) which will then be oxidised to the insoluble ferric state before being transported by transferrin to the peripheral tissues (Theil, 2012). Normally, the saturation of iron with plasma transferrin is 30% (Hentze *et al.*, 2010) making the iron-binding capacity of plasma is always more than iron concentrations. Approximately 30-40mg/kg of human body weight is being concentrated with iron, with hepatocytes acting not only as the main storage but also regulate iron movement via the secreted hepcidin (Nemeth *et al.*, 2004)

The excretion of iron from body is predominantly attributed by losses from exfoliation of epithelial at the gastrointestinal tract, skin and genitourinary tract accounting for only 1mg/day (Hunt *et al.*, 2009) meanwhile menstrual iron lost contributed 1.5mg/day to

2.1mg/day for females (Cole et. al., 1971). Because of that, requirement of iron in the reproductive females are 15mg/day, more than the requirement for adult male and postmenopausal which is 10mg/day (Bothwell et. al., 1979). Another way of depleting the iron storage in the body is via phlebotomy which can reduced approximately 200mg/unit (Bokhoven et. al., 2011). This is important as human will not be able increase the iron excretion just to compensate for the iron overload and this repeated therapeutic iron removal method is currently being practice for a hereditary haemochromatosis patient.

2.2 Serum Ferritin

Ferritin is the major protein storage for iron in the body, storing up to 4,500 ferric iron atoms (Fishbach, 1965) however in vivo, approximately 20% of ferritin are saturated with iron. At this rate, 800 out of 4500 iron sites will be occupied (Cook *et al.*, 1982). When 4000 atoms of iron per ferritin molecule are filled, it will be degraded to form haemosiderin and the iron in it are less chemically reactive and less available for mobilisation (Crichton *et al.*, 1992). Serum ferritin concentration reflects the storage of iron compartment in the body with each 1mg/L representing 10mg of storage iron in the body. The normal value of serum ferritin ranges between 20-300mg/L. However, serum ferritin is also an acute phase protein, raises dramatically with both acute and chronic inflammations, vitamin B12 and folic acid deficiency, liver disease, leukaemia, Hodgkin's disease, alcohol intake and hyperthyroidism (Bothwell *et al.*, 1979). Because of these influential factors other than iron status, the usefulness in assessing iron overload is limited although it is rather inexpensive, simple, widely available and clinically indicated. This is proved by studies by Brittenham and colleague in 1993 which stated that in Thalassaemia Major patient, there can be up 57% of the variability in plasma ferritin in body iron storage, mostly attributed by the fact that inflammation increases serum ferritin, and so does the distribution of liver iron between macrophages (Kupffer cells) and hepatocytes in the liver. The diagnosis of iron overload varies according to different centers. For instance, Spanish Society of Blood Transfusion and Society of Hematology and Hemotherapy, defined iron overload as ferritin levels greater than 1000 ug/L on 2 or more consecutive measurements within 15 days.

2.3 Thalassaemia and transfusion-dependent patients

Regular transfusions in transfusion-dependent thalassaemia major patients will increase the total iron storage in the body. Approximately 200mg of iron is present in each unit of packed cell or about 1.08mg of iron per 1mL of pure red blood cells. In thalassaemia major, most of the patients had the mean transfusions loading of 0.3-0.5mg/kg/day to >0.5mg/kg/day of iron transfused (Chaim 2010). Excess iron will mostly have deposited in reticuloendothelial cells (macrophages) in the spleen, hepatic Kupffer cells, and bone marrow, and in parenchymal tissues such as the myocardium, liver and endocrine organs. Repeated blood transfusions would result in increased of transferrin saturations and ultimately to the presence of iron species that are not bound to transferrin, called NTBI (Hersko *et al.*, 1978). NTBI readily enters cardiomyocytes and endocrine tissues through L-type voltage dependent calcium channels that are not present on all tissues making the pattern of iron deposition in transfusion iron overload (Oudit *et al.*, 2003). In comparison with hepatic uptake, myocardium iron uptake is much slower thus myocardial iron overload tends to develop at a later stage in comparison with liver toxicity (Wood, 2008). Diverse types of chronically transfused patients such as sickle cell disease or myelodysplastic syndrome are susceptible to develop cardiac toxicities (Buja LM *et al.*, 1971) however β -thalassaemia major has been the most extensively studied transfusion dependent disorder in regards with cardiac, endocrine and extrahepatic iron toxicities (Schafer AI *et al.*, 1981).

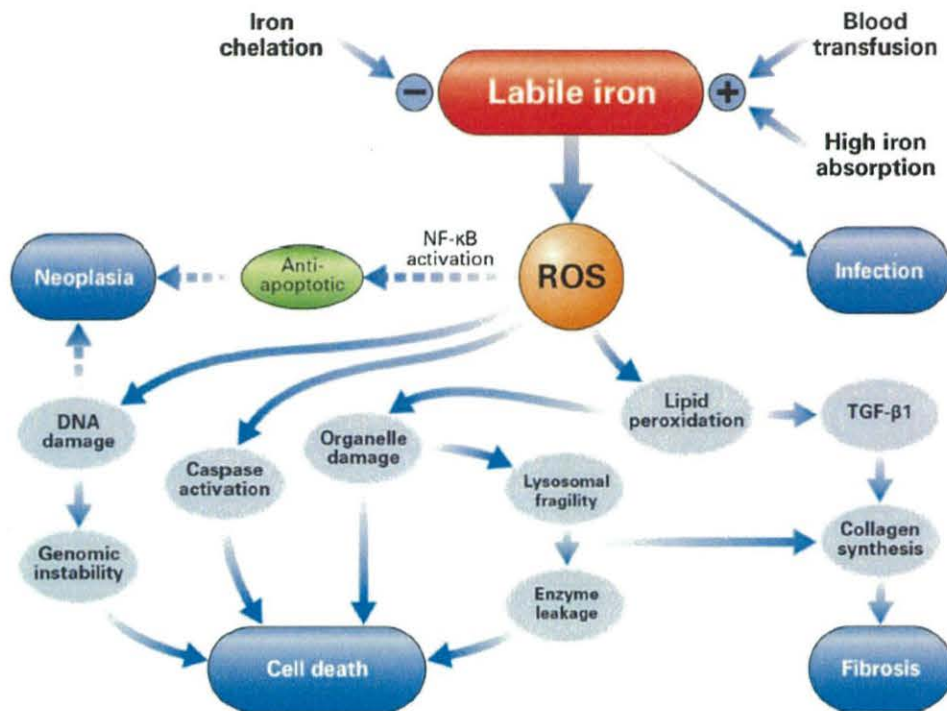


Figure 2.1: Mechanism of iron overload toxicity which showed how reactive oxygen species through labile iron leading to cell death, neoplasia, fibrosis and infection.

Obtained with permission from (Porter 2014)

2.4 Iron overload in the heart

In cardiac iron overload, iron accumulates in the ventricular wall, the epicardium, the papillary muscles and the ventricular septum. Iron accumulation occurs initially in the ventricular followed by the atrial myocardium (Buja *et al.*, 1971) which could manifest as first-degree heart block and supraventricular arrhythmias. Inside the myocytes, iron will be stored in the form of ferritin, haemosiderin, and labile cellular iron (free iron) which is the most active form and can cause the formation of reactive oxygen species and toxic hydroxyl radical (Murphy *et al.*, 2010).

Iron that was bounded to ferritin will be transported to lysosomes for degradation and long-term storage, which can be visualised by CMR and this form the basis of non-invasive monitoring in cardiac iron CMR T2* sequence. It has been proven that the concentration of heart iron is increased upon reaching serum ferritin levels higher than 1,800 µg/L, and the prevalence of cardiac events will be significantly increased when serum ferritin levels are more than 2,500 µg/L (Jensen *et al.*, 2003). In a retrospective study in Japan, 90% of patients with either cardiac or hepatic complications had high serum ferritin levels of more than 1,000 µg/L and are associated with liver and cardiac complications (Takatoku *et al.*, 2007). Other susceptible endocrine organs affected by iron toxicity includes anterior pituitary, thyroid, and liver which would results in multiple sequelae including hypogonadism, poor growth, diabetes, hypothyroidism, liver fibrosis, liver cirrhosis and hepatoma (Brognia-Pignatti, 2004). As iron store increases with age as the disease progress, factor such as aged mitochondria leading to reduce antioxidant mechanism thus leading to development of cardiac hypertrophy and age related cardiac damage (Fairweather-Tait SJ *et al.*, 2014).

2.5 Role of CMR in assessing cardiac siderosis

Contrary to popular believes, cardiac iron uptake and toxicity can occur despite adequate and normal total body iron balance (Porter 2001). Because of that, measurements such as electrocardiography, echocardiogram, or radionuclide angiography will not appear as toxic until severe cardiac iron deposition has occurred (Wood *et al.*, 2005). Subclinical iron overload can be measured through an invasive procedure of cardiac biopsy however it is prone for error in sampling or unacceptable sampling. A non-invasive assessment of cardiac iron via MRI technique called Cardiac Magnetic Resonance T2* sequence (CMR T2*) has the reliability to identify patients with subclinical cardiac iron overload and stratify their risk of subsequent cardiac dysfunction by measuring the half-life, T2*. The darkening cardiac muscle image produced by this magnetic imaging method means presence of active stored cardiac iron (Anderson *et al.*, 2001). The cardiac T2* measurement is inversely correlated with cardiac iron concentration (Wood *et al.*, 2005).

The relaxation time which was introduced a decade ago (Anderson *et al.*, 2001) has been the mainstay for the quantitative assessment of cardiac iron deposition in a CMR-derived T2* sequence (Wood, 2009). The survival improvement of thalassaemia major is attributed by the introduction of CMR-T2* imaging (Modell *et al.*, 2008). The principle of CMR T2* lies within the fact that within the heart, the iron was being stored in the form of haemosiderin and not by ferritin or labile cellular iron. These 3 constituents are within a continuous reflux between each other's forms in keeping the stored iron. This technique accurately predicts the tissue iron content (Wood 2009). As for the global representative of myocardial iron loading, the full thickness of interventricular septum had been usually measured in T2* sequence (Carpenter. *et al.*, 2011). The occurrence of

heart failure within 1 year was predicted to be of 47% with $T2^* < 6\text{ms}$, 21% in 6-10ms and 0.2% in $> 10\text{ms}$ (Kirk P. *et al.*, 2009).

The measurement of CMR $T2^*$ mainly reflects safely stored iron within the heart itself, explaining why an abnormal cardiac $T2^*$ values can occur despite a normal cardiac function in any given patient. This safely stored iron pool will result in a higher incidence/prevalence of cardiac decompensation at low $T2^*$ values as it is in dynamic equilibrium with labile myocyte iron (Anderson *et al.*, 2001). $T2^*$ values below 10ms predict a potentially lethal cardiac iron levels and cardiac dysfunction (Cheung *et al.*, 2008). Cardiac iron overload begins after as few as 75 units of blood that have been transfused in a chronically transfused patient without iron chelation therapy (Jensen *et al.*, 2003). In patients with iron chelation therapy, a minimum of 35g of exposures to transfusional iron (approximately 175 units of blood) will give rise to a cardiac iron loading especially at the second decade of life (Wood *et al.*, 2008). This calculation is based on the content of 0.5mg iron per mL of erythrocyte concentrate which makes a 400 unit of donated erythrocytes to contain about 200mg of iron. In β -thalassaemia major patients, cardiac iron overload correlates with extrahepatic iron toxicities such as diabetes or endocrines complications with a proportion of men to succumb to cardiac iron overload is higher than female, given the same transfusions history as well as serum ferritin (Borgna-Pignatti *et al.*, 2006). Almost half of the adult patient with β -thalassaemia major have a detectable cardiac iron despite being asymptomatic (Wood *et al.*, 2004). A level of 44-51g of iron were associated with identified end-point cardiac death. Other than the myocardial portrayal of iron loading, CMR $T2^*$ has a direct correlation with endothelial dysfunction and arterial stiffness causing increased in peripheral vascular resistance and

subsequently decreases left atrial size, left ventricular size and thus cardiac output (Tanner *et al.*, 2007).

Cardiac T2* values and the iron balance are complex as the mechanism and kinetics of cardiac iron uptake and clearance differ from the liver. There was no significant correlation with serum ferritin concentration and liver iron concentration (LIC) in cross sectional analysis done by Anderson and friends back in 2001. Because of these distinct aspects of iron storage pool, all the parameters of serum ferritin, LIC as well as CMR T2* should be measured thoroughly to monitor the total body iron and cardiac iron load. Myocardial T2* is said to be normal when the level of measurement is >20/ms, present when T2* is <20/ms, and considered as severe with T2* <10/ms. This is because the rate of heart failure increases dramatically below the threshold of <10ms (Anderson *et al.*, 2001). Iron overload cardiomyopathy diagnosis is made according to the following criteria namely; evidence of diastolic LV dysfunction with restrictive filling or LV remodelling with chamber dilatation and reduced LVEF, coexists with iron overload (serum ferritin >300ng/mL, transferrin saturation >55%) and cardiac siderosis (cardiac T2* <20ms) (Kremastinos *et al.*, 2010, Anderson *et al.*, 2001).

2.6 Left ventricular ejection fraction in Cardiac Magnetic Resonance

The superiority of CMR T2* when it comes to the diagnostic accuracy as well as reproducibility in comparison with echocardiography, has made CMR a gold standard in measuring the cardiac systolic function, LV volumes and mass despite the cost effectiveness of this method. Rather than depending on geometric models, the standard CMR-derived LVEF is calculated via volumetric coverage of the left ventricular chamber, which increases its accuracy as well as reproducibility. This is true not only for the evaluation of ejection fraction, but also for the LV volumes and masses. In 2012, a study by Joshi and colleagues showed that the potential clinical impact of using CMR to calculate EF upon comparing the EF calculated by CMR and by echocardiography in 52 patients undergoing assessment for implantable cardioverter defibrillator placement. CMR resulted in 11 out of 52 (21%) and 5 out of 52 (10%) of patients being reclassified from the echocardiographic-determined categories regarding implantable cardioverter defibrillator eligibility at the EF thresholds of 35 and 30% respectively. Another study by Bellenger and colleagues examined the reproducibility of LV masses, volumes, and EF in 20 heart failure patients and 20 controls and compare them between CMR versus echocardiography values. The results showed the variabilities obtained from the CMR values were less compare to echocardiography

The usage of CMR for measurement of LVEF is convenient as it is non-invasive, involves no exposure to radiation, has no poor echocardiographic window issue that can be faced during echocardiography and the feasibility of the procedure which can be done within 10 minutes time. The CMR cut off value for LVEF systolic dysfunction that can be used is <56% according to a study by Maceira *et al.* in 2006. The usage of CMR for LV

function as well as evaluation of specific infiltrative myopathy has been indicated as appropriate by American College of Cardiology Foundation 2006 as shown in Figure 2.1.

Table 4. All subjects: Left ventricular volumes, systolic function and mass (absolute and indexed to body surface area) by age decile (mean, 95% confidence interval)

All subjects	20–29 years	30–39 years	40–49 years	50–59 years	60–69 years	70–79 years
Absolute values						
EDV [mL] SD 21	153 (112, 193)	149 (108, 189)	144 (104, 185)	140 (100, 181)	136 (96, 177)	132 (91, 172)
ESV [mL] SD 10	53 (32, 73)	50 (30, 71)	48 (28, 69)	46 (26, 67)	44 (24, 65)	42 (21, 62)
SV [mL] SD 14	100 (72, 128)	98 (70, 126)	96 (68, 124)	94 (66, 122)	92 (64, 120)	90 (62, 118)
EF [%] SD 4.6	66 (57, 74)	66 (57, 75)	67 (58, 76)	67 (58, 76)	68 (59, 77)	69 (60, 77)
Mass [g] SD 19	127 (90, 164)	127 (90, 164)	127 (90, 164)	127 (90, 164)	127 (90, 164)	127 (90, 164)
Indexed to body surface area						
EDV /BSA [mL/m ²] SD 8.8	84 (67, 101)	81 (64, 98)	79 (62, 96)	76 (59, 93)	74 (57, 91)	71 (54, 88)
ESV /BSA [mL/m ²] SD 5.1	29 (19, 39)	28 (18, 38)	26 (16, 36)	25 (15, 35)	24 (14, 34)	22 (12, 32)
SV /BSA [mL/m ²] SD 6.2	55 (43, 67)	54 (42, 66)	52 (40, 65)	51 (39, 63)	50 (38, 62)	49 (37, 61)
Mass /BSA [g/m ²] SD 8.1	69 (53, 85)	69 (53, 85)	69 (53, 84)	68 (53, 84)	68 (52, 84)	68 (52, 84)

EDV, End-Diastolic Volume; ESV, End-Systolic Volume; SV, Stroke Volume; EF, Ejection Fraction; BSA, Body Surface Area; SD, Standard Deviation.

Figure 2.2: LV mass and volume reference values by CMR obtained from Maceira *et al.*,

2006

Table 19. Appropriate Indications (Median Score 7–9)

Indication		Appropriateness Criteria (Median Score)
Detection of CAD: Symptomatic—Evaluation of Chest Pain Syndrome (Use of Vasodilator Perfusion CMR or Dobutamine Stress Function CMR)		
3.	- Intermediate pre-test probability of CAD - ECG uninterpretable OR unable to exercise	A (7)
Detection of CAD: Symptomatic—Evaluation of Intra-Cardiac Structures (Use of MR Coronary Angiography)		
8.	Evaluation of suspected coronary anomalies	A (8)
Risk Assessment With Prior Test Results (Use of Vasodilator Perfusion CMR or Dobutamine Stress Function CMR)		
13.	- Coronary angiography (catheterization or CT) - Stenosis of unclear significance	A (7)
Structure and Function—Evaluation of Ventricular and Valvular Function Procedures may include LV/RV mass and volumes, MR angiography, quantification of valvular disease, and delayed contrast enhancement		
18.	- Assessment of complex congenital heart disease including anomalies of coronary circulation, great vessels, and cardiac chambers and valves - Procedures may include LV/RV mass and volumes, MR angiography, quantification of valvular disease, and contrast enhancement	A (9)
20.	- Evaluation of LV function following myocardial infarction OR in heart failure patients - Patients with technically limited images from echocardiogram	A (8)
21.	- Quantification of LV function - Discrepant information that is clinically significant from prior tests	A (8)
22.	- Evaluation of specific cardiomyopathies (infiltrative [amyloid, sarcoid], HCM, or due to cardiotoxic therapies) - Use of delayed enhancement	A (8)
23.	- Characterization of native and prosthetic cardiac valves—including planimetry of stenotic disease and quantification of regurgitant disease - Patients with technically limited images from echocardiogram or TEE	A (8)
24.	- Evaluation for arrhythmogenic right ventricular cardiomyopathy (ARVC) - Patients presenting with syncope or ventricular arrhythmia	A (9)
25.	- Evaluation of myocarditis or myocardial infarction with normal coronary arteries - Positive cardiac enzymes without obstructive atherosclerosis on angiography	A (8)
Structure and Function—Evaluation of Intra- and Extra-Cardiac Structures		
26.	- Evaluation of cardiac mass (suspected tumor or thrombus) - Use of contrast for perfusion and enhancement	A (9)
27.	Evaluation of pericardial conditions (pericardial mass, constrictive pericarditis)	A (8)
28.	Evaluation for aortic dissection	A (8)
29.	- Evaluation of pulmonary veins prior to radiofrequency ablation for atrial fibrillation - Left atrial and pulmonary venous anatomy including dimensions of veins for mapping purposes	A (8)
Detection of Myocardial Scar and Viability—Evaluation of Myocardial Scar (Use of Late Gadolinium Enhancement)		
30.	- To determine the location, and extent of myocardial necrosis including 'no reflow' regions - Post acute myocardial infarction	A (7)
32.	- To determine viability prior to revascularization - Establish likelihood of recovery of function with revascularization (PCI or CABG) or medical therapy	A (9)
33.	- To determine viability prior to revascularization - Viability assessment by SPECT or dobutamine echo has provided "equivocal or indeterminate" results	A (9)

Figure 2.3: 2006 Appropriateness Criteria for Cardiac Magnetic Resonance Imaging

from American College of Cardiology Foundation

2.7 Other modalities to assess cardiac siderosis

As the diastolic dysfunction of the heart may precede systolic dysfunction, the readily available echocardiography indices of diastolic function correlated poorly with EF and myocardial T2* in the transfusion-dependent thalassaemia patients and is not a well-suited risk stratification (Leonardi *et al.*, 2008). Findings that can be obtained from echocardiography used in screening patients with iron-loading conditions for heart disease include left and right ventricular systolic and diastolic dysfunction, pericardial and valvular involvement. Early findings in echocardiography includes impaired diastolic LV function featuring pseudo normalised or restrictive filling patterns, with or without left atrial enlargement meanwhile the advanced-stage disease is characterised by left and right cardiac chamber dilatation and reduced LVEF (dilated phenotypes), restrictive LV filling with left atrium and right ventricular dilatation, increased pulmonary artery pressure and preserved LVEF (restrictive phenotypes) (Aessopos *et al.*, 2004).

Other supportive investigations which might help to identify the patient with cardiac iron overload include electrocardiogram which allows the identification of supraventricular and ventricular arrhythmias and conduction system abnormalities (Jessup *et al.*, 1998) and also non-specific repolarisation abnormalities seen associated with intensive chelation therapy (Aessopos *et al.*, 2007).

Dilated cardiomyopathy secondary to iron overload is one of the sequelae of cardiac siderosis which can be detected via a plain chest X-ray, reflected as left ventricular enlargement. On the other hand, other cardiac complications of iron overload such as